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1

THE IMPACT OF AN INTRODUCED HOMING ENDONUCLEASE GENE ON A REGIONAL MOSQUITO POPULATION

Ace North, Charles Godfray

University of Oxford, Oxford, United Kingdom

Homing endonuclease genes (HEGs) exist naturally in many single-celled organisms and can show extremely strong genetic drive allowing them to spread through populations into which they are introduced. They are being investigated as tools to manipulate the populations of important vectors of human disease, in particular the mosquitoes that transmit malaria. Before HEGs can be deployed, it is important to study their spatial spread in order to design efficient release strategies. We recently analysed an individual-based simulation model which demonstrated that a HEG which acts to either male-bias the mosquito sex-ratio or reduce female fecundity could potentially eliminate local mosquito populations, as reported previously. Questions remain, however, about how readily this gene-drive mechanism will spread across larger landscapes comprised of numerous interacting local mosquito populations. In this talk I present a metapopulation model which explores how gene-drive, which facilitates the spread of a HEG, interacts with the local elimination of populations, which curtails spread. The model predicts that a HEG may persistently reduce the number of mosquito populations in a region although without causing global extinction. The load imposed by the HEG increases in a correlated landscape where mosquito population sites are clumped in space. Since the HEG effectively acts as a contagious and virulent pathogen on the mosquito population, the model can be summarised as an 'SIER' (Susceptible-Infected-Eliminated-Recolonised) metapopulation model. Implications of the model to other infectious diseases are discussed.

2

OPTIMIZING FIELD RELEASES OF ENGINEERED MALE MOSQUITOES

Peter Winskill[1], Andrew R. McKemey[2], Luke Alphey[2], Christl A. Donnelly[1]

[1]Imperial College London, London, United Kingdom, [2]Oxitec Ltd, Oxford, United Kingdom

Control of *Aedes* spp, the mosquito vectors of the dengue virus, remains the primary approach in the prevention of dengue. However, there is a lack of effective tools for controlling the vector, emphasising the importance of novel techniques. Engineering male mosquitoes that are functionally genetically sterile and releasing them to compete with wild males for a female mate is a modern alternative to the traditional sterile insect technique (SIT). Successful implementation of this approach in the field relies on efficacious release methods. One key objective of a sterile insect release is to obtain a well-balanced distribution of the released sterile insects at the required density across the targeted treatment area. Approaches that facilitate operational optimisation of releases are investigated and applied to a range of theoretical and real field scenarios. The distribution of sterile male release points in a theoretical arena is examined, leading to further investigation of the optimal placement of release points at a field site. For field releases, the primary objective of maximising the coverage of sterile males competes with secondary, limiting objectives such as the number of mosquitoes released or the number of release points that must be visited. Multiple objective particle-swarm optimisation (MOPSO) techniques are used to investigate the trade-offs between coverage and the cost of a release using a range of measures. For large-scale transgenic sterile insect programmes, releases may be conducted from moving vehicles. MOPSO is combined with an ant-colony optimisation procedure to estimate the most efficient driven route for sterile male releases at a field site.

3

INSIGHT ON OVIPOSITION CHOICE OF *ANOPHELES GAMBIAE* SENSU LATO PRESENTS A NEW FRONT FOR VECTOR CONTROL

Manuela Herrera-Varela[1], Jenny Lindh[2], Steve Lindsay[3], Ulrike Fillinger[4]

[1]International Centre for Insect Physiology and Ecology, Mbita, Kenya, [2]Royal Institute of Technology, Stockholm, Sweden, [3]Durham University, Durham, United Kingdom, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom

The two riskiest parts of a female mosquito's life are taking a blood meal and laying her eggs. Whilst there is a considerable body of work that has demonstrated that *Anopheles gambiae* is attracted to human subjects by the volatiles produced by humans, next to nothing is known about how they locate a breeding site. Here we presented the results of a case-control study of potential breeding sites to identify the characteristics of habitats associated with the presence of *Anopheles gambiae* sensu lato, the principal African malaria vector. A cross-sectional study of aquatic habitats with anopheline larvae (cases) and without larvae (controls) was carried out in Rusinga Island, on Lake Victoria, Kenya. Factors evaluated include biological characteristics of the sites, zooplankton, invertebrate fauna, physical parameters, nutrients, bacteria communities and volatile chemicals released from the water. Characteristics of 120 habitats (74 cases and 46 controls) were analyzed between March and July 2012. Data were analysed using a random forest model. The presence of early instar larvae was associated with habitats located within 100 meters of the lake. Preferred habitats of *An. gambiae* s.l. were characterised by increasing content of phosphates, conductivity and turbidity beyond 200 NTU. Late instar culicines and small crustaceans of the orders Cyclopoida and Cladocera were also abundant in these sites. Contrary to previous report invertebrate predators of the orders Odonata, Coleoptera and Heteroptera were common in anopheline habitats. Volatile chemicals released from the water headspace were less diverse and released in lower concentration from cases than from controls. This study demonstrates that *Anopheles gambiae* select breeding sites with specific characteristics These factors can potentially be used to target breeding habitats for larval control or manipulated to attract and kill gravid females.

4

HIGH LINKAGE DISEQUILIBRIUM IN *ANOPHELES GAMBIAE* FROM KILIFI, KENYA IS CONSISTENT WITH A RECENT REDUCTION IN POPULATION SIZE

Samantha M. O'Loughlin

Imperial College, London, United Kingdom

We used RADseq to genotype ~6000 SNPs in An. gambiae collected from Kilifi, Kenya and Muheza Tanzania. Linkage disequilibrium (LD) was higher but genetic diversity only slightly lower in Kilifi compared with Muheza. We investigated the relationship between rho (population recombination parameter) and theta (genetic diversity). In the Kilifi samples the ratio rho/theta departed from neutral expectations. Simulations showed that a recent, severe population reduction gives a significant reduction in rho without an equivalent change in theta. This is consistent with the rho/theta observed in Kilifi. It has been previously observed that abundance of An. gambiae has been reduced in the Kilifi district in the last 10-15 years; our results suggest that the population size change can be detected in the population genomics of the remaining mosquitoes. This result shows that monitoring linkage disequilibrium in vector populations may be an effective way of tracking recent changes in population size, due to mosquito control interventions or climate change.

5

UNBIASED CHARACTERIZATION OF *ANOPHELES* MOSQUITO BLOODMEALS BY TARGETED HIGH-THROUGHPUT SEQUENCING OF 16S RRNA GENES

Kyle Logue[1], John Bosco[2], Edward Walker[2], Peter Siba[3], Peter Zimmerman[1], David Serre[4]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Michigan State University, East Lansing, MI, United States, [3]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea, [4]The Cleveland Clinic, Cleveland, OH, United States

Understanding mosquito blood feeding behavior is important for assessing vector competence or identifying possible reservoir hosts. To date, there is no unbiased method of evaluating mammalian host bloodmeals from mosquitoes and most current molecular assays are designed to test whether a mosquito fed on *a priori* selected species. Here, we describe a targeted high-throughput sequencing method that utilizes a universal primer pair, which amplifies mammalian mitochondrial 16S ribosomal genes, to identify host bloodmeals from female mosquitoes. Our assay enables analyzing 96 mosquitoes simultaneously and, with more than 1,000 sequences generated per mosquito, provides a comprehensive and quantitative perspective on each blood meal composition. We applied our approach to 529 blood-fed female *Anopheles* collected from five villages in Papua New Guinea. 483 mosquitoes (91%) yielded a successful PCR product and, after sequencing, showed that these *Anopheles* mosquitoes fed almost exclusively on humans, dogs and pigs. Interestingly, 68 mosquitoes (14%) showed clear evidence of having fed on two or more species. In one village, where similar number of mosquitoes were collected on both sides of an erected barrier screen, we observed that mosquitoes fed more often on humans on the village versus the bush side of the net (p=0.001). Our analyses also showed that *An. farauti s.s* . fed more often on humans in the village of Matukar than in Mirap (p=8.7e-9), revealing potential behavioral differences between these populations. Overall, our study shows that this assay enables us to objectively identify host bloodmeals from female *Anopheles* mosquitoes and to discover feeding behavior differences. This approach is generalizable to any insects that feed on mammalian blood and can be applied to improve our understanding of a wide variety of infectious diseases.

6

SOUTH AUSTRALIA'S FIRST LARGE SCALE ARBOVIRUS SURVEY: SPATIAL ANALYSIS OF SENSITIVE FTA® CARD METHOD

Emily Johnston[1], Cheryl Toi[2], Philip Weinstein[1], Stephen Doggett[2], Craig Williams[1]

[1]University of South Australia, Adelaide, Australia, [2]Westmead Hospital, Westmead, Australia

Every year in Australia, over 6000 people become infected with a mosquito-borne virus (arbovirus) including Ross River virus (RRV) and Barmah Forest virus (BFV), among others. The most prevalent of these viruses is RRV which causes prolonged symptoms of fatigue, muscle soreness and polyarthritic joint pain and costs Australians tens of millions annually in diagnosis, prevention and treatment. Approximately 250 cases of human infection are reported every year in the state of South Australia. Until recently, the methods for conducting arbovirus surveillance made large scale surveys logistically and economically difficult. A modern development has capitalized on the nectar-feeding behaviour of mosquitoes to collect abroviruses on nucleic acid-preserving cards (FTA® cards), thus streamlining the virus surveillance process. We adapted this technique to an existing mosquito surveillance program and conducted the first ever large-scale arbovirus survey in South Australia. During two months of the peak virus season (January/February 2014), we set CO2-baited EVS light traps at 100 locations around South Australia with a honey-baited FTA® card inside. After collection, mosquitoes were kept in a humid environment and given one week to feed on the honey-soaked card. Cards were then tested for arboviruses using a nested PCR. We made 23 virus detections from around South Australia including 14 of RRV, 6 of BFV and 3 of Stratford virus (not previously reported from South Australia). This level of detection suggests that our low-budget method is highly sensitive at detecting infectious mosquitoes. In this presentation, we report on our methods, the resulting detections (including pending results from March) and the mosquitoes associated with the detections. We also will present a spatial analysis of how these detections correlate with human case data from the last 20 years and explore the implications of our method and findings.

7

IDENTIFICATION OF PROXIMATE INDICATORS OF MALE MATING PERFORMANCE IN *ANOPHELES GAMBIAE*

Rosemary S. Lees[1], Mark Q. Benedict[2]

[1]Polo d'Innovazione Genomica, Genetica e Biologia S.C.a.R.L., Perugia, Italy, [2]Department of Experimental Medicine, Perugia, Italy

Releases of male mosquitoes for population control or replacement strategies rely on those males successfully mating with wild females, in competition with wild male counterparts. Currently performance of strains is tested in competition assays, in laboratory cages then in increasingly large and complex mating arenas. The extent to which these results reflect how well a strain will perform in the field is unclear. A quick, small-scale assay to predict which strains will fail to meet the required standard to compete with wild males would save time and cost in this lengthy process. I have optimised a method to measure the mating performance of single male *Anopheles gambiae*, allowing the characteristics of more and less successful males to be compared on an individual basis. Cohorts of males were were reared, selected or categorized according to characteristics thought to be important for the mating success or competitiveness of male mosquitoes, for example longevity or size. The relative performance of phenotypically distinct males was measured, and these correlations validated in competition assays on a larger scale, in an attempt to identify those male characteristics that are most predictive of male performance. I have also addressed through competition assays the question of whether assortative mating occurs based on, for example, adult size. If evidence for this is seen it would indicate that an understanding of the wild female population and the production of compatible males would give an advantage over the current strategy of simply producing the largest and nutritionally best prepared release cohort possible under mass rearing conditions.

8

CHIKUNGUNYA INCIDENCE AND CORRELATION WITH PROTECTION IN A PROSPECTIVE LONGITUDINAL COHORT IN THE PHILIPPINES

In-Kyu Yoon[1], Maria T. Alera[1], Stefan Fernandez[1], Jens Levy[1], Catherine Lago[1], Butsaya Thaisomboonsuk[1], Chonticha Klungthong[1], Ananda Nisalak[1], Louis Macareo[1], Anon Srikiatkhachorn[2]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]University of Massachusetts Medical School, Worcester, MA, United States

Chikungunya is a re-emerging arboviral disease that has caused increasing epidemics and recently appeared for the first time in the Americas. However, the true incidence and clinical spectrum of chikungunya virus (CHIKV) infection have not been well established. In addition, although CHIKV antibodies have been suggested as being protective, no correlation with protection has been demonstrated in humans. In March 2012, we initiated a longitudinal prospective cohort of approximately 1000 subjects aged ≥6 months in Cebu, Philippines, which underwent community-based active surveillance for febrile episodes. Acute and 3-week convalescent blood samples were obtained and tested by CHIKV RT-PCR and CHIKV hemagglutination inhibition assay (HAI). Enrollment and 12-month follow up samples were tested by CHIKV HAI to identify subclinical

seroconversion. During one year of surveillance, the annual incidence of total and symptomatic CHIKV infection in the cohort was approximately 8% and 2%, respectively. The total and symptomatic incidence in the 6 month-5 year old age group was 7% and 3%; 6-15 years was 8% and 4%; 16-30 years was 9% and 1%; 31-50 years was 9% and 1.5%; >50 years was 5% and 0.5%. The total and symptomatic incidence among 672 subjects with negative CHIKV HAI titer (≤10) at enrollment was 9% and 2.5%; and among 181 subjects with positive CHIKV HAI titer (>10) was 0.5% and 0%. Subjects with negative CHIKV HAI at enrollment were more likely than those with positive HAI to have CHIKV infection (Fisher's exact, p=0.0001) and symptomatic CHIKV infection (Fisher's exact, p=0.019). Our results demonstrate that CHIKV infection is a common endemic infection across all age groups in the Philippines, but a greater proportion of those ≤15 years old are symptomatic. CHIKV HAI titer >10 is correlated with protection against both symptomatic and subclinical CHIKV infection. Our findings have important implications for assessment of CHIKV disease burden, understanding of virus transmission, and development of candidate vaccines.

9

HIGH RATES OF O'NYONG NYONG AND CHIKUNGUNYA VIRUS TRANSMISSION IN COASTAL KENYA

A. Desiree LaBeaud[1], Tamara Banda[1], Francis Mutuku[2], Julie Brichard[1], Peter Mungai[3], Sarah Pfeil[1], Erin M. Borland[4], Ann Powers[4], Ginny Gildengorin[1], Crystal Teng[1], Eric Muchiri[3], Kristin Long[5], Mark Heise[5], Uriel Kitron[6], Charles King[7]

[1]Children's Hospital Oakland Research Institute, Oakland, CA, United States, [2]Technical University of Mombasa, Mombasa, Kenya, [3]Division of Vector Borne and Neglected Tropical Diseases, Msambweni, Kenya, [4]Centers for Disease Control and Prevention, Division of Vector-Borne Diseases, Fort Collins, CO, United States, [5]University of North Carolina, Chapel Hill, NC, United States, [6]Emory University, Atlanta, GA, United States, [7]Case Western Reserve University, Cleveland, OH, United States

Chikungunya virus (CHIKV) and o'nyong nyong virus (ONNV) are mosquito-borne alphaviruses endemic in Kenya that cause acute febrile illness and arthritis. The objective of this study was to measure the seroprevalence of CHIKV and ONNV in coastal Kenya and link it to demographics and other risk factors. Demographic and exposure questionnaires were administered to 1,862 participants recruited from two village clusters (Milalani-Nganja and Vuga) in 2009. Sera were tested for alphavirus exposure using standardized CHIKV IgG ELISA protocols and confirmed with plaque reduction neutralization tests (PRNT). Logistic regression models were used to determine the variables associated with seropositivity. G statistic and kernel density mapping were used to identify locations of high seroprevalence. Weighted K test for global clustering of houses with alphavirus positive participants was performed for distance ranges of 50-1,000 meters. 486 (26%) participants were seropositive (1-79 years, mean 27 years). Of 443 PRNT confirmed positives, 24 samples (5%) were CHIKV, 246 samples (56%) were ONNV, and 173 samples (39%) had equally high PRNT titers for both CHIKV and ONNV. Age was significantly associated with seropositivity (OR 1.01 per year, 95% C.I. 1.00-1.01). Males were less likely to be seropositive (p<0.05; OR 0.79, 95% C.I. 0.64-0.97). Adults who owned a bicycle (p<0.05; OR 1.37, 95% C.I. 1.00-1.85) or motor vehicle (p<0.05; OR 4.64, 95% C.I. 1.19-18.05) were more likely to be seropositive. Spatial analysis demonstrated hotspots of transmission within each village and clustering among local households in Milalani-Nganja, peaking at the 200-500m range. Alphavirus exposure is common in coastal Kenya with ongoing interepidemic transmission of both ONNV and CHIKV. Women, adults and those with higher socioeconomic status were more likely to be seropositive. Household may be one of the defining locations for the ecology of alphaviral transmission in this region, given our spatial analysis results and the fact that anophelines transmit ONNV. Human disease caused by ONNV and CHIKV in this region is being missed in clinical settings.

10

DETERMINANTS OF CHIKUNGUNYA EMERGENCE POTENTIAL IN SOUTH FLORIDA

Rebecca C. Christofferson[1], Helen J. Wearing[2], Christopher N. Mores[1]

[1]Louisiana State University, Baton Rouge, LA, United States, [2]University of New Mexico, Albuquerque, NM, United States

Arbovirus transmission is dependent on the transmission efficiency between infectious and susceptible hosts. Heterogeneity of virus acquisition from a viremic human to a susceptible mosquito is often assumed to be nearly perfect and almost always uniform across the infectious period. Chikungunya transmission models often do not account for the variation in infectiousness of a single individual, and subsequent infection of naïve mosquitoes. Understanding the contribution of human infectious heterogeneity is especially important in the context of introduction events where an infected individual carries the virus into a population(s) of competent vectors. We construct a stochastic, compartmental model to describe the heterogeneity of human viremia and calculate the probability of a successful introduction, taking into account the viremia level (and thus acquisition potential) of the index case on, and after, the day of introduction into a susceptible population. We inform our model based on the experience of dengue emergence in South Florida, 2009-2013. We further account for viral genotype and predominant vector (Ae. aegypti versus Ae. albopictus) by altering the transmission efficiency rate between the human and specific mosquito populations by exploring 1) differential vector competence between genotypes of chikungunya and 2) differential contact rates within and between the two mosquito populations. We find that the infectivity of the index case as well as the parameters affecting transmission efficiency affected the probability of emergence, but that the effects among these parameters were not equal. To predict the likelihood and magnitude of a potential chikungunya outbreak in the United States, it is critical to understand the interplay between individual human heterogeneity of infectiousness and the transmission efficiency of the virus and vector populations.

11

PRECLINICAL EVALUATION OF A LIVE ATTENUATED CHIKUNGUNYA VACCINE

Jill A. Livengood[1], Charalambos D. Partidos[2], Haiyan Chu[2], Subash Das[2], Chad Roy[3], Laszlo Varga[1], Dan T. Stinchcomb[1], Jorge E. Osorio[2], Scott C. Weaver[4]

[1]Takeda, Fort Collins, CO, United States, [2]Takeda, Madison, WI, United States, [3]Tulane, Covington, LA, United States, [4]University of Texas Medical Branch, Galveston, TX, United States

Recently, Chikungunya virus (CHIKV) has re-emerged to cause major epidemics in the Indian Ocean, India and Southeast Asia, infecting millions. Autochthonous transmission in Italy and France after CHIKV introductions via travellers and the recent outbreak in the Caribbean highlight the threat of wide spread chikungunya transmission. We used a molecular attenuation approach, inserting a picornavirus internal ribosome entry site (IRES) to replace the subgenomic promoter, to generate a live attenuated vaccine strain (CHIKV/IRES). The CHIKV/IRES vaccine provides robust immunity and protection in murine models, and is incapable of infecting mosquito vectors, an important safety feature for use in nonendemic locations. The CHIK vaccine elicited a robust memory $CD4^+$ and $CD8^+$ T cell response. However, adoptively transferred immune T cells did not protect against wtCHIKV-LR challenge. In contrast, passive immunization with anti-CHIKV/IRES immune serum provided protection, and a correlate of a minimum protective neutralizing antibody titer was established. Vaccination of cynomolgus macaques with a single dose produced no signs of disease but was highly immunogenic. After challenge with wild-type CHIKV, the vaccine prevented the development of detectable viremia and signs of clinical CHIKV disease. We also have established processes and assays for high-titer manufacture at a large scale. The CHIKV/IRES

vaccine candidate is safe, immunogenic and efficacious in multiple animal models, supporting its potential as a human vaccine to protect against CHIKV infection and reduce transmission and further spread. Current pre-clinical development efforts are aiming to complete the Investigational New Drug (IND)-enabling studies necessary to begin human clinical testing.

12

CLINICAL RESULTS OF NOVEL CHIKUNGUNYA VACCINE TESTED IN PHASE 1/2 TRIAL: NEUTRALIZING ANTIBODIES AND ANTI-VECTOR IMMUNITY

Katrin Ramsauer[1], Philippe Despres[2], Christa Firbas[3], Matthias Müllner[1], Frederic Tangy[2], **Erich Tauber**[1]

[1]Themis Bioscience GmbH, Vienna, Austria, [2]Institute Pasteur, Paris, France, [3]Medical University of Vienna, Vienna, Austria

We show here a first in man phase - 1/2 clinical trial to demonstrate the immunogenicity, safety and tolerability of a novel chikungunya virus vaccine. Chikungunya Virus is an emerging mosquito borne virus that causes a severe polyarthritis in humans. The virus is endemic in nearly 40 countries in Asia, Africa, Europe, and recently also in the Americas posing a major threat to public health. So far there is no vaccine approved for human use that prevents the disease. Nucleotide sequences encoding the Chikungunya virus capsid and envelope structural proteins were inserted into the Schwarz vaccine strain of measles virus to produce the candidate vaccine MV-CHIK. To evaluate the optimal dose of MV-CHIK in regard to immunogenicity, safety and tolerability we performed an observer blinded, block-randomized, active and placebo-controlled, dose escalation, phase 1 trial in 42 healthy volunteer subjects. All subjects received three i.m. injections (days 0, 28, 90), the first injection was a vaccine. Subjects were block-randomized to MV-CHIK or to the control-vaccine Priorix® (MV-CHIK: n=12, Priorix: n=2/ Cohort). All volunteers were additionally randomized to one of two treatment sequences, vaccination in day 0 and 28 or 0 and 90. The Immunogenicity on day 28 after vaccination was confirmed by the presence of functional antibodies as determined by the plaque reduction neutralization test (PRNT50). Preliminary findings point at excellent safety and immunogenicity profile in all three doses tested. Data are currently under final evaluation and auditing, and will be presented here for the first time.

13

A MULTIAGENT ENCEPHALITIC ALPHAVIRUS DNA VACCINE DELIVERED BY ELECTROPORATION ELICITS PROTECTIVE IMMUNITY AGAINST AEROSOL CHALLENGE WITH EASTERN AND WESTERN EQUINE ENCEPHALITIS VIRUSES IN NONHUMAN PRIMATES

Lesley C. Dupuy[1], Callie E. Bounds[1], Michelle J. Richards[1], Drew Hannaman[2], Connie S. Schmaljohn[1]

[1]U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, United States, [2]Ichor Medical Systems, San Diego, CA, United States

Next-generation vaccines that can safely and effectively protect against Venezuelan, eastern, and western equine encephalitis virus (VEEV, EEEV, and WEEV) are needed. Previously, we demonstrated that monovalent or a trivalent combination of DNA plasmids expressing codon-optimized envelope glycoprotein genes of VEEV, EEEV, or WEEV delivered by intramuscular (IM) electroporation (EP) elicited high levels of virus-neutralizing antibodies in multiple animal species and provided protective immunity against lethal aerosol homologous viral challenge in mice. Currently, we have completed studies to further assess the monovalent and trivalent DNA vaccines delivered by IM EP in nonhuman primate (NHP) models of aerosol EEEV and WEEV challenge. Although some neurological signs of disease were observed after aerosol EEEV challenge in three of four NHPs that received the monovalent EEEV DNA vaccine, these were of lesser severity than those observed in the negative control animals and all survived. Interestingly, no significant clinical signs of disease were observed after challenge in the NHPs that received the trivalent DNA vaccine. While one of four NHPs that received the monovalent WEEV DNA vaccine displayed clinical signs of disease similar to those observed in the majority of the negative control animals after aerosol WEEV challenge, the remaining animals from this group only displayed mild clinical manifestations. Similar to results observed for the EEEV NHP challenge study, no significant clinical signs of disease were observed in any of the NHPs that received the trivalent DNA vaccine. We are currently investigating the apparent synergistic protective effect achieved with the trivalent DNA vaccine in further detail. Taken together, the results of our current studies further demonstrate that IM EP delivery of a multiagent formulation of VEEV, EEEV, and WEEV DNA vaccines represents a potent means of protecting against aerosolized encephalitic alphavirus infections and support its continued development into a mature vaccine candidate suitable for future clinical testing.

14

A MURINE MODEL FOR ACUTE AND CHRONIC, FLARING CHIKUNGUNYA VIRUS-INDUCED DISEASE

Christina L. Gardner, Chengqun Sun, Matthew B. Rogers, Hanqi Tang, Derek W. Trobaugh, Alan M. Watson, Elodie Ghedin, William B. Klimstra, Kate D. Ryman

University of Pittsburgh, Pittsburgh, PA, United States

Chikungunya virus (CHIKV) has gained notoriety in recent years with the explosive pandemic outbreak that has afflicted of over six million people in Africa, Indian Ocean, Asia, southern Europe, and recently the Caribbean and South American mainland. CHIKV is a mosquito-borne, enveloped, single-stranded, positive-sense RNA virus that causes fever, rash, and chronic polyarthralgia/polyarthritis in humans, often accompanied by incapacitating joint and muscle pain that can last anywhere from weeks to years. Currently there are no licensed vaccines or antiviral therapies against CHIKV. The development of a murine model that mimics both the acute and chronic, flaring phases of disease is critical to our understanding of this disease but has proven difficult. Current murine models mimic features of the acute phase but overt chronic disease has not been observed. To improve the model, we adapted to mice a wild-type strain of CHIKV by *in vivo* passage in musculoskeletal tissue. During the acute disease, the mouse-adapted strain of CHIKV causes swelling of both the inoculated and opposite footpad, which does not occur with the wild-type CHIKV in immunocompetent mice. Furthermore, mouse-adapted CHIKV induced chronic disease in the mice with flaring of musculoskeletal disease and swelling around three weeks post infection with chronic swelling lasting for months. Preliminary data indicate that markers of human chronic CHIKV disease are also present in our model (e.g. CCL2 and CXCL8). Recent identification of a single nucleotide change that confers this phenotype suggests possible mechanisms by which chronic disease may arise which are currently being investigated. Importantly, this model will also aid in the testing of candidate vaccines and antiviral therapeutics targeting long-term sequelae of CHIKV infection.

15

A MOUSE MODEL OF HIV AND CEREBRAL MALARIA CO-INFECTION

Sarah Hochman, Harris Goldstein, Sunhee Lee, Kami Kim

Albert Einstein College of Medicine, Bronx, NY, United States

Experimental cerebral malaria (ECM) models are controversial because leukocytes and platelets accumulate in rodent brain vasculature, but sequestration of parasitized red blood cells as described in human cerebral malaria (CM) has been inconsistently observed. We have found that HIV is a risk factor for pediatric CM and that intravascular platelets and monocytes are prominent in the brains of children with fatal CM. We revisited the ECM model to study co-infection, infecting mice transgenic for a HIV-1 provirus and for the human cyclin T1 promoter with *Plasmodium berghei* ANKA. 56 mice (31 transgenic "HIVJR-CSF", 25 littermate controls) age 6 weeks-4 months were infected IV with 105

P. berghei ANKA. Mice were assessed for development of CM daily using a standard behavioral scale and were sacrificed when severely impaired. Mice that did not develop CM were sacrificed when they became less active and severely anemic. Brains were harvested for histopathologic analysis. All mice developed peripheral parasitemia. During early infection HIVJR-CSF mice had higher mean parasitemia than controls (0.79% vs. 0.32% on day 4 post-infection, p=0.014), but there was no difference by day 7 when signs of CM develop. HIV viral load increased during malaria infection, from 80,000 copies/ml at baseline to 1.7 million copies/ml on day 7 (p=0.05). Evaluation of H&E stained sections on a subset of mice with CM found that all control mice and only 41% of HIVJR-CSF mice had ≥2 microhemorrhages/100 high power fields while 33% of HIVJR-CSF mice had no visible hemorrhages, similar to pathology patterns noted in our pediatric CM studies. Quantification of intravascular platelets and monocytes is ongoing. While there was no difference in incidence of CM (68% for HIVJR-CSF mice and 52% for controls, p=0.23) in young mice, older adult HIV transgenic mice continued to develop CM, unlike age-matched littermates. The mouse model may be a powerful tool to understand the pathophysiology of pediatric CM and HIV co-infection.

16

CD47 MEDIATED PARASITE CLEARANCE IN *PLASMODIUM YOELII* MALARIA

Rajdeep Banerjee[1], Sanjay Khandelwal[2], Yukiko Kozakai[1], Sanjai Kumar[1]

[1]Food and Drug Administration, Kensington, MD, United States, [2]Duke University, Durham, NC, United States

Many *Plasmodium* species, including a few human malarias that cause a relatively less severe infection have a strong preference to invade and grow inside young red blood cells (RBC). We utilized the murine GFP-*Plasmodium yoelii* 17XNL, which predominantly invades young RBC, to identify the host factors responsible for the preferential RBC infection and a possible mechanism for the potential cellular advantage for this preferential infection. CD47 or Integrin Associated Protein is a marker of self on most cells including RBC that, in conjugation with its receptor SIRP-α, prevents the phagocytosis of RBC. By injections of biotin in C57Bl/6 mice, we were able to differentiate between the young and old RBC by flowcytometry. On day 9 post infection (PI) with GFP-PyNL, young RBC had significantly higher parasitemia than old RBC (young RBC 45.1 ± 11.8 vs. old RBC 1.05 ± 0.8, p<0.001) and old RBC, young RBC had significantly higher CD47 (mean fluorescence intensity, young RBC 2373 ± 139 and old RBC 1156 ± 24, p<0.001). Importantly, infected RBC had higher CD47 levels than non-infected RBC throughout the course of GFP-PyNL infection and higher CD47 levels on RBC also associated with the higher parasitemia. C57Bl/6 $CD47^{-/-}$ mice resolved their infection at an accelerated rate (wt on 17 PI vs. $CD47^{-/-}$ on 15 PI) and developed significantly lower parasite burden (day 9, 2.3 ± 0.7 % vs. 26.48 ± 3.9 %, p<0.001). FACS analysis revealed a higher percentage of splenic F4/80 population in $CD47^{-/-}$ mice (day 7, 4.2 ± 0.4% $CD47^{-/-}$ vs. 2.3 ± 0.9 % wt , p< 0.05) that also had a higher percentage of phagocytized infected RBC (13.6 ± 1.7 % CD47-/- vs. 3.7 ±0.7 % wt, p<0.01). Furthermore, Injection of CD47 neutralizing antibody caused a significantly reduction in parasite burden in wt C57Bl/6 mice. Together, these results strongly suggest that CD47 is important for parasite growth and survival and, through the mechanism of lower phagocytic clearance of infected RBC, may be mediator of immune escape to avoid the splenic clearance of infected RBC.

17

PLASMODIUM SPOROZOITES DIRECTLY TARGET HEPATOCYTE EPHA2 RECEPTOR FOR SUCCESSFUL HOST INFECTION

Alexis Kaushansky, Nadia Arang, Alyse Douglass, Heather Kain, Vladimir Vigdorovich, Laura Austin, D. Noah Sather, Stefan H. Kappe

Seattle Biomedical Research Institute, Seattle, WA, United States

After mosquito transmission, selection of a suitable host hepatocyte to support the intracellular liver stage malaria parasite is an essential early step in infection. Yet, the molecular recognition conduits by which invasive *Plasmodium* sporozoites select a suitable target cell remain largely unknown. Here, we show that sporozoites engage the hepatocyte EphA2 receptor for infection, preferring $EphA2^{high}$ hepatocytes as their hosts. This is mediated by a direct interaction between the parasite ligand P52 and EphA2, which occurs at the point of invasion and is critical to establish a permissive intracellular replication niche. When EphA2 is deleted, liver stage infection is dramatically reduced in a mouse model of malaria. Deletion of P52 in the parasite mirrors this host phenotype resulting in the loss of selectivity for hepatocytes that express high levels of EphA2 and are permissive to productive parasite development. Taken together, the data provide mechanistic insights into host cell selection at the point of infection.

18

GLUCOSE STARVATION REVEALS EVIDENCE OF A NOVEL FATTY ACID OXIDATION PATHWAY IN *PLASMODIUM FALCIPARUM*

Allison Demas[1], Selina Bopp[1], Pamela Magistrado[1], Sarah Volkman[1], Ulf Ribacke[2], Dyann F. Wirth[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Uppsala University, Uppsala, Sweden

Plasmodium falciparum has finely tuned metabolic pathways to support growth in the various nutrient environments of its complex lifecycle. Despite its metabolic importance, relatively little is known about fatty acid (FA) metabolism in *Plasmodium*. Using ^3H hypoxanthine incorporation assays and SYBR Green-based FACS, we show reduced growth of 3D7 in limited FA media, and titrating glucose and glutamine to 40-45% of normal levels causes additional growth restriction. Knockdown of members of the acyl CoA synthetase (ACS) gene family further reduces growth in restricted glucose and FAs, an intriguing finding, as the ACS enzymes are known to mediate FA scavenging and activation. This observation prompted us to investigate a metabolic link between the exogenous FA substrates of ACSs and carbon sources used to generate ATP in the parasite. We determined that glucose-deprived parasites exhibit FA oxidation, as measured by release of $^3H_20$ from parasites grown in ^3H labeled FAs. Following two cycles of glucose restriction, there was an 18-24 fold increase in FA oxidation as compared to uninfected red blood cells. Parasites grown in the standard glucose concentration of RPMI1640 did not exhibit FA oxidation, suggesting that typical *in vitro* culture conditions mask this metabolic phenomenon. To our knowledge, this is the first reported experimental evidence for FA oxidation in *Plasmodium*. The canonical FA oxidation enzymes are not annotated in *P. falciparum*, though the pathway is believed to be present in *Toxoplasma gondii*. Our data suggests that glucose restriction redirects FAs into a beta-oxidation pathway to generate ATP. Furthermore, this important role for exogenous FAs offers a potential explanation for the previously reported expansion and positive selection of the ACS gene family in *P. falciparum*. Ongoing experiments will reveal the physiological importance of this catabolic pathway and shift in energy metabolism. Additional characterization of the molecular mechanisms may reveal novel targets for development of antimalarials.

19

GENOMIC ASSESSMENT OF *PLASMODIUM FALCIPARUM* POPULATION STRUCTURE AND DIVERSITY DURING AN ENHANCED MALARIA CONTROL PROGRAM IN WESTERN KENYA

Harold Ocholla[1], Simon Kariuki[1], Feiko ter Kuile[1], Sarah K. Volkman[2]

[1]KEMRI/Centers for Disease Control and Prevention, Kisumu, Kenya, [2]Havard School of Public Health, Boston, MA, United States

Malaria Control and Elimination Partnership in Africa is working toward aggressive malaria transmission reduction in Siaya County (western Kenya). The application of population genetic approaches to understand and estimate transmission dynamics in the context of malaria elimination in this setting will be crucial. To monitor changes in *Plasmodium falciparum* population structure in this population, we are applying estimates of parasite genetic diversity and relatedness, and assessing key biomarkers, such as those related to drug resistance that may impact efforts to reduce the malaria burden toward eradication. Additionally, we aim to provide a genetic map of parasite movement between Siaya County, and other parts of Kenya (e.g., Nairobi and central regions) and eastern Uganda where substantial movement of parasites have been spatially quantified through population movement studies.

20

SUSCEPTIBILITY WEIGHTED IMAGING IN PEDIATRIC CEREBRAL MALARIA AT 1.5T

Samuel Kampondeni[1], Gretchen L. Birbeck[2], Danny Milner[3], Mark Haacke[4], S. Sinyangwe[5], M. Mwenechanya[5], Karl Seydel[6], E. Zeli[7], Colleen Hammond[6], D. Utrianen[8], Terrie E. Taylor[6], Michael J. Potchen[2]

[1]Queen Elizabeth Central Hospital, University of Malawi, Malawi, [2]University of Rochester, Rochester, NY, United States, [3]Harvard Medical School, Boston, MA, United States, [4]Wayne State University, Detroit, MI, United States, [5]University Teaching Hospital, Lusaka, Zambia, [6]Michigan State University, East Lansing, MI, United States, [7]Cancer Disease Hospital, Lusaka, Zambia, [8]Magnetic Resonance Innovations, Detroit, MI, United States

The pathogenesis of Pediatric Cerebral Malaria (PCM), a leading killer of children in sub-Saharan Africa, is unknown. The pathological hallmark of the disease is sequestration of parasitized red blood cells in brain microcirculation. Intravascular hemozoin, a ferromagnetic breakdown product of hemoglobin metabolism and microhemorrhages in various structures, including the white matter and grey-white junction, have been reported. We imaged Zambian children with cerebral malaria on a 1.5T magnet to investigate the distribution of iron breakdown products from hemoglobin metabolism and BBB breakdown. Twelve children with strictly defined PCM (peripheral blood slide positive and retinopathy positive) were scanned at 1.5T. Eleven received intravenous gadolinium enhancement. SWI (susceptibility weighted imaging), T2, T1 pre and post, DWI with ADC and FLAIR imaging sequences were obtained. The children had mild PCM: none died and none had severe brain swelling. Normal intravascular gadolinium enhancement was noted but there was no parenchymal enhancement to suggest BBB breakdown. SWI imaging confirmed the present of a ferromagnetic substance in distributions similar to those of parasite sequestration and hemozoin seen at autopsy. A ferromagnetic substance, possibly hemozoin, the metabolic end product of hemoglobin digestion, was identified. Additional studies using R2* and SWI will assist in quantifying hemozoin, and parasite sequestration distribution *in vivo*. In this small population of non-fatal PCM cases, extravasation of contrast indicative of BBB breakdown was not evident using 1.5T MRI. Additional evaluations with dynamic enhancement techniques are warranted to further assess the integrity of the BBB, as this has relevance to adjuvant treatment strategies.

21

SEVERE AND CEREBRAL MALARIA IN CHILDREN LEADS TO A SIGNIFICANT IMPAIRMENT OF TRANSITORY OTOACOUSTIC EMISSIONS - A MULTICENTER PROSPECTIVE STUDY

Joachim Schmutzhard[1], Peter Lackner[1], Raimund Helbok[1], Helene Verena Hurth[1], Fabian Cedric Aregger[1], Veronika Muigg[1], Josua Kegele[1], Sebastian Bunk[1], Lukas Oberhammer[1], Natalie Fischer[1], Leyla Pinggera[1], Allan Otieno[2], Charles Newton[3], Tsiri Agbenyega[4], Akim Adegnika[5], Patrick Zorowka[1], Carsten Köhler[6], Sanjeev Krishna[7], Benjamin Mordmüller[6], Erich Schmutzhard[1], Peter Kremsner[6]

[1]Medical University Innsbruck, Innsbruck, Austria, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]Centre for Geographic Medicine (Coast) Kenya Medical Research Institute, Kilifi, Kenya, [4]School of Medical Sciences at the University of Science and Technology, Kumasi, Ghana, [5]Medical Research Unit, Albert Schweitzer Hospital (MRUG), Lambarene, Gabon, [6]Eberhard Karls Universität Tübingen, Tübingen, Germany, [7]St. George´s University of London, London, United Kingdom

Lately, severe and especially cerebral malaria have been suspected to influence the inner ear function. Cerebral malaria has been associated with hearing loss in 9 of 23 patients, as reported previously. However, prospective studies evaluating the inner ear function in severe and cerebral malaria are missing. An objective way to quickly evaluate the inner ear function is to measure otoacoustic emissions - a technique which is used worldwide to screen newborns for potential hearing impairment. This prospective multicenter study analyses the function of the inner ear in patients with severe and cerebral malaria up to the age of 10 years. In three study sites (Ghana, Gabon, Kenya) 144 patients with severe and cerebral malaria and 108 age-matched local control children were included. All patients were treated with artemisinin combination therapies. Of the severe malaria patients 42 % failed the initial otoacoustic emissions testing at the baseline measurement, i.e. prior to therapy, on day 28 only 27.1%. The cerebral malaria group showed a comparable number of failures with 38 % at the baseline. In contrast to the severe malaria the number of fails increased up to 66 % at day 7 after diagnosis. A slight improvement to 55% fails could be noted on day 28. Negative transient otoacoustic emissions are associated with a threshold shift of 20 dB and above. The present data shows a pathologic involvement of the inner ear in severe and cerebral malaria of around 40% prior to the initiation of schizontocidal therapy, a figure which improves in severe malaria but deteriorates over the next weeks in cerebral malaria. Therefore, especially in children with cerebral malaria, hearing screening and post-malaria audiologic work up has to be recommended after severe malaria infection.

22

ARTESUNATE-MEFLOQUINE VERSUS CHLOROQUINE IN PATIENTS WITH ACUTE UNCOMPLICATED *PLASMODIUM KNOWLESI* MALARIA: AN OPEN-LABEL RANDOMIZED CONTROLLED TRIAL IN SABAH, MALAYSIA (ACT KNOW TRIAL)

Matthew J. Grigg[1], Timothy William[1], Jayaram Menon[1], Bridget E. Barber[2], Prabakaran Dhanaraj[3], Giri S. Rajahram[1], Lorenz von Seidlein[2], Ric N. Price[2], Nicholas M. Anstey[2], Tsin W. Yeo[2]

[1]Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia, [2]Menzies School of Health Research, Darwin, NT, Australia, [3]Kudat District Hospital, Kudat, Sabah, Malaysia

Plasmodium knowlesi is the most common cause of malaria in Malaysia, and has been reported throughout South-East Asia. There are no recommendations in the current 2010 WHO malaria treatment guidelines for the optimal treatment of uncomplicated knowlesi malaria, which replicates every 24 hours and has the potential to cause severe disease and death. *P. knowlesi* is commonly microscopically misreported as other *Plasmodium* species, including *P. falciparum* and *P. vivax,* a high proportion of which are chloroquine-resistant in Malaysia. Artemisinin combination

therapies (ACT) and chloroquine have each been used to successfully treat *P. knowlesi* malaria in the past, however a unified ACT treatment protocol would support effective blood stage malaria treatment for all *Plasmodium* species. Malaysia's national policy for malaria pre-elimination involves mandatory admission for all patients with confirmed malaria, therefore a more rapidly acting anti-malarial agent also has health cost benefits. ACT KNOW, the first RCT ever performed in knowlesi malaria, is a 2-arm open label trial with enrolments over a 2-year period at 3 district sites in north-western Sabah, powered to show a difference in proportion of patients negative for malaria by microscopy at 24 hours between the treatment arms (clinicaltrials.gov #NCT01708876). Enrolments commenced in December 2012 and are expected to be completed by September 2014. As at March 2014 a total of 153 patients meeting inclusion criteria have been enrolled, with a total sample size of 228 required to give 90% power (alpha 0.05) to determine the primary endpoint using intention-to-treat analysis. Secondary endpoints include parasite clearance time, rates of recurrent infection/treatment failure to day 42, gametocyte carriage throughout follow-up, and rates of anemia at day 28, as determined by survival analysis. Results will be presented at the 2014 ASTMH annual meeting.

23

REPEATED TREATMENT OF YOUNG CHILDREN 4-11 MONTHS WITH ARTEMETHER-LUMEFANTRINE AND DIHYDROARTEMISININE-PIPERAQUINE OVER A TWO-YEAR FOLLOW UP PERIOD IN REAL-LIFE SETTINGS: NEURO-OTOTOXICITY DRUG SAFETY ASSESSMENTS

Dianne J. Terlouw[1], Carmen Gonzalez[1], Joachim Schmutzhard[2], Ian Mackenzie[3], Anna Maria van Eijk[4], Brian Faragher[4], Virginia Ramos[4], Kamija Phiri[5], David Lalloo[4]

[1]Malawi-Liverpool-Wellcome Trust Clinical Programme, Blantyre, Malawi, [2]Medical University Innsbruck, Innsbruck, Austria, [3]University of Liverpool, Liverpool, United Kingdom, [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [5]College of Medicine, Blantyre, Malawi

Artemisinin-combination therapies (ACTs) are widely seen as safe drugs. Early animal data of artemisinins raised the possibility of neurotoxicity with high doses of the lipophilic (oil-based) derivatives such as arte(m) ether in the form of selective damage of the brainstem involved in auditory and vestibular function, and recent animal data suggested the possibility of a cumulative effect. While the implication for human toxicity is controversial and audiology assessments in adults in Asia and single-use trials in older children over 5 years in Africa have been reassuring so far, this has never been confirmed in the most vulnerable group of young children during a phase of substantial brain maturity and high drug exposure. We conducted a cluster-randomized effectiveness trial in an area of high malaria transmission in Malawi. 814 children aged 4-11 months, recruited at community-level, were randomized at village level into either receiving artemether-lumefantrine (ArLu, lipophilic) or dihydroartemisinin-piperaquine (DHA-PPQ, hydrophilic), for any consecutive clinical malaria episodes during a 2 year follow-up period, in a 'real-life'setting. To explore any neuro-ototoxic adverse effect of repeated ACT exposure, two audiology safety components were conducted. Short-term and potentially reversible effects were assessed in a subset of 176 clinical malaria episodes with auditory brainstem response (ABR) tests before treatment on Day 0, and day 7 and day 42 post treatment. Long-term irreversible, cumulative effects were assessed with ABR readings at trial baseline, mid-point and end-of-study. Primary ABR endpoint was the interpeak latency between wave I and V(IPL). Overall trial analyses are ongoing and will be finalized by September. We will present the intention-to-treat and per-protocol analyses results, adjusting for treatment exposure, artemisinin intake dose, child age, concomitant treatments, and history of exposure of known determinants of audiological changes, including relevant birth and disease history, and other reported exposure to known ototoxic drugs.

24

BOUTS OF MALARIA ILLNESS AS MEDIATED BY ANEMIA DIMINISHES COGNITIVE DEVELOPMENT IN VERY YOUNG UGANDAN CHILDREN

Michael J. Boivin[1], Alla Sikorskii[1], Itziar Familiar-Lopez[1], Horacio Ruisenor-Escudero[1], Victor Bigira[2], James Kapisi[2], Mary Muhindo[2], Paul Bangirana[2], Noeline Nakasujja[2], Moses Kamya[2], Robert O. Opoka[2], Grant Dorsey[3]

[1]Michigan State University, East Lansing, MI, United States, [2]Makerere University, Kampala, Uganda, [3]University of California San Francisco, San Francisco, CA, United States

We evaluated child development in an open-label RCT of anti-malarial chemoprevention in children living in an area of intense, year-round transmission in Eastern Uganda. Infants 4 to 5 months of age were enrolled and randomized to one of four treatment arms: no chemoprevention, monthly sulfadoxine-pyrimethamine (SP), daily trimethoprim-sulfamethoxazole (TS), or monthly dihyrdroartemisinin-piperaquine (DP). At 2 yrs of age chemoprevention was stopped and children were followed for 1 additional year. Number of malaria episodes and anemia (hgb<10) were summarized up to cessation of chemoprevention (2 yrs of age) and again from 2 to 3 yrs of age. The Mullen Early Learning Scales (MELS) was administered to 471 children at 2 yrs and 452 children at 3 yrs. 69% were HIV-unexposed and 31% were HIV-exposed, equally distributed across treatment arms. The number of malaria episodes differed at the end of chemoprevention by trial arm (P <.0001) with DP having a high protective efficacy against malaria and anemia; TS having a moderate protective efficacy against malaria only, and SP offering no protection against malaria or anemia. Following cessation of chemoprevention, all treatment arms displayed levels of malaria (~7.5 episodes per year) and anemia comparable to that of the no chemoprevention arm. To determine the effects of the number of malaria episodes on cognitive development, linear mixed effects model related 2 repeated measures of Mullen scores (at 2 and 3 yrs) to trial arm, HIV exposure status, sex, socio-economic status, weight for age z-score, duration of breast feeding, and the number of malaria episodes in each year. Trial arm was not significantly related to any of the Mullen development outcomes. The number of bouts of malarial illness was significantly predictive of Mullen cognitive outcomes both at 2 and 3 yrs of age (P = 0.02). This relationship was mediated by the number of anemia episodes. Anemia episodes over and above those related to malaria was independently associated with poorer Mullen working memory (P = 0.02). HIV exposure was associated with lower Mullen receptive language development over and above malaria and anemia. We are the first to document within an RCT study that early malaria illness and anemia can lead to adverse developmental outcomes in very young children. Concurrent intervention for both of these risk factors in early childhood is important for enhancing developmental outcomes in at-risk children.

25

SEASONAL MALARIA CHEMOPREVENTION AND MICRONUTRIENT SUPPLEMENTATION IN EARLY CHILDHOOD: EFFECT ON ASYMPTOMATIC PARASITAEMIA, ANEMIA AND COGNITION

Sian E. Clarke[1], Yvonne Griffiths[2], Yahia Dicko[3], Modibo Bamadio[3], Seybou Diarra[3], Philippe Thera[3], Rebecca Jones[1], Diahara Traore[4], Modibo Traore[5], Bonaventure Maiga[6], Alassane Dicko[7], Moussa Sacko[8], Natalie Roschnik[9]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Institute of Education, London, United Kingdom, [3]Save the Children, Bamako, Mali, [4]Programme National de Lutte contre le Paludisme, Bamako, Mali, [5]Division Nutrition, Direction Nationale de la Sante, Bamako, Mali, [6]Direction Nationale de la Pedogogie, Ministry of Education, Bamako, Mali, [7]Malaria Research and Training Centre, University of Bamako, Bamako, Mali, [8]Institut National de Recherche en Santé Publique, Ministry of Health, Bamako, Mali, [9]Save the Children, Washington, DC, United States

Early childhood is a time of rapid growth and development and public health interventions during this period could yield substantial benefits across several developmental areas: physical, cognitive, and linguistic. Iron is important in brain function, and interventions that reduce iron-deficiency and anemia may improve cognitive function and learning. A randomized trial was undertaken to examine the combined impact of two newly-recommended interventions in early childhood: seasonal malaria chemoprevention and home fortification with micronutrient powders. Although each intervention has previously been shown to be associated with improvements in malaria morbidity, anemia and/or physical growth, the benefits for cognitive and linguistic development are comparatively unknown. The combined effect of these two interventions has not previously been examined. A cluster-randomized controlled trial was conducted in 60 rural communities with pre-school programs in southern Mali. Children aged less than 5 years living in the 30 intervention communities received two rounds of seasonal malaria chemoprevention in Oct and Nov 2013, followed by daily supplementation of micronutrients for four months from January-April 2014. Delivery of the two interventions at community-level was organized by pre-school management committees. The impact of the interventions will be evaluated in May 2014 through cross-sectional surveys to compare malaria infection, nutrition and cognitive performance in children aged 3 and 5 years living in intervention and control communities. Study outcomes will include asymptomatic parasitaemia, hemoglobin concentration, nutritional indices (height-for-age, weight-for-age), and cognitive foundation skills for early literacy. Findings on the combined effect of the malaria and nutrition interventions after the first year of implementation will be presented.

26

POPULATION PHARMACOKINETICS OF PIPERAQUINE IN YOUNG UGANDAN CHILDREN TREATED WITH DIHYDROARTEMISININ-PIPERAQUINE FOR UNCOMPLICATED MALARIA

Nancy Sambol[1], Li Yan[1], Darren Creek[2], Shelley McCormack[1], Emmanuel Arinaitwe[3], Victor Bigira[3], Humphrey Wanzira[3], Abel Kakuru[3], Jordan Tappero[4], Joel Tarning[5], Niklas Lindegardh[5], Francois Nosten[6], Francesca Aweeka[1], **Sunil Parikh**[7]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Monash University, Parkville, Australia, [3]Makerere University, Kampala, Uganda, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]Mahidol University, Bangkok, Thailand, [6]SMRU, Mae Sot, Thailand, [7]Yale School of Public Health, New Haven, CT, United States

Dihydroartemisinin-piperaquine (DP) is currently recommended as first-line treatment for malaria. Doses of DP to treat malaria in young children were initially chosen by scaling adult doses based on weight. This approach does not adequately address physiologic changes in early childhood that affect pharmacokinetics (PK). There is little information about the PK of piperaquine in children, and particularly those < 2 years of age. The objective of this study is to describe the PK of piperaquine in children 6 months to 2 years of age using a population approach that also explores covariate effects. We undertook a prospective population PK study of piperaquine (PQ) in 107 children 6 months to 2 years old who were treated with standard weight-based three-dose DP for uncomplicated malaria in Uganda. Participants underwent 5-7 finger sticks for 28 days following each treatment, providing 1282 evaluable capillary plasma samples from 218 treatments for a mixed effects model analysis with the program NONMEM®. Capillary plasma concentrations on day 7 in most patients, but especially in lower weight children 1-2 years of age, were below the value generally believed to be associated with a longer time to infection. PK data of piperaquine were well described by a 3-compartment open model with first-order absorption. Age, and not weight or other covariates, had a statistically significant effect ($p<0.005$) on clearance/bioavailability (CL/F) or on relative bioavailability (F) alone. This finding provides a possible contributing explanation for the low exposure in low-weight 1-2 year old children relative to higher-weight 1-2 year olds and 6 month-1 year olds, all of whom were dosed according to weight. Based on this population model we then simulated pharmacokinetic profiles of alternative treatment doses, as well as candidate PQ chemoprevention regimens, including monthly administration of standard therapeutic doses, a regimen currently being evaluated. Our results suggest that higher doses, especially in lower-weight 1-2 year olds, will increase the fraction of young children with piperaquine exposure greater than a predefined target, whether it is a previously published one or otherwise chosen. This study supports a growing body of evidence that there is a need to re-evaluate dosing of piperaquine in children.

27

ARTEMETHER-LUMEFANTRINE EFFICACY: POTENTIAL FOR FURTHER DOSE OPTIMIZATION

Christian Nsanzabana, On Behalf of the WWARN AL Dose Impact Study Group

WorldWide Antimalarial Resistance Network, Oxford, United Kingdom

Artemether-lumefantrine (AL) is the first line antimalarial treatment in 49 countries, administered according to four weight bands. Patients at the margins of these bands can receive significant deviation from the target dose. To assess the impact of weight adjusted (mg/kg) dose variations in therapeutic efficacy, individual patient data were shared with the WorldWide Antimalarial Resistance Network (WWARN) and collated using standardised methodology. Risk factors associated with recrudescence were evaluated using Cox's regression model with shared frailty on study sites. Data from 14,327 patients (61 efficacy studies between 1996 and 2012 in Africa, Asia and South America) with uncomplicated *P. falciparum* malaria were included in the analyses. A total of 386 Polymerase Chain Reaction (PCR)-confirmed recrudescent infections were reported. The PCR adjusted cure rate was 97.6% [95% CI: 97.4-97.9%] at day 28 and 96.0% [95% CI: 95.6-96.5%] at day 42. After controlling for age and parasitemia, every unit increase in daily artemether dose reduced the risk of being parasitaemic on day 1 by 8% [95% CI:1-14%, p=0.024] and every unit increase in total artemether dose reduced the risk of gametocyte carriage by day 14 by 8% [95% CI:1-15%, p=0.037]. Overall the dose of AL did not correlate with recrudescence, however the risk of recrudescence was higher in Asian children weighing 10 to 15 kg who received a total lumefantrine dose less than 60 mg/kg (Adjusted Hazards Ratio of 2.73 [95% CI: 1.40-5.32]), accounting for 41% of all treatment failures; p=0.003. In Africa, the risk of recrudescence was greatest in malnourished children from 1 to 3 years old (PCR adjusted cure rate 94.3% [95% CI: 92.3-96.3%]). The currently recommended dose of AL provides reliable efficacy in most patients with uncomplicated malaria. However, cure rates were lowest in young children in Asia and young underweight children from Africa. A higher dose regimen should be evaluated in these groups.

28

ARE PUBLIC OR RETAIL SECTOR PATIENTS MORE LIKELY TO COMPLETE TREATMENT? AN ANALYSIS OF PATIENT ADHERENCE TO ARTEMETHER-LUMEFANTRINE IN SOUTHERN TANZANIA

Katia Bruxvoort[1], Admirabilis Kalolella[2], Matthew Cairns[1], Charles Festo[2], S. Patrick Kachur[3], David Schellenberg[1], Catherine Goodman[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [3]Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States

Artemisinin combination therapies (ACTs) are first-line treatment for malaria in the public sector of most endemic countries and increasingly available in the private sector. Most studies on ACT adherence have been conducted in the public sector, with minimal data from private retailers. We conducted parallel adherence studies in Mtwara, Tanzania, in which patients obtaining artemether-lumefantrine (AL) at 40 randomly selected public health facilities and 37 private drug shops were followed up at home and questioned about each dose taken. The effect of health sector on adherence was assessed with random effects logistic regression controlling for potential confounders. Factors associated with adherence in each sector were examined in separate models. Of 572 health facility patients and 451 drug shop patients, 75% and 70% respectively completed treatment (p=0.2) , and 46% and 35% took each dose at the correct time (p=0.003). Drug shop patients were wealthier, more educated, older, sought care later in the day, and were less likely to test positive for malaria than health facility patients. Controlling for patient characteristics, the adjusted odds of drug shop patients completing treatment and taking each dose at the correct time were 0.63 and 0.67 times that of health facility patients (p=0.030 and p=0.044). Factors associated with adherence in drug shop patients were higher socioeconomic status and recalling correct dose instructions. Patients seeking care in the evening were half as likely to be adherent as those who sought care in the morning. In the public sector, having fever and recalling correct instructions were associated with completed treatment, while seeking care within two days, being tested for malaria, and taking the first dose at the facility were associated with timely completion. Patients attending drug shops differ from those at public health facilities, but when controlling for these characteristics, adherence was lower in drug shops. Better understanding is needed of which aspects of patient care are most important for adherence.

29

IMMUNE REGULATION BY HELMINTH PARASITES, INVOLVES MODULATION OF HUMAN DENDRITIC CELL METABOLISM INCLUDING INDUCTION OF INDOLEAMINE 2,3-DEOXYGENASE, DOWNREGULATION OF MTOR SIGNALING, AND INDUCTION OF AUTOPHAGY

Roshanak Tolouei Semnani, Sasisekhar Bennuru, Renee McDonald-Fleming, Rachel N. Cotton, Narasimhan Prakash Babu, Thomas B. Nutman

National Institutes of Health, Bethesda, MD, United States

Helminth infection has been associated with dysregulation of professional antigen presenting cells (APC) such as Langerhans cells (LC) and dendritic cells (DC) with DC being the cell type most affected by parasite exposure, particularly when exposed to the micrrofilarial (mf) stage of the *Brugia malayi* one of the 2 major species causing lymphatic filariasis in humans. To gain further insights into nature of the mf-induced alterations in DC function and viability (previously shown), we used LC-MS/MS to elucidate the entire proteome of mf-exposed monocyte derived human DC (n=4) in comparison to mf-unexposed human DC. Our proteomics data indicated that multiple components of the Mammalian Target of Rapamycin (mTOR) signaling pathway, including mTOR, and Eukaryotic Initiation Factor 4 (eIF4) A were downregulated by mf, suggesting that mf target this pathway for immune modulation in DC. Utilizing western blot analysis we showed that similar to rapamycin (a known mTOR inhibitor), mf downregulate the phosphorylation of mTOR regulatory proteins p70S6K1 and 4E-BP1, a process essential for DC protein synthesis. As active mTOR signaling regulates autophagy by inhibiting an autophagy induction complex, we examined whether mf exposure alters autophagy-associated processes. Expression of p62, a ubiquitin-binding protein that aggregates protein in autophagosomes and is degraded upon autophagy, was reduced dramatically by mf exposure ($P<0.05$), suggesting that mf induce autophagy in DC. Furthermore, as amino acid deficiency is one mechanism of mTOR activation and inhibition of autophagy, upregulation of kynureninase observed in our proteomics data suggested that mf also induce tryptophan catabolism in human DC; this was corroborated by showing that the expression of Indoleamine 2,3-deoxygenase (IDO) and activity was increased dramatically by exposure to mf ($p<0.05$) compared to mf-unexposed cells. Together, these results suggest that *Brugia malayi* mf employ mechanisms of metabolic modulation in DC to influence the regulation of the host immune response by downregualting mTOR signaling resulting in increased autophagy.

30

HYPERREACTIVE ONCHOCERCIASIS IS CHARACTERIZED BY AN ANTIGEN INDEPENDENT BIAS TOWARDS TH17 COMBINED WITH STRONG TH2 IMMUNE RESPONSES

Tomabu A. Adjobimey, Gnatoulma Katawa, Charlotte von Horn, Laura Layland, Achim Hoerauf

Institute for Medical Microbiology, Immunology and Parasitology, Bonn, Germany

Onchocerciasis or river blindness is the second leading infectious cause of blindness after trachoma. In human, the disease is caused by infections with the filarial nematode *Onchocerca volvulus* and presents two major polar forms: the hyporeactive form or generalized onchocerciasis (GEO) and the hyperreactive form (hyperreactive onchocerciasis: HO). The latter is associated with more aggressive disease manifestations and disfiguring dermatopathology and eye lesions that can lead to blindness, whereas GEO is characterized by tolerance to the parasite and mild skin disease. The immunological determinants of this polarization of the disease are still not fully clarified. Using multicolor FACS analysis, multiplex cytokine arrays and Real Time PCR, we compared the immune profiles of a group of 16 endemic normals (EN), individuals who had no clinical or parasitological evidence of infection despite ongoing exposure to the parasite, with a group of 16 individuals with GEO and a group of 6 individuals presenting the rare but sever hyperreactive form of the disease. We could show that individuals with HO presented higher frequencies of CD4+ cells expressing Th17 related cytokines (IL-17A, IL-17C, IL-6, IL-22) and markers (RORC, STAT3), while those with GEO presented significantly higher CD25+Foxp3+ and IL-10 secreting T cells. Concordantly, TCR activation using anti-CD3/CD28 induced high amounts of IL-17 in culture supernatants of peripheral blood mononuclear cells (PBMCs) from patients with HO, whereas IL-17 was barely detectable in PBMCs from EN and GEO individuals in same conditions. Strikingly, antigen specific stimulation using Onchocerca volvulus extracts did not elicit IL-17 secretion, either in PBMCs of GEO or in those of HO individuals. In contrast, a robust Th2 response was induced upon antigen specific activation of PBMCs from HO patients, as shown by prominent expression of GATA3, IL-4, IL-5 and IL-13 in PBMCs of these individuals. These findings suggest that, strong parasite specific Th2 responses combined with robust parasite-independent Th17 polarization in the absence of adequate immune regulation is associated with HO, revealing a novel Th17/Th2 axis as potential target in the development of new therapeutical strategies to prevent HO in human.

31

HELMINTH INFECTIONS DURING PREGNANCY IS ASSOCIATED WITH IMPAIRED VACCINE EFFICACY IN KENYAN INFANTS

Indu Malhotra[1], Peter L. Mungai[1], Maxim J. McKibben[1], Laura J. Sutherland[1], Eric Muchiri[2], Xuelei Wang[1], Charles H. King[1], Christopher L. King[1], Angelle Desiree LaBeaud[3]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Division of Neglected Tropical Diseases, Nairobi, Kenya, [3]Children's Hospital Oakland Research Institute, Oakland, CA, United States

Both animal models and human studies suggest that parasitic infections can result in decreased vaccine efficacy. Maternal parasitic infections during pregnancy prime the fetal immune response and induce an immunomodulatory phenotype at birth that may affect subsequent immune responses to childhood vaccines. We investigated whether prenatal exposure to helminth infections affect the pattern of infant immune response to standard vaccination against Haemophilus influenzae (Hib), hepatitis B (Hep B), tetanus toxoid (TT) and diphtheria toxoid (DT). 450 Kenyan women were tested for LF, urogenital schistosomiasis, malaria, and intestinal helminths during pregnancy. Their newborns were followed biannually to age 36 months and tested for levels of IgG against Hib, Hep B, TT, and DT at each time point. Overall, one third of the mothers were infected with LF, urogenital schistosomiasis, malaria or hookworm. Using a generalized estimating equation analysis, the presence of multiple maternal infections were associated with lower immune response to Hib PRP-specific IgG (p=0•001, 0•002, 0•045 with one infection; p=0•028, 0•022, 0•051 with two infections at 12, 18 and 24 months of age), compared to no maternal infection. There was a significant difference in response to DT in infants of mothers with three or more infections (p= 0•001 and 0•02 at 6 and 12 months) compared to no maternal infection. Response to Hib was also associated with immunophenotype; offspring putatively tolerized to filarial antigens (LF infected mothers but lacking filarial-specific Th1/Th2-type response in cord blood, N=94) compared to unexposed (no evidence of maternal LF infection nor antigen responsiveness in cord blood, N=119) had a lowest vaccine-induced antibody response to Hib-specific IgG (p= 0•052, 0•033 and 0•035 at 12, 18 and 24 months). Antenatal helminth infections are associated with lower immune response to Hib and DT vaccine antigens. Thus, in developing countries, eradication of chronic helminthic infections may be imperative to the success of future global vaccination efforts.

32

BLADDER UROTHELIAL CELL CYCLE RESPONSES TO *SCHISTOSOMA HAEMATOBIUM* INFECTION ARE MODULATED BY IL-4 RECEPTOR-α

Chi-Ling Fu, **Michael Hsieh**

Stanford University School of Medicine, Stanford, CA, United States

The bladder urothelium, a normally quiescent epithelial organ, undergoes dynamic changes during *Schistosoma haematobium* infection (urogenital schistosomiasis). These alterations include hyperplasia, ulceration, dysplasia, and frank carcinogenesis, and likely involve shifts in urothelial cell cycle status. Defining the pathways underpinning these urothelial responses will contribute to a deeper understanding of how *S. haematobium* egg-induced expulsion, hematuria, and bladder cancer develop in humans. Akin to their responses to many different helminth infections, mammals mount an IL-4 and -13-associated type 2 immune response during *S. haematobium* infection. These cytokines share the IL-4 receptor-α subunit as one of their cognate receptor subunits. To determine whether IL-4 receptor-α plays a role in urothelial cell cycle alterations in urogenital schistosomiasis, we injected *S. haematobium* eggs or control vehicle into the bladder walls of wild type, IL-4 receptor-α-deficient, and myeloid-associated IL-4 receptor-α-deficient mice. Three weeks later, mice were sacrificed and their bladder urothelium isolated and prepared as single cell suspensions. These suspensions were stained with DAPI and antibodies to CD45 and EpCAM. CD45-EpCAM+ urothelial cells were gated and their DAPI staining analyzed to assess cell cycle status. Relative to vehicle controls, wild type mice injected with eggs demonstrated increased proportions of urothelial cells in S phase and decreased proportions in G2/M phase. Although egg-injected, macrophage-associated IL-4 receptor-α-deficient mice featured similar urothelial responses as their egg-injected wild type counterparts, egg-injected conventional IL-4 receptor-α-deficient mice exhibited fewer urothelial cells in S phase. Thus, IL-4 receptor-α signaling through non-myeloid cells indeed seems to affect urothelial cell cycle status; this effect may specifically target DNA synthesis, a crucial process in carcinogenesis. Ongoing work seeks to determine which IL-4 receptor-α-expressing cells are crucial to these phenomena, and how these cells mediate alterations in the cell cycle status of the bladder urothelium.

33

IMMUNOGENICITY AND ANTI-FECUNDITY EFFECT OF NANOPARTICLE COATED GLUTATHIONE S-TRANSFERASE (SJGST) DNA VACCINE AGAINST MURINE *SCHISTOSOMA JAPONICUM* INFECTION

Evaristus C. Mbanefo[1], Takashi Kumagai[2], Yukinobu Kodama[3], Nguyen T. Huy[1], Nobuo Ohta[2], Hitoshi Sasaki[3], Kenji Hirayama[1]

[1]Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan, [2]Section of Environmental Parasitology, Tokyo Medical and Dental University, Tokyo, Japan, [3]Department of Hospital Pharmacy, Nagasaki University Hospital, Nagasaki, Japan

There is still urgent need for a vaccine against schistosomiasis, especially in *Schistosoma japonicum* endemic areas where even a vaccine that will interrupt zoonotic transmission will be potentially effective as an intervention tool. We had developed a novel nanoparticle gene delivery system, which has proven efficacious in gene transfection to target immune cells with complementary adjuvant effect and high protective efficacy in several diseases. Here, we have applied this nanoparticle system in combination with *S. japonicum* glutathione S-transferase (SjGST) DNA vaccine to show improved immunogenicity and anti-fecundity effect of the nanoparticle coated vaccine formulation against murine schistosomiasis. The nanoparticle-coated DNA vaccine formulation induced desired immune responses with significantly increased humoral response, T-helper 1 polarized cytokine environment, higher proportion of IFN-γ producing CD4+ T-cells and the concomitant decrease in IL-4 producing CD4+ T-cells. Although there was no effect on worm burden, the proportion of immature worms was higher in the SjGST vaccinated groups with a lower proportion of mature paired worms. We recorded an unprecedented reduction in tissue egg burden. There was 71.9% decrease in liver egg burden, 71.6% decrease in intestinal egg burden, and 54.7% reduction in the fecundity of female adult worms. In conclusion, our data showed that the combination of nanoparticle gene delivery system with SjGST DNA vaccination significantly improved the characteristic anti-fecundity effect of SjGST, thereby proving this DNA vaccine formulation as a promising candidate for anti-pathology and transmission blocking application.

34

THE MOLECULAR CONSEQUENCES OF INTRA-PATIENT PHAGE PREDATION ON *VIBRIO CHOLERAE* POPULATION DIVERSITY

Kimberley D. Seed[1], Minmin Yen[1], Jesse Shapiro[2], Isabelle J. Hilaire[3], Richelle C. Charles[4], Jessica E. Teng[5], Louise C. Ivers[6], Jacques Boncy[7], Jason B. Harris[8], Andrew Camilli[1]

[1]Tufts University School of Medicine and Howard Hughes Medical Institute, Boston, MA, United States, [2]Université de Montréal, Montréal, QC, Canada, [3]Partners In Health, Boston, MA, United States, [4]Massachusetts General Hospital, Boston, MA, United States, [5]Partners In Health and Brigham and Women's Hospital, Boston, MA, United States, [6]Partners In Health, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, United States, [7]National Public Health Laboratory, Port-au-Prince, Haiti, [8]Massachusetts General Hospital and Harvard Medical School, Boston, MA, United States

Vibrio cholerae is a globally important water-borne pathogen, which is the causative agent of the severe acute diarrheal disease cholera. We analyzed geographically disparate cholera patient samples from Haiti and Bangladesh and found that ICP2-like phage can impact the evolutionary trajectory of intra-patient *V. cholerae* populations. Evidence of intra-patient selection of phage-resistant mutants was found in one Haitian patient sample in which >99% of *V. cholerae* isolates harbored mutations in the major outer membrane porin, OmpU. Mutant *ompU* alleles were sufficient for ICP2 resistance when moved into a clean background, indicating that ICP2 uses OmpU as a receptor to initiate infection. Phage-resistant and phage-sensitive isolates from this sample were subjected to whole genome sequencing and were found to be isogenic except for single site mutations in *ompU*. The mutations within *ompU* between phage-resistant isolates were heterogeneous, indicating that phage predation and selection of resistant mutants occurred multiple independent times during infection. High levels of ICP2 were also observed in a Bangladeshi patient sample found to harbor a mixture of *toxR* mutants. Similar to what was observed in the Haitian patient sample; multiple unique *toxR* mutants were observed within this single sample. ToxR directly activates expression of *ompU* and critical virulence factors. ICP2-sensitivity was restored to the clinical *toxR* mutants by expressing *ompU* in trans, demonstrating that resistance is mediated through decreased OmpU expression. The clinically isolated *ompU* and *toxR* alleles had differing impacts on *V. cholerae* virulence, with the *toxR* mutants being severely attenuated *in vivo*. We demonstrate that *V. cholerae* faces phage-mediated predatory interactions during the natural course of infection that can impose a strong selective pressure. This work highlights the notion that host-pathogen interactions are often embedded in a diverse microbial ecosystem that can impact the virulence and transmission of the pathogen.

35

CIRCULATING MUCOSAL ASSOCIATED INVARIANT T (MAIT) CELLS ARE ACTIVATED IN *VIBRIO CHOLERAE* O1 INFECTION AND ASSOCIATED WITH LIPOPOLYSACCHARIDE ANTIBODY RESPONSES

Daniel T. Leung[1], Taufiqur R. Bhuiyan[2], Naoshin S. Nishat[2], Mohammad Rubel Hoq[2], Amena Aktar[2], M. Arifur Rahman[2], Taher Uddin[2], Ashraful I. Khan[2], Fahima Chowdhury[2], Richelle C. Charles[1], Jason B. Harris[1], Stephen B. Calderwood[1], Firdausi Qadri[2], Edward T. Ryan[1]

[1]Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Mucosal Associated Invariant T (MAIT) cells are innate-type T cells that account for up to 10% of circulating T cells and are found in the intestinal mucosa, liver, and mesenteric lymph nodes. MAIT cells are activated in response to the presentation of riboflavin metabolites, which are produced by various bacteria including Vibrio cholerae. MAIT cells are thought to play a role in bridging the innate-adaptive interface, and a recent study demonstrated that activation of MAIT cells is associated with B cell responses to a Shigella vaccine. We collected blood from patients presenting with culture-confirmed severe cholera to the icddr,b Dhaka Hospital at days 2, 7, 30, and 90 of illness. We characterized MAIT cells by multicolor flow cytometry, and assessed antibody responses to V. cholerae O1 lipopolysaccharide (LPS) and cholera toxin B subunit (CTB) by ELISA. We defined MAIT cells as $CD3^{+}CD4^{-}CD161^{hi}$ cells that are positive for the invariant T cell receptor segment $V\alpha7.2^{+}$, and used CD38 as a marker of MAIT cell activation. We found that MAIT cells were maximally activated at day 7 of cholera in all age groups. In adults, MAIT frequencies did not change over time, whereas in children, MAITs were significantly decreased at day 7 compared to day 2. This decrease persisted to day 90, the last time point examined. Notably, the magnitude of increase in MAIT cells from day 2 to day 7 of infection correlated with fold changes in LPS IgA and IgG responses. Such a correlation was not found with LPS IgM or antibody responses to CTB. In this study, we show that MAIT cells are activated during severe cholera, suggesting that they may play an important role in the innate response against V. cholerae infection. Given that increases in MAIT cells are correlated with increases in class-switched antibodies against LPS, but not CTB, we hypothesize that MAIT cells may be involved in mechanisms underlying class switching of antibodies to T-independent antigens. The persistent decrease in MAIT cells following cholera in children, not seen in adults, needs further investigation.

36

IMMUNOGENICITY OF A KILLED ORAL BIVALENT WHOLE CELL CHOLERA VACCINE IN ADULTS WITH HIV INFECTION

Louise C. Ivers[1], Richelle C. Charles[2], Isabelle J. Hiliare[3], Leslie Mayo-Smith[2], Jessica E. Teng[1], Jenna Rychert[2], Edward T. Ryan[2], Firdausi Qadri[4], Molly F. Frank[5], **Jason B. Harris**[2]

[1]Brigham and Womens Hospital, Boston, MA, United States, [2]Massachusetts General Hospital, Boston, MA, United States, [3]Partners In Health, Boston, MA, United States, [4]International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh, [5]Harvard Medical School, Boston, MA, United States

Cholera epidemics often occur in settings where human immunodeficiency virus (HIV) infection is prevalent. While some evidence suggests that individuals living with HIV are more susceptible to cholera than those that are HIV negative, little is known about how HIV alters the immune response to oral cholera vaccines. We evaluated immune responses following bivalent oral cholera vaccination (BivWC; Shanchol) in a cohort of adults in Haiti, some of which were known to have HIV infection. Blood samples were obtained prior to immunization and 7 days after each of two doses of BivWC vaccine were administered. A total of 25 adults with HIV infection were enrolled in the study; 23 of these individuals received both doses of BivWC and completed the three-week observation period including all blood draws. Compared to 22 adults without known HIV infection who completed the three-week observation period, HIV-infected individuals in this cohort had significantly lower vibriocidal antibody responses against both the *V. cholerae* O1 Inaba and Ogawa serotypes. Among the HIV-infected vaccinees, we found an inverse relationship between $CD4^{+}$ T-cell count and the subsequent vibriocidal antibody response following vaccination. However, despite lower vibriocidal antibody responses, there was still a substantial vaccine take rate in this cohort adults living with HIV infection. Both O- polysaccharide antigen specific IgA and vibriocidal antibody titers increased significantly after oral cholera vaccination in HIV-infected adults. Seroconversion, defined as four-fold or greater increase in vibriocidal antibody titer, occurred at a rate of 65% against the Ogawa serotype and 74% against the Inaba serotype in adults with HIV infection. These results suggest that killed oral cholera vaccines retain substantial immunogenicity in adults with HIV infection and may provide a significant benefit in a population that is otherwise vulnerable to cholera.

37

EFFECTIVENESS OFAN ORAL KILLED BIVALENT WHOLE-CELL CHOLERA VACCINE IN RURAL HAITI

Louise C. Ivers[1], Jessica E. Teng[2], Johanne Hilaire[3], J. Gregory Jerome[3], C. Patrick Almazor[3], Ralph Ternier[3], Megan Murray[1], Jason B. Harris[1], **Molly Franke**[1]

[1]Harvard Medical School, Boston, MA, United States, [2]Brigham and Women's Hospital, Boston, MA, United States, [3]Partners In Health, Port-au-Prince, Haiti

Between April and June 2012, a reactive oral cholera vaccine campaign was conducted in a rural district of Haiti using the oral killed bivalent whole-cell vaccine, Shanchol®. We conducted a case-control study to estimate vaccine field effectiveness in the context of this campaign. Cases had acute watery diarrhea, sought treatment at 1 of 3 participating cholera treatment units between October 24, 2012 and March 9, 2014, and had a stool sample positive for cholera by Crystal VC® rapid test and culture. For each case, four controls (individuals who did not experience or seek treatment for acute watery diarrhea between the date of study initiation and the date of their corresponding case's symptom onset) were matched by neighborhood, calendar time, and age. We also conducted a bias-indicator case-control study to assess the likelihood of bias in the vaccine effectiveness case-control study by examining the relationship between vaccination and non-cholera diarrhea. In the absence of bias we expected no association between vaccination and non-cholera diarrhea in the bias-indicator study. During the study period, 115 individuals presented with acute watery diarrhea. After excluding 8 individuals who lacked specimen for culture, 22 with discordant rapid test and culture results, and 1 cholera case that lacked interview data, 41 were included as cases in the vaccine effectiveness case-control study and 43 as cases in the bias-indicator study. In univariable analysis, both self-reported vaccination and verified vaccination (via vaccination card or registry) were associated with a statistically significant reduction in the risk of cholera. In adjusted analyses vaccine effectiveness was 66% [95% confidence interval: 15%-86%; p-value: 0.02] by self-report and 60% [95% confidence interval: 14%-82%; p-value: 0.02] for verified vaccination. Neither self-reported nor verified vaccination was significantly associated with non-cholera diarrhea in univariable or multivariable analyses. The oral cholera vaccine is an effective component of the comprehensive response to cholera in Haiti.

38

THE CASE FOR USING A SINGLE DOSE OF ORAL CHOLERA VACCINE IN EMERGENCIES

Andrew S. Azman[1], Francisco J. Luquero[2], David A. Sack[1], Justin Lessler[1]

[1]Johns Hopkins School of Public Health, Baltimore, MD, United States, [2]Epicentre, Paris, France

A global stockpile of oral cholera vaccine (OCV) was recently established for emergency use, but with less than 2 million doses available worldwide officials will likely have to contend with limited vaccine. Previous work shows that speed of response is the strongest predictor of the impact of reactive vaccination campaigns. Two OCVs are internationally licensed for use as a two-dose formulation administered 1-6 weeks apart, and the delay between vaccination rounds may compromise the benefits of receiving the second dose. With evidence from clinical trials and immunological studies suggesting that a single dose may be moderately protective, there is a strong argument that more may be gained by distributing a single dose to more people quickly, than a full course to a smaller population, thereby administering as many doses of vaccine as quickly as possible. Whether this is the case will be driven by the efficacy of a single dose, the mechanism of vaccine protection, and local cholera transmission dynamics. Using computational models of cholera transmission with varying assumptions about the epidemiologic context and vaccine mechanism, we explore the minimum single-dose efficacy needed to avert at least as many cases as a two-dose regimen in a reactive vaccination campaign with limited vaccine. In a simple model not taking into account indirect effects (i.e. herd protection), we find that early vaccination with a single-dose that confers about at least 42% protection against clinical disease would avert more cases than a two-dose regime with the same quantity of vaccine. As vaccination is delayed and indirect effects are taken into account, using a single dose with even lower efficacy will avert more cases than a two-dose campaign. When considering logistical delays and epidemic specific transmission dynamics from recent large outbreaks in Haiti, Zimbabwe, and Guinea, we find that with realistic quantities of vaccine, use of a single-dose regimen would have averted more cases than a two-dose regimen. Through synthesis of clinical and immunological evidence with data from recent cholera outbreaks, we show that a single dose of OCV may be an important tool for emergency response. If future empirical studies are consistent with current single-dose efficacy evidence, our results help create a case for the international licensing of a single-dose OCV regimen.

39

SINGLE-DOSE LIVE ATTENUATED ORAL CHOLERA VACCINE (CVD 103-HGR) PROTECTS AGAINST CHOLERA AT 10 DAYS FOLLOWING VACCINATION: RESULTS OF A *VIBRIO CHOLERAE* O1 EL TOR INABA CHALLENGE STUDY

Wilbur H. Chen[1], Mitchell B. Cohen[2], Beth D. Kirkpatrick[3], Rebecca Brady[2], David Galloway[2], Marc Gurwith[4], Robert H. Hall[5], Robert A. Kessler[1], Michael Lock[4], Caroline E. Lyon[3], Jakub K. Simon[4], Flora Szabo[2], Myron M. Levine[1]

[1]University of Maryland School of Medicine, Baltimore, MD, United States, [2]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, [3]University of Vermont College of Medicine, Burlington, VT, United States, [4]PaxVax, Inc., Menlo Park, CA, United States, [5]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States

No cholera vaccines are currently licensed and available in the U.S.A; two killed oral cholera vaccines licensed in other countries require 2 spaced doses. A single-dose oral cholera vaccine that rapidly elicits protection is needed for immunization of short-notice travelers to high risk areas and for use in explosive outbreaks, where practical administration and accelerated onset of protection are also desirable. Attenuated *Vibrio cholerae* O1 classical Inaba live oral vaccine strain CVD 103-HgR harbors a deletion of 94% of the gene encoding the cholera toxin A subunit. CVD 103-HgR, manufactured by PaxVax Inc., typically elicits vibriocidal antibody seroconversion (a correlate of protection) within 10 days of vaccination. To evaluate vaccine efficacy, we challenged healthy volunteers, 18-45 years old, with virulent *V. cholerae.* Consenting healthy cholera-naïve adults (n= 98, including n=50 of blood group O, high risk for cholera gravis), were randomly allocated 1:1 to receive vaccine (10^8 CFU) or placebo. Ten days after vaccination, 68 subjects ingested 10^5 CFU wild type *V. cholerae* O1 El Tor Inaba strain N16961. Subjects were observed on a Research Isolation Ward for measurement of stool output and management of cholera illness. The vaccine was well tolerated: adverse events, generally mild to moderate, were reported in 13% vaccine and 14% placebo recipients. The primary endpoint, moderate (≥3.0 liter cumulative purge) to severe (≥5.0 liter cumulative purge) diarrhea occurred in 20/33 (61%) placebo and 2/35 (6%) vaccine recipients (point estimate of vaccine efficacy [VE] =91%, p<0.0001). 13/33 (39%) placebo recipients and 1/35 (3%) vaccinees had severe diarrhea (point estimate of VE =93%, p=0.0002). Additional subjects will be challenged at 90 days post-vaccination and overall VE will be calculated in May 2013 with pooled placebo recipients serving as the control. The encouraging preliminary analysis of VE observed 10 days after receipt of a single 10^8 CFU dose of CVD 103-HgR supports an accelerated clinical development path towards FDA licensure.

40

OPTIMAL ALLOCATION OF ORAL CHOLERA VACCINE IN ENDEMIC AND EPIDEMIC SETTINGS

Sean M. Moore, Justin Lessler

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

In 2013 the World Health Organization (WHO) established a global stockpile of two million doses of oral cholera vaccine (OCV) to be deployed reactively during cholera epidemics. With an estimated 3--5 million cases, 100,000 deaths annually, and 1.4 billion people at risk of infection, the current cholera vaccine supply can only prevent a small percentage of cases. Due to the limitations in vaccine supply, it is important to determine how to best allocate existing vaccine between populations in endemic settings versus stockpiling the vaccine for reactive use during epidemics, where attack rates and case fatality ratios are often higher. We used a mathematical model of cholera transmission to examine how the allocation of a limited OCV supply can maximize the total number of cholera cases prevented in both epidemic and endemic settings. We found that under a broad range of expected epidemic sizes the number of cases prevented is maximized by allocating a fairly high proportion of the existing OCV supply to reactive vaccination in the epidemic setting. This optimal OCV allocation is sensitive to the timing of the reactive vaccination campaign. For less explosive epidemics with lower initial growth rates the optimal allocation to reactive vaccination increases as the start of the reactive vaccination campaign is delayed (up to a limit of 120-150 days). However, in the case of larger epidemics with high initial growth rates, moderate delays to the start of reactive vaccination (>50 days after the start of outbreak) lead to allocating OCV doses away from reactive vaccination and towards endemic populations because the reactive vaccination campaign will miss the peak of the epidemic and prevents relatively few cases. Our results indicate that the strategy of maintaining the stockpile for reactive campaigns rather than using these doses in an endemic setting is optimal with the current limited supply as long as vaccine deployment is timely.

41

MODELING THE COST EFFECTIVENESS OF MALARIA CONTROL INTERVENTIONS IN THE HIGHLANDS OF WESTERN KENYA

Erin M. Stuckey[1], Jennifer Stevenson[2], Katya Galactionova[1], Amrish Y. Baidjoe[3], Teun Bousema[3], Wycliffe Odongo[4], Simon Kariuki[4], Chris Drakley[5], Thomas A. Smith[1], Jonathan Cox[5], Nakul Chitnis[1]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [4]Kenya Medical Research Institute/Centers for Disease Control and Prevention, Kisumu, Kenya, [5]London School of Hygiene & Tropical Medicine, London, United Kingdom

Tools that allow for in silico optimization of available malaria control strategies can assist the decision making process for prioritizing interventions. The OpenMalaria stochastic simulation modeling platform can be applied to simulate the impact of interventions singly and in combination as implemented in Rachuonyo South District, western Kenya, to support this goal. Combinations of malaria interventions were simulated using a previously-published, validated model of malaria epidemiology and control in the study area. An economic model of the costs of case management and malaria control interventions in Kenya was applied to simulation results and cost-effectiveness of each intervention combination compared to the corresponding simulated outputs of a scenario without interventions. Uncertainty was evaluated by varying health system and intervention delivery parameters. While an intervention with long lasting insecticide treated net (LLIN) use by 80% of the population, 90% of households covered by indoor residual spraying (IRS) with deployment starting in April, and intermittent screen and treat (IST) of school children using Coartem® with 80% coverage twice per term had the greatest simulated health impact, the current malaria control strategy in the study area of LLIN use of 56% and IRS coverage of 70% was the most cost effective at reducing DALYs over a five year period. All the simulated intervention combinations can be considered cost effective in the context of available resources for health in Kenya. Increasing coverage of vector control interventions has a larger simulated impact compared to adding IST to the current implementation strategy, suggesting that transmission in the study area is not at a level to warrant replacing vector control to a school-based screen and treat program. These results have the potential to assist malaria control program managers in the study area in adding new or changing the implementation of current interventions.

42

THE IMPORTANCE OF HISTORICAL DATA IN SIMULATING MALARIA EPIDEMIOLOGY AND CONTROL INTERVENTIONS: APPLICATION TO MULTIPLE SITES IN MADAGASCAR

Emilie Pothin[1], Olivier Briët[1], Thomas Kesteman[2], Milijaona Randrianarivelojosia[2], Christophe Rogier[2], Thomas Smith[1]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Institut Pasteur de Madagascar, Antananarivo, Madagascar

Planning malaria interventions requires prediction of likely impacts of different intervention strategies. Simulation models can provide such predictions but weak information about pre-control levels of transmission, intervention coverage and access to care often makes it challenging to correctly parameterize them. We consider a number of low malaria transmission sites in Madagascar, with available historical prevalence and entomological inoculation rate (EIR) estimates (by mosquito sampling), giving disparate estimates of historical exposure. Information about implementation of Long Lasting Insecticide Nets (LLINs) and Indoor Residual Spraying (IRS), and access to healthcare, collated from malaria surveys and on-going cross-sectional studies were used to parameterise simulations of malaria transmission, prevalence and burden within the OpenMalaria platform. Multiple parameterisations were considered using various sources of data for pre-intervention transmission level, intervention coverage and access to healthcare. In some sites the simulated impact of existing vector control programs matches reasonably well the malaria prevalence measured in a recent national survey. In others it predicts lower than observed prevalence, very likely because the models do not capture residual local transmission foci. The simulations suggest that the most cost-effective vector control strategy would be to scale-up LLINs or IRS only, depending on the transmission level. Indeed, preliminary results show no additional benefit of IRS where LLINs were used. These preliminary results suggest that historical prevalence data, combined with current coverage information are potentially adequate for planning intervention strategies. The outcome of intervention scale-up is essentially unpredictable if baseline information is poor. Reproducing the observed epidemiology of malaria through simulations both provides confidence in the use of the model but serves as a basis for prospective studies that support decision-making, including cost-effectiveness analyses.

43

ASSESSING THE POTENTIAL IMPACT OF ARTEMISININ RESISTANCE IN SUB-SAHARAN AFRICA

Hannah C. Slater, Jamie Griffin, Azra C. Ghani, Lucy Okell

Imperial College, London, United Kingdom

Artemisinin and partner drug resistant malaria parasites have emerged in Southeast Asia. If resistance were to emerge in Africa it could have a devastating impact on the morbidity and mortality associated with ongoing malaria transmission. We estimate the potential impact of artemisinin and partner drug resistance on disease burden in Africa if it were to emerge. Using data from Asia and Africa, we characterise five possible artemisinin and partner drug resistance scenarios. Artemisinin

resistance is characterised by slow parasite clearance (SPC). Partner drug resistance is associated with late clinical failure (LCF) or late parasitological failure (LPF). An individual-based malaria transmission model is used to estimate the impact of each resistance scenario on clinical incidence, severe incidence and parasite prevalence across Africa. We find that scenarios with high levels of recrudescent infections (LCF/LPF) resulted in far greater increases in clinical incidence compared to scenarios with high levels of SPC. Across Africa, we estimate that partner drug resistance at levels similar to those observed in parts of Africa for AS+SP could result in 39 million additional cases over a five year period, a 2.7% increase compared to a scenario with no resistance. Artemisinin resistance similar to levels observed in Pailin, Cambodia could result in an additional 29 million cases over the same period. Artemisinin resistance is potentially a more pressing concern than partner drug resistance due to the lack of viable alternatives. However, widespread partner drug resistance, characterised by SP resistance in parts of Africa, would result in greater increases in malaria morbidity than if widespread artemisinin resistance were to develop at levels currently observed in Pailin, Cambodia.

44

MODELING THE IMPACT OF COINFECTION ON PERSISTENCE AND INFECTIVITY OF *PLASMODIUM FALCIPARUM*

Lauren M. Childs, Caroline O. Buckee

Harvard School of Public Health, Boston, MA, United States

Each year nearly 200 million people are infected with the malaria parasite, *Plasmodium falciparum*. One of its most notable features is the variable course and duration of infection experienced by different individuals, ranging from high parasite density, acute and often severe infections to persistent, chronic infections that are often undetectable by microscopy. Levels of acute and chronic infections vary across different transmission settings, and what disturbs the delicate balance between parasite growth and immune control, leading to bursts of parasite growth or clearance of an infection, remains an open question. Field studies examining persistence of infection have used a variety of different genotyping methods, but due to limitations, it is difficult to determine the extent of mixed infections, and nearly impossible to determine if the reemergence of parasitemia is due to a new infection or recrudescence of an existing one. Mathematical models, despite limited knowledge of mechanistic details of host-parasite interactions, have qualitatively reproduced single parasite dynamics observed in patient data. Here we adapt a discrete model by Recker et al. (PLoS Pathogens, 2011) of blood-stage parasite dynamics including innate and adaptive immune responses. We analyze simulated output to examine how coinfecting strains, particularly from similar clones that elicit overlapping immune responses, impact infection length and infectiousness. We find that the level of both innate and adaptive immune responses present at the time of coinfection as well as the similarity of the coinfecting strains significantly alters the duration of both the resident and coinfecting strains, particularly during chronic infections. Timing of coinfection also influences the infectivity of the coinfecting strains, likely altering transmission patterns at a population level. Duration of infection and infectivity are critical epidemiological parameters for predicting the efficacy of control strategies, particularly with the looming problem of emerging drug resistance.

45

MEASURING THE PATH TOWARDS MALARIA ELIMINATION

Thomas S. Churcher[1], Justin M. Cohen[2], Joseph Novotny[2], Nyasatu Ntshalintshali[3], Simon Kunene[3], Simon Cauchemez[4]

[1]Imperial College London, London, United Kingdom, [2]Clinton Health Access Initiative, Boston, MA, United States, [3]National Malaria Control Program, Mbabane, Swaziland, [4]Institut Pasteur, Paris, France

As an area approaches local elimination it becomes increasingly difficult to quantify transmission accurately. This is because countries that manage to control local transmission to relatively low levels but receive large numbers of imported cases from outside its borders are likely to see a considerable number of autochthonous cases. The reproduction number R (the average number of persons infected by a malaria case) is a new method of measuring malaria transmission though, to date, its use has remained largely theoretical and it has had limited practical impact. Here, we present an operational framework to determine whether programs are successful at controlling local transmission of falciparum malaria. It is based on a new and simple method to test the hypothesis R≥1 from standard surveillance data consisting of the numbers of local and imported malaria cases. We apply this approach to Swaziland, a country which embarked on an elimination campaign in 2008. Thirty six percent (52/143), 45% (170/377) and 67% (153/229) of investigated cases were imported in 2010, 2011 and 2012 respectively. This indicates that the status of controlled non-endemic malaria was reached in 2011 and 2012 but not in 2010. This provides evidence that since 2010, Swaziland has halted endemic transmission at the national level, and that malaria would be eliminated if the current level of control was continued and importations ceased. The method offers a simple and practical solution to quantify transmission as the disease becomes increasingly rare. Instead of just aiming for zero local cases, programs should use these metrics to set a series of intermediate milestones which are easy to test and show progress towards disease elimination. Evidence like this will be essential to enable the successes of countries like Swaziland to be maintained and replicated elsewhere.

46

SPATIAL DYNAMICS OF MALARIA TRANSMISSION IN THE EMOD MODEL FOR CAMPAIGNS TARGETING SUSTAINED REGIONAL ELIMINATION IN SOUTHERN ZAMBIA

Edward A. Wenger, Alexander M. Upfill-Brown, Jaline L. Gerardin, Philip A. Eckhoff

Institute for Disease Modeling, Bellevue, WA, United States

As malaria control programs move towards regional elimination campaigns, it will be increasingly important to understand the dynamics of malaria transmission within and between adjacent regions with different characteristics. Within the framework of the EMOD model, we have constructed a network of 116 interconnected simulated populations corresponding to the spatial distribution of households in Gwembe and Sinazongwe districts in southern Zambia. We use a gravity model that includes local and regional roadways to infer the relative rates of migration between nodes. For each simulated population, we use geographically-specific values for larval habitat, acquired immunity, ITN usage, case management rates, drug-regimen compliance, and mass-screen-and-treat (MSAT) coverage levels to the extent that they may be inferred from surveillance data. Our simulations are able to reproduce the geographically variable pattern of reduced rapid diagnostic test (RDT) detectable parasite prevalence from 2012 to 2013. Finally, we extend this spatial-simulation framework to predict the impact of different drug-delivery, vector-control, and case-management parameters on the potential to sustain regions of local elimination. When anti-malarial treatment, with both curative and short-term prophylactic effects, is distributed regardless of RDT status, substantial regions of the simulated geography are parasite-free within a few years. However, sustained success almost certainly requires significant improvements in case-management and vector-control activities.

47

THE MATHEMATICAL EPIDEMIOLOGY OF *PLASMODIUM VIVAX* MALARIA - INSIGHTS INTO THE SLOW TIMELINE TO ELIMINATION

Michael White

Imperial College London, London, United Kingdom

There is a rich history of using mathematical models of malaria transmission to inform the design of programmes for the control and elimination of *Plasmodium falciparum* malaria, building on the pioneering work of Ross and MacDonald. However, the valuable lessons learned

from these models do not always apply to *P. vivax* due to the occurrence of relapse infections months to years after the primary infection. These models are extended to consider relapsing malaria using intuitive, high-school level mathematics to illustrate the qualitative relationships between parameters of epidemiological importance: *P. vivax* parasite prevalence, the basic reproduction number R0, the proportion of infections due to relapses, and the density of *Anopheles* mosquitoes. These relationships are illustrated via examples from malaria control campaigns in The Solomon Islands, Vanuatu and Papua New Guinea where notably different trends were observed in *P. vivax* and *P. falciparum* transmission following the introduction of vector control and mass drug administration. Evidence is presented for three key results, and their implications are discussed with examples from past malaria control programmes. (i) At similar parasite prevalence, *P. vivax* has a greater basic reproduction number than *P. falciparum* ensuring more stable transmission and greater difficulty in reducing R0 < 1. (ii) Although vector control is effective at reducing the transmission of all malarias, it is predicted to be approximately twice as effective at reducing *P. falciparum* prevalence compared to *P. vivax* prevalence, depending on the expected number of relapses. (iii) In the absence of drugs such as primaquine for preventing relapses, *P. vivax* elimination timelines are predicted to be substantially longer than *P. falciparum* timelines due to the reduced effectiveness of vector control and the extended time to relapse infections. As a relapsing malaria, *P. vivax* presents a number of epidemiological challenges that must be considered if it is to be successfully controlled and eliminated.

48

BREATHING MY NEIGHBOR'S AIR: AIR POLLUTION DISPERSION FROM BIOMASS COOKSTOVES IN MIRPUR, DHAKA, BANGLADESH

Anne M. Weaver[1], Emily S. Gurley[2], Christina Crabtree-Ide[1], Henrik Salje[3], Eun-Hye Yoo[1], Lina Mu[1], Nasrin Akter[2], Pavani K. Ram[1]

[1]University at Buffalo, Buffalo, NY, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]John Hopkins School of Public Health, Baltimore, MD, United States

Exposure to high indoor concentrations of fine particulate matter (PM2.5) is a major risk factor for pneumonia and other respiratory disease. Biomass cookstoves emit high concentrations of PM2.5. However, high concentrations of PM2.5 (>1000 µg/m3) have been observed in Dhaka homes that do not use biomass cookstoves. We aimed to describe dispersion of PM2.5 from biomass cookstoves into nearby homes in a low-income urban area of Dhaka, Bangladesh. We recruited 10 clusters of homes consisting of one biomass-burning (index) home, and 3-4 neighboring homes that used electricity or kerosene cookstoves and had no obvious major sources of PM2.5. We administered a questionnaire and recorded physical features of all homes. We recorded PM2.5 concentrations inside each home, near each stove, and outside one neighbor home per cluster for 24 hours; during 8 of these 24 hours, we directly observed daily activities, such as cooking. We calculated geometric mean PM2.5 concentrations at 5-6am (baseline), during biomass cooking times, and during the entire monitoring period, for each monitor. We recruited a total of 44 homes from 10 clusters. Geometric mean PM2.5 concentrations for all monitors were near the limit of detection (50 µg/m3) at baseline. During biomass cooking in the index home, median geometric mean PM2.5 concentrations were highest in monitors near the biomass stove (341 µg/m3), followed by those inside the index home (174 µg/m3), then neighbor homes that share a wall with the index home (132 µg/m3), then neighbor homes that do not share a wall with the index home (78 µg/m3). Biomass burning in one home can be a source of indoor air pollution for several homes, increasing geometric mean PM2,5 concentrations to over 5 times the World Health Organization standard of 25 µg/m3. The effect of biomass cookstoves is greatest in homes that share a wall with a biomass-burning home. Eliminating just one biomass cookstove can potentially improve air quality for several households in a community.

49

SOLAR-POWERED OXYGEN DELIVERY

Sophie Namasopo[1], Andrea Conroy[2], Robert Opoka[3], Kevin C. Kain[2], Michael Hawkes[4]

[1]Jinja Regional Referral Hospital, Jinja, Uganda, [2]University of Toronto, Toronto, ON, Canada, [3]Makerere University, Kampala, Uganda, [4]University of Alberta, Edmonton, AB, Canada

Hypoxemia is a grim prognostic sign in pediatric pneumonia and other potentially lethal conditions, for which oxygen therapy may be life-saving. In resource-limited settings, where the majority of pneumonia deaths occur, capacity to deliver oxygen may be limited. Compressed oxygen cylinders are subject to frequent stock-outs and oxygen concentrators require a reliable electrical power source. Here we describe a strategy using solar energy to drive an oxygen concentrator, a technological innovation that could be applied in remote settings off the hydro-electric grid. The system design consisted of commercially available products: 26x80W solar panels, a bank of 8x220Ah batteries, and a 300W oxygen concentrator capable of delivering up to 5L/min of pure oxygen (total cost USD$18,000). Pilot testing of the equipment indicated that the solar panels generated a median (range) of 7.0 (3.6 to 9.0) kWh of energy daily (47% of maximum theoretical output). Between September 2013 and February 2014, 28 critically ill patients with hypoxemia presenting to the emergency ward at Jinja Regional Referral Hospital were treated with solar-powered oxygen. The median (range) age was 6 months (3 months to 3 years) and 45% were female. Common symptoms at presentation included difficulty breathing (89%), cough (82%), fever (74%), and inability to eat/drink (36%). At presentation, all were hypoxemic (SaO2<90%), 74% were tachypneic, and 33% had temperature >37.5°C. Diagnoses included pneumonia (79%), malaria (21%), and sepsis (14%). Treatment with solar-powered oxygen increased the peripheral saturation to >95% in 25 patients (89%); for the remaining 3 patients, oxygenation improved to >90%, but the patients died before recovery of lung function. Duration of hospitalization was median (range) 3 (1-28) days. Outcomes were as follows: 19 patients discharged without disability, 1 discharged with sequellae (cerebral palsy), 2 transferred to another facility, and 6 (23%) died. These data demonstrate proof-of-concept that solar energy can be used to concentrate oxygen from ambient air and oxygenate critically ill patients with hypoxemia using unlimited and freely available inputs, the sun and the air.

50

BIOMARKERS DIAGNOSE THE PATHOGEN OF FEBRILE RESPIRATORY DISTRESS

Clarissa Valim[1], Yan Tan[2], Rushdy Ahmad[2], Miguel Lanaspa[3], Sozinho Acácio[4], Michael Gillette[2], Lola Madrid[3], Katherine Almendinger[2], Danny Milner[1], Karell Pellé[1], Jaroslaw Harezlak[5], Jacob Silterra[2], Pedro Alonso[3], Steve Carr[2], Jill Mesirov[2], Dyann Wirth[1], Roger Wiegand[2], Quique Bassat[3]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Broad Institute, Cambridge, MA, United States, [3]Barcelona Centre for International Health Research (CRESIB), Barcelona, Spain, [4]Manhiça Health Research Center (CISM), Manhiça, Mozambique, [5]Indiana University, Indianapolis, IN, United States

Blood-markers that rapidly and accurately differentiate the bacterial, viral or malarial etiology of febrile acute respiratory distress could reduce the morbidity of respiratory disease, by facilitating diagnosis and appropriate treatment. Using 56 markers measured in a multiplex immunoassay, we sought to identify proteins and protein combinations that could discriminate these three diagnoses. We selected 80 patients with clinical pneumonia (according to the World Health Organization) meeting criteria for bacterial, viral or malarial disease based on clinical, radiographic, and laboratory criteria, including bacteria blood cultures. Patients were subdivided into a training set (17 malaria, 20 virus, and 15 bacteria) and a validation set (10 malaria, 10 virus, and 8 bacteria).

Markers that accurately differentiated any two of the three groups included haptoglobin, IL-10, IL-6, and TNF-alpha. Among markers that accurately classified bacterial disease in the training set, only haptoglobin (AUC-ROC > 0.85 in all comparisons) performed well in the validation set, where it misclassified only one bacterial patient. The overall sensitivity of haptoglobin (> 0.995 mg/mL) for bacteria was 96% with a +Likelihood-Ratio of 3. In a Classification Tree (CT) signature, TNFR2 (or equivalently IL-10) and TIMP-1 were added to haptoglobin to further classify non-bacterial patients into malarial or viral etiologies. Signatures based on Support Vector Machine (SVM) and regression models showed comparable performance and included haptoglobin, IL-10, MMP9, and CK-MB. The overall sensitivity of the CT/SVM signatures for bacteria were 96%/91%, for malaria 81%/96%, and a total of 20%/17% virus patients were misclassified. Blood-proteins can constitute biomarkers for pediatric respiratory disease amenable to deployment as rapid diagnostic tests. These biomarkers are sensitive for the crucial diagnosis of bacterial infection while achieving moderate to good specificities for viral and malarial infections. These markers should allow diagnosis in malaria endemic and non-endemic areas.

51

LOW-COST NON-IMAGING ULTRASOUND (LOCONIUS) FOR PNEUMONIA DETECTION IN RESOURCE-CONSTRAINED SETTINGS

Malena Correa[1], Monica J. Pajuelo[1], Cynthia F. Anticona[1], Holger Mayta[1], Franklin P. Barrientos[2], Ronald B. Campos[2], Benjamin Castañeda[3], Kristians Diaz[3], William Checkley[4], Robert H. Gilman[4], Mirko Zimic[2], Richard A. Oberhelman[1]

[1]Tulane University, New Orleans, LA, United States, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Pontificia Universidad Católica del Perú, Lima, Peru, [4]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Pneumonia is the leading cause of child mortality. Its diagnosis is currently based on chest X-rays (CXR) and clinical/laboratory criteria, which require trained professionals and a complex infrastructure. In low-resource settings clinical criteria are the only available resources, but these lack sensitivity and specificity. New, simple, low-cost methods for diagnosis of pneumonia are urgently needed. Ultrasound (US) has been increasingly used to diagnose pneumonia in children. Although US has a high sensitivity and specificity when compared to CXR, its major limitation is the lack of specialists to interpret US images in resource-poor settings. The goal of our study is to develop a specialist-independent system that uses real time US information interpreted through mathematical algorithms for diagnosis of pneumonia in children. Pulmonary US data were obtained from 30 pneumonia and 30 healthy children presenting to local hospitals in Lima, Peru. Images from US videos were processed to obtain vectors of illumination density (10 pixels per vector) along the depth-axes (475 pixels per image). Vectors skipping one column and corresponding to pulmonary infiltrates on US images were classified as pneumonia as our gold standard. 76,964 vectors were used to train and test a single layer neural network classification system. This neural network was used to classify 38,482 vectors from images not used in developing the algorithm, with an observed accuracy of 92% for recognizing pneumonia. The same data were analyzed in a logistic and multinomial multiple statistical regression. On preliminary analysis, we found 94% accuracy in the classification by the neural network. A preliminary multiple regression model achieved a 93.5% sensitivity and 91.4% specificity, with an accuracy of classification expressed by the area under the ROC curve of 0.97 (i.e. 97% of probability to correctly classify a pair of random samples). We expect that further refinement of this model will lead to an automated algorithm for pneumonia that will permit accurate diagnoses using US in low resource settings.

52

RISK FACTORS FOR SEVERE OUTCOMES AND IMPACT OF VACCINATION ON PNEUMONIA AND INFLUENZA WITHIN ACTIVE US MILITARY POPULATIONS 2000-2012

Michael J. Cooper

Armed Forces Health Surveillance Center, Silver Spring, MD, United States

Respiratory infections are responsible for up to 350,000 of medical encounters each year among US military personnel. Our objective in this study was to quantify risk factors associated with severe acute respiratory infection (SARI) in a US Military Cohort admitted to hospital with respiratory illness. We obtained data on 15,210 hospitalizations for pneumonia and influenza (P&I) between 01/01/00 and 31/12/12. From these, we identified 335 SARI episodes using standard case definitions. We evaluated the effect of demographic and occupational characteristics, comorbid conditions, and history of influenza vaccination on the risk of a hospitalized P&I case becoming a SARI case. We also evaluated the risk of SARI and the length of time since vaccination. The median age of subjects was 22 years (range, 17-64) and subjects were predominantly male (89.5%). Risk factors for developing SARI included age (≥45 years, RR=1.8, 95% CI 1.2-2.7), American Indian or Alaskan Native ethnicity (RR=3.0, 95% CI 1.0-8.7), and service in the Air Force (RR=1.7, 95% CI 1.3-2.3). Being male, born in mainland US (vs outside of mainland US) and recent vaccination (within 180 days of episode) were protective against developing SARI. Among comorbid conditions, risk factors for SARI included chronic liver disease (RR=5.7, 95% CI 4.5-7.1), diabetes (RR=2.8, 95% CI 1.7-4.6) and, immune disorders (RR=2.6, 95% CI 1.1-6.1). Male gender (RR=0.7, 95% CI 0.5-0.9) and influenza vaccination (RR=0.7, 95% CI 0.5-0.97) remained significantly protective. Our results also suggest that the closer the vaccination was to hospitalization, the less likely the individual was to develop SARI, with substantially reduced odds of SARI for delays of 1 vs. 6 months). These data suggest that timing of influenza vaccination is critical in reducing severity of P&I hospitalizations; additional analysis of delays in vaccination may impact future military immunization policy.

53

IMPACT OF INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY WITH AZITHROMYCIN-CONTAINING REGIMENS ON MATERNAL NASOPHARYNGEAL CARRIAGE AND ANTIBIOTIC SENSITIVITY OF *STREPTOCOCCUS PNEUMONIAE*, *HEMOPHILUS INFLUENZAE* AND *STAPHYLOCOCCUS AUREUS* AT DELIVERY

Holger W. Unger[1], Celestine Aho[2], Maria Ome[2], Regina Wangnapi[2], Wanda Jack[2], Alice Lafana[2], Audrey Michael[2], Sarah Hanieh[1], Peter Siba[2], Ivo Mueller[3], Andrew R. Greenhill[4], Stephen J. Rogerson[1]

[1]University of Melbourne, Melbourne, Australia, [2]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea, [3]Walter and Eliza Hall Institute, Melbourne, Australia, [4]Federation University, Churchill, Australia

Sulfadoxine-pyrimethamine (S/P) combined with azithromycin (AZI) has the potential for use as intermittent preventive treatment in pregnancy (IPTp) to prevent malaria, treat sexually transmitted infections, and reduce low birthweight. However, the intervention may increase circulation of antibiotic-resistant bacteria associated with severe paediatric infections. We evaluated the effect of IPTp with S/P+AZI compared to a single course of S/P plus chloroquine (S/P+CQ) on maternal nasopharyngeal carriage and antibiotic susceptibility of *Streptococcus pneumoniae, Haemophilus influenzae* and *Staphylococcus aureus* at delivery amongst 854 women participating in a randomised controlled trial in Papua New Guinea. Serotyping was performed, and susceptibility to azithromycin and other antibiotics tested by disk diffusion and Etest. Potential risk factors for carriage were examined. Significantly lower proportions of women who received S/P+AZI had nasopharyngeal carriage of S. pneumoniae (S/

P+AZI: 7.2% [30/418] vs S/P+CQ: 19.3% [84/436], P < 0.001) and H. influenzae (2.9% [15/418] vs 6.0% [26/436], P = 0.028), but not S. aureus (23.7% [99/418] vs 24.7% [105/436], P = 0.892). The number of macrolide-resistant pneumococcal isolates was small, but increased in the S/P+AZI group (13.3% [4/30]) compared to S/P+CQ (2.2% [2/91], P < 0.033). The proportion of S. pneumoniae isolates with serotypes covered by the 13-valent pneumococcal conjugate vaccine was not statistically different by IPTp arm (S/P+AZI: 10.3% [3/29] vs S/P+CQ: 17.6% [16/91], P = 0.352). Many pneumococcal isolates exhibited reduced sensitivity to penicillin (59.5% [72/121]) and trimethroprim-sulfamethoxazole (42.1% [51/121]), irrespective of treatment arm. Although numbers were small, S/P+AZI increased the proportion of macrolide-resistant pneumococci, whilst significantly reducing maternal carriage of *S. pneumoniae* and *H. influenzae*. Future studies on S/P+AZI should evaluate the persistence of maternal macrolide-resistant *S. pneumoniae* and assess the clinical significance of their circulation.

54

UNEXPECTED HIGH FREQUENCY OF VIRAL AGENTS IN ASYMPTOMATIC CHILDREN AND LOW INFLUENZA REPRESENTATION IN PNEUMONIA CASES AMONG CHILDREN IN RURAL NORTH PAKISTAN

Julia M. Baker[1], Zeba A. Rasmussen[1], Assis Jahan[1], Asad Ali[2], Fatima Aziz[2], Shahida M. Qureshi[2], Uzma Bashir Aamir[3], Syed Iqbal Azam[2], Khalil Ahmed[4], Cecile Viboud[1], Wladimir J. Alonso[1], Syed Sohail Zahoor Zaidi[3], Stacey Knobler[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Aga Khan University, Karachi, Pakistan, [3]National Institute of Health, Islamabad, Pakistan, [4]Karakoram International University, Gilgit, Pakistan

Pneumonia is a major cause of child mortality and morbidity in developing countries; the contribution and dynamics of various viral pathogens is not clear, affecting the implementation of optimal prevention and treatment strategies. Oshikhandass, a rural village in NE Pakistan, was previously studied from 1989-1996, when pneumonia was the main cause of mortality in children <5 years (disease incidence 44/100 child years). This study was repeated (after HIB vaccine introduction) with weekly surveillance of children <5 from Apr. 2012-Mar. 2014 to determine changes in epidemiology and describe the frequency of viral agents in nasopharyngeal swabs. PCR from Apr. 2012-Nov. 2013 used the Luminex® platform to detect 20 viruses (RSV, influenza, enterovirus/rhinovirus, parainfluenza, adenovirus, corona virus, metapneumovirus, bocavirus), while the Taqman® method was used Dec. 2013-Mar. 2014 to detect 4 viruses (RSV, influenza). From Feb. 2013, age, sex and neighborhood matched controls with no respiratory symptoms were added to the study. On average, 809 children were followed monthly with 238 pneumonia episodes detected (14.7/100 child years, average age 22.5 months). Pneumonia incidence was highest during winter months with significant variation between the 2012-13 and 2013-14 winters (54.6 and 10.1 episodes/100 child years, respectively). Among all 232 cases tested, 77% had a detectable virus. Detection rates were (cases/controls): RSV 25.0/15.6, influenza 3.4/1.6, enterovirus/rhinovirus 70.3/41.9, parainfluenza 8.8/7.0, adenovirus 4.4/9.3, corona virus 1.1/4.7, metapneumovirus 1.1/0.0, bocavirus 3.3/2.3. Unexpected findings include: 1) a substantial portion (54.7%) of asymptomatic children carried viruses, only slightly below the rate for their matched counterparts with pneumonia (69.0%); 2) the low prevalence of influenza viruses despite high sensitivity of the assay used. Further investigation on severity of disease is underway, but it seems that public health interventions aiming to reduce pneumonia burden should not focus on influenza vaccination as a priority.

55

SCHISTOSOMIASIS HAEMATOBIA AND INFERTILITY IN COAST PROVINCE, KENYA

Sarah C. Miller-Fellows, Vanessa Hildebrand, Jennifer Furin, **Charles H. King**

Case Western Reserve University, Cleveland, OH, United States

We examined reproductive patterns, cultural practices surrounding reproduction, and the potential effects of childhood urogenital *Schistosoma haematobium* infection and treatment on adult subfertility among women in Coast Province, Kenya. Previous research has documented an increased risk of subfertility in areas of sub-Saharan Africa due to high rates of pelvic infection, as well as an ecological association between urogenital schistosomiasis prevalence and decreased fertility. This project analyzed findings from 162 interviews with women of childbearing age in a rural, coastal community, linking them to their individual treatment records from a previous 30+ year longitudinal study of parasitic infections. Both quantitative and qualitative findings were included. Reproductive histories suggested a much higher rate of subfertility (43.8%) than worldwide averages (8-12%). Qualitative analysis regarding reproductive practices demonstrated a high saturation of public health messages regarding proper pregnancy care, co-existing with continuing ethnomedical beliefs. Although no significant relationship was demonstrated between Schistosoma infection history and adult subfertility due to the high regional prevalence of schistosomiasis, significant associations were found between age at first treatment and fertility in adulthood, with those treated before age 21 less likely to have subfertility (p=0.001). The high subfertility rate documented in this study suggests the importance of public health programs to prevent and treat pelvic infections in their early stages to prevent reproductive tract damage. The qualitative findings suggest the successful saturation of some public health messages regarding pregnancy care, such as the importance of sleeping under bednets in malaria-endemic regions. However, other messages, such as the importance of seeking prenatal care, were less frequently mentioned. Finally, the findings suggesting the importance of early treatment to prevent the fertility-damaging effects of urogenital schistosomiasis lend further support for programs for universal treatment of children in endemic regions.

56

THE IMPACT OF PRAZIQUANTEL GIVEN AT 12-16 WEEKS GESTATION ON PREGNANCY OUTCOMES: RESULTS OF A DOUBLE BLIND, PLACEBO CONTROLLED TRIAL

Jennifer F. Friedman[1], Luz P. Acosta[2], Palmera Baltazar[2], Veronica Tallo[2], Emily A. McDonald[1], Haiwei Wu[1], Sunthorn Pond-Tor[1], Nora L. Watson[3], Jonathan D. Kurtis[1], Remigio M. Olveda[2]

[1]Center for International Health Research, Lifespan Hospital, Providence, RI, United States, [2]Research Institute of Tropical Medicine, Manila, Philippines, [3]The EMMES Corporation, Rockville, MD, United States

Praziquantel was released in 1979, but was never studied in pregnant or lactating women, necessitating its designation as an FDA Pregnancy Class B drug. Given this, and the lack of well-controlled trials evaluating its safety and efficacy in this population, pregnant and lactating women are excluded from treatment programs in many countries where schistosomiasis is endemic. In 2002, based on post-market experience and a concern that Praziquantel would never be formally evaluated in human pregnancy, a WHO informal conference concluded that all schistosomiasis infected pregnant and lactating women should be considered a high-risk group and be offered treatment. Though many nations, particularly in sub-Saharan Africa, adopted this approach, many continue to withold treatment pending more data on safety and efficacy. The objectives of this randomized, double blind placebo controlled trial were to evaluate the safety and efficacy of Praziquantel given to pregnant women infected with *S. japonicum* at 12-16 weeks gestation. Women were enrolled into the study if they provided informed consent and were over age

18, infected with *S. japonicum*, otherwise healthy, and pregnant at 12-16 weeks gestation. Women (N=380) were enrolled and treated with overencapsulated Praziquantel (60 mg/kg in split dose) or placebo and admitted for 24 hours. The following efficacy outcomes were ascertained: birthweight (primary), maternal hemoglobin and iron status at 32 weeks gestation, maternal gestational weight gain, newborn hemoglobin and birth weight. In addition, safety data were collected including toxicology pre and post dosing, abortion and miscarriage rates, and congenital anomalies. Though all analyses for these efficacy and safety outcomes are complete and data locked, we cannot report these until pharmacokinetic studies are completed (expected summer of 2014). We will present the impact of treatment on the aforementioned outcomes. Results from this trial will provide important data from a well controlled study to inform policies regarding treatment of this high risk group.

57

IMPACT OF SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHS ON VACCINE-INDUCED IMMUNE RESPONSES

Diana K. Riner[1], Emmy Kavere[2], Eric M. Ndombi[2], Nupur Kittur[1], W. Evan Secor[3], Diana Karanja[2], Daniel G. Colley[1]

[1]University of Georgia, Athens, GA, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

In light of recent global health initiatives to increase vaccine rates for the world's most vulnerable populations, there is an urgent need to address the issue of vaccine efficacy in populations where helminth infection rates are high. Helminths such as schistosomes are remarkable in their ability to modulate host immune responses, which helps to promote their survival. Immunoregulation begins early in schistosome infection and is characterized by hyporesponsiveness to parasite antigens and other bystander antigens, suggesting that a schistosome infection at the time of vaccination could negatively impact the induction of a protective response to the vaccine. To investigate the impact that concurrent helminth infection might have on how individuals respond to vaccine antigens, we recruited participants from Kisumu Polytechnic College in Kisumu County, western Kenya. At study enrollment participants were screened for schistosomiasis and soil transmitted helminths (STHs) and assigned to a group based on helminth status. The vaccines were then administered: tetanus toxoid (single dose), hepatitis B (doses at 0, 1 and 6 months), and meningococcus A+C (doses at 0 and 2 months). Helminth infections were treated a week after the second hepatitis B boost. A baseline blood draw, a blood draw 2 months after the start of vaccinations and 2 months after the final hepatitis B boost were obtained for evaluation of humoral and cellular immune responses to the vaccine antigens. CD3+/CD4+/CD25high T regulatory cell levels were also determined at each time point to assess their impact on vaccine responsiveness. Preliminary data analysis shows that participants with schistosomiasis had significantly higher proportions of circulating CD3+/CD4+/CD25high T regulatory cells compared to uninfected controls at baseline. At the second blood draw a week after treatment, CD3+/CD4+/CD25high T regulatory cell levels in the schistosomiasis group were significantly elevated compared to baseline levels.

58

EVALUATION OF ORGANOMEGALY AND OTHER MORBIDITIES FOLLOWING TWO ROUNDS OF PRAZIQUANTEL MASS DRUG ADMINISTRATION AMONG KENYAN SCHOOL CHILDREN. THE SCORE PROJECT

Isaac Onkanga

KEMRI-Centers for Disease Control and Prevention, Kisumu, Kenya

One of the goals of schistosomiasis control among school age children is to reduce morbidity. However, monitoring and evaluation of the impact of deworming projects usually is limited to parasitological outcomes as there are not well defined markers of schistosomiasis morbidity other than for persons with severe hepatosplenic disease. In a subset of a larger study comparing mass drug administration approaches in school children, we measured organomegaly and anemia at baseline and after two years to determine whether praziquantel treatment approaches made a difference in morbidity outcomes. Children from 12 schools were enrolled; children from 6 of the schools received school-based treatment (SBT) once after baseline assessment while children from the other 6 schools lived in villages that received community-wide treatment (CWT) once a year. Children were examined for *Schistosoma mansoni* infection using the Kato-Katz method, venous blood was collected to test for malaria parasites and hemoglobin levels, and abdominal ultrasound was performed by a trained ultra-sonographer. The differences between baseline and follow up schistosomiasis prevalence and intensity were not significant for either SBT or CWT schools, or between the treatment approaches. However, ultrasound-detected liver pathology ($P< 0.001$) and anemia ($P< 0.001$) were significantly higher at the 2 year follow up for both groups, as was the percent of students with malaria infection ($P<0.001$). Because anemia and the type of liver pathology we detected are both associated with malaria, we believe that the increased morbidity that we observed at follow-up was likely due to the increased malaria and could have masked any benefits of the schistosomiasis treatments. Our results indicate a need for continued monitoring of morbidity markers in deworming projects, including assessment of co-infecting organisms that can affect morbidity, to determine the best intervention strategies to control *S. mansoni* infection as a public health problem among school age children.

59

GLYCAN MICROARRAY-ASSISTED ANALYSIS OF ANTI-GLYCAN ANTIBODY RESPONSES UPON INFECTION OR VACCINATION WITH *SCHISTOSOMA MANSONI*

Cornelis H. Hokke[1], Linh Nguyen[1], Cornelis H. Smit[1], Alan Wilson[2], David W. Dunne[3], Angela van Diepen[1]

[1]Leiden University Medical Center, Leiden, Netherlands, [2]University of York, York, United Kingdom, [3]University of Cambridge, Cambridge, United Kingdom

Schistosomiasis is a chronic and potentially deadly parasitic disease that affects millions of people in (sub)tropical areas. Immunity to *Schistosoma* can be acquired, but this takes many years of exposure, multiple infections and treatments, and maturation of the immune system. Most antibodies generated are directed against the numerous schistosome glycans, but the precise structure of the glycan antigens and the relation to immunity are poorly understood. Anti-glycan antibodies can be studied efficiently using glycan microarrays. We have generated a microarray containing hundreds of naturally occurring glycans isolated from different life stages of *S. mansoni*. To study the specificity and nature of anti-glycan antibodies in schistosomiasis, we have applied this microarray to the analysis of anti-glycan IgG and IgM in sera from different age groups within an *S. mansoni*-endemic community in Uganda. The most intense IgG responses are against highly fucosylated glycans associated with cercarial and egg glycoproteins and glycolipids. We observed age-dependent differences in anti-glycan responses, especially when considering changes induced by treatment with PZQ. Also within age groups we observed differences between groups with high and low infection intensities. In addition we have used sera from baboons protected against challenge infection after vaccination with irradiated *S. mansoni* cercariae to study longitudinally the development of anti-glycan responses. The strong responses against cercarial lipid-derived glycans as well as cercarial O-glycans are gradually induced by repeated vaccination raising the question if these responses contribute to the high level of protection observed in the vaccinated baboons. Shotgun glycan microarrays allow the definition of groups of schistosome-infected individuals as well as glycan element clusters to which antibody responses are generated in different cohorts and settings.

60

MULTIPLEX SEROLOGICAL ASSESSMENT OF SCHISTOSOMIASIS IN MBITA DISTRICT, WESTERN KENYA

Kimberly Won[1], Henry Kanyi[2], Faith Mwende[2], Ryan Wiegand[1], E. Brook Goodhew[1], Jeffrey Priest[1], Yeuk-Mui Lee[1], Sammy Njenga[2], W. Evan Secor[1], Patrick Lammie[1], Maurice Odiere[3]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Kenya Medical Research Institute, Nairobi, Kenya, [3]Kenya Medical Research Institute, Kisumu, Kenya

Schistosomiasis control programs centered on mass drug administration (MDA) reduce parasite prevalence and morbidity in treated individuals. Currently, program impact in areas endemic for *Schistosoma mansoni* is measured primarily by assessing changes in infection prevalence and intensity as measured by stool examination. Antibody-based tests potentially have an advantage over currently used antigen-based tests, and a newly developed multiplex bead assay allows for the analysis of multiple antigens at one time. A total of 935 serum samples collected from individuals (1-85 years) living in communities on the shores of Lake Victoria in western Kenya were tested for antibody responses to 36 antigens, including two *Schistosoma* spp. antigens (soluble egg (SEA); adult worm microsomal (Sm25)). Stool and serum samples were collected at three time points: May-July 2012; December 2012; May-July 2013. One MDA was conducted in August 2012. Parasitological data were available for 916 (98.0%) of the samples. Overall prevalence of *S. mansoni* at baseline was 35.6% by stool examination, and modestly decreased to 29.6% 9-11 months after MDA with praziquantel. However, intensity of infection significantly decreased after treatment ($p=0.005$), and importantly, heavy intensity infections decreased from 16.1% to 7.5% ($p<0.05$). Antibody responses to both SEA and Sm25 were associated with age ($p<0.001$), and older individuals were more likely to have higher antibody responses than children under five years. Antibody responses to both antigens were significantly associated with intensity of infection, with responses increasing as egg burdens increased ($p<0.001$). Overall responses to Sm25 significantly decreased after one round of MDA ($p<0.001$). The observed decrease in Sm25 response after treatment has not been previously reported. Additional samples from this study area will be tested to further evaluate this observation. Detection of antibodies to Sm25 or other defined antigens have the potential to be a useful tool for monitoring the impact of schistosomiasis treatment programs.

61

THE ADIPOSE TISSUE DERIVED STEM CELLS (ASC) CHANGED THE ACTIVATION PROFILE OF IMMUNE SYSTEM CELLS IN *SCHISTOSOMA MANSONI* ACUTE INFECTION

Adriana Bozzi[1], Ana Thereza Chaves[2], Kelly Alves Bicalho[3], Dirli Emerick Eller[1], Lorena de Cássia Ferraz[1], Carlos Eduardo Calzavara-Silva[1], Andrea Teixeira Carvalho[1], Olindo Assis Filho[1], Alfredo Miranda Goes[2], Rodrigo Correa-Oliveira[1]

[1]Centro de pesquisas René Rachou/FIOCRUZ-MG, Belo Horizonte, Brazil, [2]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [3]Universidade Federal de Ouro Preto, Belo Horizonte, Brazil

Mesenchymal stem cells (MSC) have the ability of self-renewal and differentiation into various mesodermal cell lineages. Recently, it has been observed that MSC have potent anti-proliferative and anti-inflammatory effects in autoimmune and inflammatory diseases as a new strategy for immunosuppression. The control of inflammation in infectious and parasitic diseases by MSC was not evaluated so far. The present study aimed to evaluate if the adipose tissue derived stem cells (ASC) could be able to regulate the inflammation in an experimental acute *Schistosoma mansoni*-infection model by immune system activation analysis. The ASC were isolated from C57BL/6 mice, expanded *in vitro* and characterized phenotypic and functionally. These cells were injected by the tail vein into C57BL/6 mice (n=5) in two, four, six and eight weeks post-infection with *S. mansoni*. Fifteen and thirty days after the ASC injection, the splenocytes were obtained and lymphocytes activation evaluated by the expression of CD25, CD69, CD28 and CTLA-4 molecules. The IL-2, IFN-γ, TNF-α, IL-17, IL-4, IL-6 and IL-10 cytokine levels were measured in the serum blood by flow cytometry. The results showed a decrease ($p<0.05$) in TCD4+ regulation as determined by CD25, CD69 and CTLA-4, mainly six weeks post-infection and after fifteen or thirty days post-injection of ASC. Interesting, the cytokines analysis revealed a different profile in the IL-2 and IL-4 levels. Both cytokines showed significant levels after eight weeks post-infection and fifteen days post-injection of ASC. In conclusion, our results shows that the ASC can modulate the immune response in *S. mansoni*, mainly after six weeks post-infection, and suggest that ASC can be evaluated for the control of the granulomatous reaction in this disease.

62

ESTIMATING ACCURACY OF PARTICIPANT RECALL FOLLOWING AN INTEGRATED MASS DRUG ADMINISTRATION FOR NEGLECTED TROPICAL DISEASES

Alaine K. Knipes[1], Salissou Adamou Bathiri[2], Mawuli Nyaku[1], Gina Chapleau[1], Els Mathieu[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Ministry of Health, Niamey, Niger

Coverage Surveys (CS) assess quality of implementation of mass drug administration (MDA) and provide feedback for ministries of health and drug donation programs. CS independently determine if individuals in targeted areas received medication during previous MDA, and rely on individuals' correct recall of medications taken. As neglected tropical disease programs integrate, multiple medications may be offered during MDA, potentially diminishing the strength of CS as a tool. We assessed individuals' ability to recall multiple medications received in integrated MDA, over time. Niger's 2012 MDA distributed ivermectin (IVM), albendazole (ALB), praziquantel (PZQ), and azithromycin (AZM) to eligible individuals. During MDA, observers accompanied distributors and created a Gold Standard Register (GSR), independent from the distributor register. All persons living in households in villages were registered, and it was noted whether or not they took medications, regardless of their eligibility. Households were systematically selected to be revisited for CS at 2, 6, or 12-months post-MDA. During CS, respondents were shown pills. During CS, surveyors and respondents were blinded to MDA register responses. Of 4423 individuals registered from 806 households at GSR, 3455 (78.1%) responded at CS. Response rate at 2, 6, and 12-months post-MDA was 84.4%, 80.7%, and 68.3%, respectively. At GSR, 93.9% (3243 of 3455) ingested one or more medications, while 93.5%, 93.5%, 88.7%, and 91.2%, ingested IVM, ALB, AZM, and PZQ respectively. At CS, 95.1% (3287 of 3455)recalled having ingested one or more medications, while 86.2%, 88.7%, 89.0%, and 82.0%, recalled ingesting IVM, ALB, AZM, and PZQ, respectively. IVM concordance (% agreement between GSR and CS) at 2, 6, and 12-months post-MDA was 82.1% (95% CI: 79.1-85.1%), 86.6% (CI 83.6-89.6%), and 80.8% (CI 77.2-84.4%), respectively. ALB concordance at 2, 6, and 12-months was 85.2% (CI 82.5-87.9%), 87.3% (CI 84.5-90.0%), and 85.0% (CI 81.8-88.3%), respectively. AZM concordance at 2, 6, and 12-months was 79.9% (CI 76.7-83.2%), 82.9% (CI 79.2-86.5%), and 82.5% (CI 79.2-85.8%), respectively. PZQ, concordance at 2, 6, and 12-months was 74.5% (CI 70.9-78.2%), 82.6% (CI 79.6-85.7%), and 80.3% (CI 77.0-83.6%), respectively. CS correctly measured overall MDA coverage, though it is less reliable for drug-specific coverage. We confirm the strength of CS as an MDA evaluation tool.

63

THE SOCIO-ECONOMIC IMPACT OF CONTROL OR ELIMINATION OF FIVE NEGLECTED TROPICAL DISEASES

Edeltraud J. Lenk[1], Marianne Luyendijk[1], Ken Redekop[1], Rian Rijnsburger[1], Wilma A. Stolk[2], Sake J. de Vlas[2], Jan Hendrik Richardus[2], Johan L. Severens[1]

[1]Institute of Health Policy and Management, Rotterdam, Netherlands, [2]Erasmus MC, Rotterdam, Netherlands

There is renewed commitment to control and eliminate neglected tropical diseases (NTDs) as defined by the WHO and endorsed by the London Declaration. We estimated the economic impact of meeting the targets for the five diseases eligible for mass drug administration: lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminthiasis, and trachoma. We performed a systematic literature review to identify information on costs related to the diseases or related health outcomes, considering out of pocket payments (OPP), productivity loss, and impoverishment. Cost estimates per person were combined with projections of the number of people suffering from clinical manifestations per NTD, country, and year, for the periods 2011-2020 and 2021-2030, comparing the ideal scenario in which the targets are met with a counterfactual scenario (assuming that the prevalence (%) of clinical manifestations remains constant at its pre-control level). The averted burden is calculated as the difference between the two scenarios. The limited information available on the costs of illness shows that the productivity loss associated with clinical manifestations are about 3% for infestation and mild symptoms, about 15% for more severe manifestations like lymphedema and hydrocele, and as high as 38% and 79% for severe vision loss and blindness. The total productivity costs averted globally by reaching the goals for the five above-mentioned diseases may amount to US$240 billion in the period 2011-2020 and US$390 billion in the period 2021-2030, even excluding OPPs as well as productivity loss due to premature mortality. Soil-transmitted helminthiasis accounted for approximately half of this amount. There is considerable uncertainty in these estimates because of scarce literature. Nevertheless, even with conservative assumptions, the averted costs of achieving the London Declaration targets are high. Results will be discussed with respect to the impact on poverty.

64

SHOULD SCHOOL BASED DEWORMING BE SUBSIDIZED? LONG RUN EVIDENCE FROM A RANDOMIZED CONTROL TRIAL IN KENYA

Sarah Baird[1], Joan Hamory Hicks[2], Michael Kremer[3], Edward Miguel[2]

[1]Milken Institute School of Public Health, Washington, DC, United States, [2]University of California Berkeley, Berkeley, CA, United States, [3]Harvard University, Boston, MA, United States

Recent research suggests that preventative and non-acute health investment in the developing world are often highly responsive to subsidies, but what level of subsidy should governments provide? Using data from a deworming randomized control trial in Kenya this paper shows that by combining data on take up elasticities with data on labor market impacts and tax rates one can establish the optimal subsidy. To derive this optimal subsidy we first develop a framework where individuals may generate a positive fiscal externality, through consumption or labor income tax, by investing in human capital. Applying this framework to the case of deworming in Kenya, we find that fully subsidizing child deworming raised adult earnings generating sufficient future gains in government revenue to make possible Pareto improving reductions in tax rates. Consistent with previous reports, we estimate differing labor market impacts of child health investments by gender. Ten years after the start of the deworming program, men who were eligible to participate as boys work 3.5 more hours each week, spend more time in entrepreneurship, are more likely to hold manufacturing jobs with higher wage earnings, and have higher living standards. Women, who were eligible as girls, have better educational outcomes, are more likely to grow cash crops, and reallocate labor time from agriculture to entrepreneurship. These results suggest that health interventions that are too late in life to affect cognition or height can still have long-run impacts on labor market outcomes by affecting the amount of time people spend in school or work.

65

THE IMPACT OF MASS DRUG ADMINISTRATION WITH ALBENDAZOLE ALONE ON LYMPHATIC FILARIASIS IN THE REPUBLIC OF CONGO

Sebastien D. Pion[1], Cédric B. Chesnais[1], Jean Bopda[2], Frédéric Louya[3], Andrew C. Majewski[4], Peter U. Fischer[4], Gary J. Weil[4], Michel Boussinesq[1], François Missamou[3]

[1]Institut de recherche pour le Développement, Montpellier, France, [2]Center for Research on Filariasis and Other Tropical Diseases, Yaoundé, Cameroon, [3]Programme National de Lutte contre l'Onchocercose, Brazzaville, Republic of the Congo, [4]Infectious Diseases Division, Department of Internal Medicine, St. Louis, MO, United States

Implementation of mass drug administration (MDA) with ivermectin plus albendazole (Alb) for lymphatic filariasis (LF) has been delayed in Central Africa, because ivermectin sometimes causes severe adverse events in people with very high *Loa loa* microfilaremia (Mf). Albendazole has activity against *Wuchereria bancrofti*, and it is safe for use in patients with loiasis, and WHO has recently recommended use of Alb MDA together with vector control for control of LF in areas with co-endemic loiasis. We are presenting early results from a planned 3-year community trial that is assessing the impact of MDA with Alb on LF in a village of the Republic of Congo. Baseline results (September 2012) from 773 subjects revealed a filarial antigenemia rate of 17.3% (ICT) and a Mf rate of 5.3% (140 µl night blood smear). The population was offered 400 mg of Alb at baseline and 6 months later. Therapeutic coverage for the population > 2 years of age was ~ 85%. A second cross-sectional evaluation at 12 months (741 tested) showed that Alb MDA had not yet reduced ICT or Mf rates in the community (16.6 and 4.2%); however, Mf counts in Mf-positive subjects were reduced by 60% (geometric mean reduced from 199.4 to 79.6 Mf/ml, $P = 0.01$). The effect of Alb was more dramatic in those 38 people who were Mf positive at baseline and retested at 12 months: 37% had total Mf clearance, and Mf counts in those with >10 Mf/ml at baseline were reduced in average by 82.1% (range: 4.3-100%). In addition, ICT scores (a semi-quantitative measure of filarial antigenemia) were also reduced after Alb MDA. MDA also dramatically reduced the hookworm infection rate from 6.5% to 0.6%, with less impressive effects on *Ascaris* and *Trichuris*. These preliminary results suggest that semiannual community MDA with Alb is a useful tool for control of LF and STH that is feasible for use in areas where loiasis is co-endemic. Evaluation at 24 months is planned for October 2014.

66

COST-EFFECTIVENESS OF COMMUNITY-WIDE, INTEGRATED PREVENTIVE CHEMOTHERAPY FOR SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHS

Nathan C. Lo[1], Isaac I. Bogoch[2], Brian G. Blackburn[1], Eliézer K. N'Goran[3], Giovanna Raso[4], Jürg Utzinger[4], Jason R. Andrews[1]

[1]Stanford University School of Medicine, Stanford, CA, United States, [2]University of Toronto, Toronto, ON, Canada, [3]Unité de Formation et de Recherche Biosciences, Abidjan, Côte D'Ivoire, [4]Swiss Tropical and Public Health Institute, Basel, Switzerland

World Health Organization (WHO) recommendations for preventive chemotherapy against helminth infections emphasize treatment of school-age children. We aimed to evaluate the cost-effectiveness of expanding integrated preventive chemotherapy to adults in high-burden settings. We developed a dynamic, age-structured transmission and cost-effectiveness model that simulates integrated preventive chemotherapy programs

for schistosomiasis and soil-transmitted helminths (STH). We utilized data on prevalence and intensity of infection with *Schistosoma mansoni* and STH from surveys in Côte d'Ivoire, and simulated a community of 15,000 children and adults. Transmission parameters were calibrated according to prevalence and dispersion of each worm. We simulated drug administration with praziquantel+albendazole among: (i) school-age children only or (ii) school-age children and adults. We assumed 75% coverage for the intervention. Treatment costs for a child and adult were estimated at US$1.09 and US$1.94, respectively. The incremental cost-effectiveness ratio (ICER) was calculated in 2014 US$ per disability-adjusted life year (DALY) averted, comparing expanded treatment of both child and adult populations against current WHO guidelines that target children alone. We defined strategies as highly cost-effective if the ICER was less than the GDP per capita of Côte d'Ivoire (US$1,244 in 2014). An integrated preventive chemotherapy program for schistosomiasis and STH was highly cost-effective in treatment of children alone (ICER: US$620/DALY averted) compared to no treatment. Expanded coverage of both children and adults (ICER: US$653/DALY averted) was highly cost-effective compared to treatment of children alone, and remained highly cost-effective even if treatment costs for adults were 4-fold greater than school-based strategies. Expanded treatment of adults yielded a 50% increase in DALYs averted for children, suggesting that treating adults can lower the prevalence and infection intensity in children. Integrated, community-wide preventive chemotherapy programs for schistosomiasis and STHs may be highly cost-effective. These results support re-evaluating global guidelines for helminth control programs and possibly expanding coverage to adults.

67

SOCIOECONOMIC INEQUALITIES IN THE BURDEN OF NEGLECTED TROPICAL DISEASES

Margarete C. Kulik, **Tanja A. Houweling**, Henrike E. Karim-Kos, Wilma Stolk, Jan Hendrik Richardus, Sake de Vlas

ErasmusMC University Medical Center Rotterdam, Rotterdam, Netherlands

It is generally assumed that neglected tropical diseases (NTDs) are concentrated in poor populations, but evidence remains scattered. We describe between and within country poor-rich inequalities in burdens of London Declaration NTDs, including NTDs controlled through preventive chemotherapy (onchocerciasis, blinding trachoma, lymphatic filariasis, schistosomiasis, soil-transmitted helminths (STH)) and those controlled through intensified disease management (Chagas disease, leprosy, visceral leishmaniasis, human african trypanosomiasis (HAT)). First, using data from the global burden of disease 2010 study, we examined to which extent each NTD is concentrated in low and lower-middle income countries based on Gross National Income per capita. Second, we conducted a systematic literature review on the socioeconomic distribution of NTD prevalence within countries, including publications between 2004 and 2013. The vast majority (84-100%) of the global burden of each NTD in 2010 is concentrated in low and lower-middle income countries, with the exception of STH (63% of the global burden) and Chagas disease (8%). The concentration in low income countries is strongest for HAT (92% of the global burden), followed by onchocerciasis (55%) and schistosomiasis (45%). For many NTDs the burden per 100.000 population has declined since 1990, but low income countries have benefitted less than lower-middle and upper-middle income countries from control initiatives. Evidence on the socioeconomic distribution of NTD prevalence within countries is scarce for some NTDs like onchocerciasis and HAT, but more readily available for schistosomiasis and STH. Most studies report a gradient in NTD prevalence, with highest prevalence among lower socioeconomic groups. The magnitude of inequalities varies, but often prevalence is at least twice as high in lower compared with higher socioeconomic groups. Future NTD control as pursued by the London Declaration will benefit the poorest countries and within these countries the poorest populations.

68

AN INTEGRATED SENTINEL SITES APPROACH TO EVALUATING THE IMPACT OF MASS DRUG ADMINISTRATION ON NEGLECTED TROPICAL DISEASES

Mawuli Nyaku[1], Alaine K. Knipes[1], Naomi Drexler[1], Roland Bougma[2], Benjamin Nwobi[3], Adams Valian[2], Sunday Isiyaku[4], Aliyu Mohammed Jabo[5], Els Mathieu[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Ministry of Health, Ouagadougou, Burkina Faso, [3]Federal Ministry of Health, Abuja, Nigeria, [4]Sight Savers, Kaduna, Nigeria, [5]Sokoto State Eye Care Programme, Sokoto, Nigeria

Preventative chemotherapy for the Neglected Tropical Diseases (NTDs) is provided through mass drug administrations (MDA). Although the interventions for different NTDs are often integrated, the protocols to evaluate MDA impact are disease specific. We field-tested an integrated impact survey to evaluate feasibility and determine if this low resource method could be used by the trachoma program to decide when to conduct their more resource-intensive impact surveys. We validated the trachoma data with the WHO recommended cluster survey. Impact evaluations were conducted in Gwadabawa district, Nigeria and Dô district, Burkina Faso (BF). We implemented sentinel sites over a 3-year period as currently done by the lymphatic filariasis (LF) program but added schistosomiasis and soil-transmitted helminthes (STH). Four sites per district were selected based on NTD prevalence and geographic representativeness. In each district, 2000 children aged 1-9 years in 4 sites were clinically examined for signs of trachoma using Trachomatous Inflammation - Follicular prevalence (TF) as indicator. In addition, 1600 children (80 per cluster, aged 1-9 years) were examined using the WHO cluster survey method. In Nigeria, TF in year 1 was 29.8%, 14.3%, 21.0%, and 16.3% per site, and cluster survey prevalence was 16.4% [95% CI: 14.8-18.0]; for year 2, TF was 4.75%, 9.25%, 6.00%, and 8.75% and 10.9% [95% CI: 9.5-12.5]; for year 3, TF was 10.0%, 16.0%, 9.0% and 15.0%, and 17.2% [95% CI: 14.8-19.7]. In BF, TF in year 1 was 9.6%, 10.2%, 15.2% and 7.9%, and cluster survey prevalence was 5.9% [95% CI: 3.8-7.6]; for year 2, TF was 1.6%, 2.6%, 5.0%, and 1.4% and 1.6% [95% CI: 0.0-3.71]; for year 3 TF was 0.8%, 3.0%, 2.8%, and 0.8%, and 1.8% [95% CI: 0.0-3.9]. Schistosomiasis prevalence for sites in BF in year 1 was 0.3%, 0.0%, 0.0%, and 0.0%; for year 2, 0.3%, 0.0%, 0.0%, and 0.0%; for year 3, 0.0% in all sites. STH prevalence for sites in BF in year 1 was 0.0%, 0.0%, 0.0%, and 0.3%; for year 2, 0.3%, 0.0%, 0.3%, and 0.0%; for year 3, 0.7%, 0.3%, 2.3%, and 0.3%. LF prevalence for sites in BF in year 1 was 0.0% in all sites and in year 3, 0.3%, 0.0%, 0.5%, and 0.0%. We conclude that integrated sentinel sites are feasible. Prevalence trends between sentinel sites and trachoma cluster surveys in both countries indicated that using sites might be a reliable indicator to determine when the prevalence is low enough to conduct the WHO recommended cluster survey.

69

CAN WE IGNORE THE INFORMAL PROVIDERS FOR THE TREATMENT OF CHILD DISEASES IN INDIA?

Camille A. Saade[1], Saikat Mukhopadhyay[2], Elizabeth Cohen[1]

[1]FHI 360, Washington, DC, United States, [2]FHI 360 India, Delhi, India

Diarrhea is one of the two leading child killers in India. When asked about care seeking behavior for a child with diarrhea, 83% of caregivers in Uttar Pradesh, India, reported going to a private "doctor". Due to the dearth of qualified health providers in rural India, the private doctor (called Rural Health Provider – RHP) is generally an unqualified provider who nevertheless, has gained the trust of the community. Childhood diarrhea is optimally treated through a short course of ORS and zinc. However, the RHP routinely dispenses unnecessary antidiarrheals and antibiotics. Under the Diarrhea Alleviation through ORS and Zinc (DAZT) project, FHI 360 partnered with local NGOs to identify the RHPs and educate them on the improved treatment of diarrhea through ORS and zinc. The trained NGO

health worker, emulating the marketing strategy of the pharmaceutical industry, repeatedly visited the RHP to provide him with the benefits of ORS and zinc treatment. The partners initially listed 27 000 RHPs and drug sellers in 12 districts representing a population of 34 million people. After categorizing the RHPs according to their work load and potential, the list was reduced to 22 000 RHPs. A tracking study in August 2013 showed that 54% of RHPs dispensed ORS and zinc treatment from a baseline of 23% ORS use and 1% zinc use in 2010. With the increasing demand for ORS and zinc, the pharmaceutical companies became motivated to supply to the rural areas. The market became large enough to attract several companies who are maintaining the demand and providing uninterrupted supply to the RHPs. As a sign of a sustainable market, 42 brands of zinc were inventoried in the area of intervention, up from seven at the start of the intervention three years ago. Changing RHPs' treating behaviors towards rational treatment of critical childhood diseases shows potential to improve health outcomes. This simple approach could be replicated for other MNCH interventions in an integrated way aiming at reducing maternal, newborn and child mortality.

70

PREVALENCE OF ANEMIA AND FACTORS ASSOCIATED WITH SEVERE ANEMIA AMONG UNDER FIVE CHILDREN ADMITTED AT BUGANDO MEDICAL CENTRE, MWANZA, NORTHWESTERN TANZANIA

Rehema H. Simbauranga

National Institute for Medical Research, Dar es Salaam, United Republic of Tanzania

Anemia is a major public health problem in developing countries, contributing significantly to morbidity and mortality among under-fives. About 43% of under-fives are anemic worldwide, and two-thirds reside in Sub Saharan Africa. Even where blood transfusion is available for treatment there is still a significant case fatality rate of 6-18%. This study aimed to determine the prevalence of anemia, factors associated with severe anemia and morphological types of anemia among anemic under-fives admitted at Bugando Medical Centre (BMC). This hospital-based, cross-sectional study was conducted between November 2012 and February 2013. Selected laboratory investigations were done using standard operating procedures. A total of 448 under-five children were eligible but detailed history taking and physical examination was available on 309. The overall prevalence of anemia was 77.2% (346/448). Mild, moderate and severe anemia were 16.5%, 33% and 27.7% respectively. Of 239 children with moderate and severe anemia 22.6% (54/239) had iron deficiency. Majority of the anemic children (37.5%) had microcytic hypochromic anemia. The factors associated with severe anemia included malaria parasitaemia [OR = 4.0 95% CI (2.1 - 7.8); p-value <0.001], presence of sickle haemoglobin [2.0 (1.1 - 3.5); 0.018] and unemployment of the parent [2.2 (1.2-4.0); 0.007]. In conclusion, the prevalence of anemia among underfives admitted at BMC was high. Iron deficiency anemia was the leading type. Factors strongly associated with severe anemia were malaria parasitaemia, presence of haemoglobin S and unemployment among caretakers.

71

EFFECT OF LOW BIRTH WEIGHT AND INTRA-UTERINE GROWTH RESTRICTION ON STUNTING AND WASTING IN A COHORT OF INFANTS IN THE PHILIPPINES

Rachel A. Blake[1], Palmera Baltazar[2], Luz Acosta[2], Remigio Olveda[2], Veronica Tallo[2], Jennifer Friedman[3]

[1]Brown University, Warren Alpert Medical School, Providence, RI, United States, [2]Research Institute for Tropical Medicine, Manila, Philippines, [3]Brown University, Warren Alpert Medical School and Center for International Health Research, Providence, RI, United States

The proportion of babies born low birth weight (LBW) and intra-uterine growth restricted (IUGR) has increased over the past few decades in both high and low-income nations. A recent meta-analysis in Europe found that after controlling for socio-economic factors, LBW had a negative effect on health outcomes during childhood. In other studies, LBW infants had lower mean weight, length, and head circumference at one year of age. However, these studies have been conducted in higher income settings, and it is expected that infants in lesser-developed settings may be even less likely to experience catch up growth. The aims of this study were to compare rates of stunting and wasting at six and 12 months of age among LBW, IUGR, and normal weight infants born in Leyte, Philippines. Pregnant women were recruited at 12-16 weeks gestation as part of an NIH-funded trial. Newborns were weighed within 48 hours of delivery, and LBW was defined as weight < 2.5 kg, and IUGR as weight <3rd percentile for gestational age. WHOAnthro was used to derive height-for-age (HAZ) and weight-for height (WHZ) Z-scores at six and 12 months old. We found that overall 20.6% (72/349) of infants were LBW, 24.6% (86/349) were IUGR, and 2.3% (8/349) were premature. These findings suggest that the primary cause of LBW was IUGR rather than prematurity. Overall, LBW and IUGR infants had significantly lower mean HAZ and WHZ at six and 12 months of age than normal infants. The odds ratio (OR) for stunting (HAZ< -2.0) at 6 months of age among LBW infants compared to normal infants was 3.56 (P = 0.0002), and for wasting (WHZ < - 2.0) was 2.13 (P= 0.0002). At 12 months of age, the ORs for stunting and wasting were 2.09 (P = 0.061) and 1.16 (P= 0.6217), respectively. These results suggest that in this low-resource setting where infants often lack sufficient macro and micronutrients required for the rapid growth of infancy, catch up growth may be significantly delayed if it occurs at all. Further follow up of these children will be conducted to determine whether these effects persist throughout childhood.

72

LOW QUALITY EVIDENCE OF EPIDEMIOLOGICAL STUDIES ON LEISHMANIASIS IN BRAZIL: THE LESS OBVIOUS OBSTACLE FOR CONTROL INITIATIVES

Bruno Trentini[1], Mariel A. Marlow[2], Mário Steindel[3]

[1]School of Medicine, Federal University of Santa Catarina, Florianópolis, Brazil, [2]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, CA, United States, [3]Department of Microbiology, Immunology and Parasitology, Federal University of Santa Catarina, Florianópolis, Brazil

According to recent estimates for Brazil, leishmaniasis accounted for 16,233 DALYs and 280 deaths in 2010, representing a higher disease burden than malaria or dengue. Currently, no implemented control efforts have proven effective given leishmaniasis incidence in the country has not declined and new foci continue to emerge. As observational epidemiological studies are a key component for developing control measures, we reviewed a decade of Brazil's scientific publications on leishmaniasis epidemiology and evaluated the quality and evidence of the studies. In a PRISMA structured literature search of PubMed, MedLine, ScieLO and LiLACS databases, we obtained 2,011 articles from the search terms "leishmaniasis" and "Brazil" published from 2002 to 2012. Only 14% (n=283) of articles were found to be epidemiological studies that incorporated human subjects. Predominant study type was descriptive (53.4%, n=151), followed by cross-sectional (20.8%, n=59), case-control (8.5%, n=24) and cohort (6.0%, n=17). Study design was not stated in 46.6% (n=120) of publications; in addition, 24 (17.5%) studies incorrectly reported study design. Majority of studies were conducted in a single municipality (62.9%, n=178) and did not include healthy controls (78.1%, n=221). Only 66.4% (n=188) of publications had an English version of the full text. Mean journal impact factor of all publications was 1.9 (± 1.6), with only 6% (n=17) of articles being published in journals with an impact factor higher than 4.0. Our findings demonstrated the majority of epidemiological studies reviewed here did not provide sufficient evidence for creating population-based interventions. The abundance of descriptive studies conducted in Brazil is not cost-effective, creates bad habits in the next generation of researchers and hinders the development of novel control strategies. Solutions include encouraging epidemiology courses

in biology and medical programs that emphasize how to evaluate quality of epidemiological studies and discouraging graduate projects based on describing leishmaniasis cases in an area.

73

PAPER-BASED YEAST BIOSENSORS FOR ANTIBIOTIC DETECTION

Abigail A. Weaver, Sarah Halweg, Michelle Joyce, Marya Lieberman, Holly Goodson

University of Notre Dame, Notre Dame, IN, United States

Paper-based tests have the potential to serve as inexpensive tools to address analytical questions both inside and outside of the laboratory setting. In this study, Saccharomyces cereviscIae were used to construct the first example of a yeast whole-cell, paper-based biosensor device. This test is sensitive to antibiotics in the tetracycline family and could potentially address questions of pharmaceutical quality as well as antibiotic contamination in liquids. This biologically-based paper analytical device or "BioPAD" can qualitatively discriminate the presence/absence of doxycycline over a range of 30 - 10,000 µg/mL. Using a BioPAD, a doxycycline dosage form (tablet) commonly used for malaria prophylaxis, was confirmed to contain the antibiotic with 92% and 95% success, evaluated by eye and computer-assisted image analysis respectively, with no false positives by either method. Stored at 4°C these tests were found to remain viable for greater than a year. This research demonstrates the utility of whole yeast cells in paper-based pharmaceutical testing, while highlighting the potential for the development of yeast-based BioPADs to address a range of qualitative analytical questions, especially in low resource settings.

74

EFFECT OF COOKING METHODS ON THE CONCENTRATION OF OXYTETRACYCLINE RESIDUE IN CHICKEN

Ekene V. Ezenduka, John A. Nwanta, Steve I. Oboegbulem, Dennis N. Onah

University of Nigeria, Nsukka, Nigeria

Antibiotics are used in poultry industry to obviate disease, enhance growth and increase production. However, the use of these veterinary drugs often results in the accumulation of violative levels of residues in tissues. Consumption of such poultry meat would potentially adversely affect human health through the development of resistant pathogenic microorganisms and hypersensitivity reactions in sensitized individuals. Although meat is always heat treated before consumption, which should ordinarily render the residues innocuous, some drugs are heat stable and therefore would persist at residue violative levels even after heat treatment. Since tetracyclines are the most frequently used antibiotics in poultry production in Nigeria, this study was therefore embarked up on to find out the effect of cooking methods (boiling, microwaving and roasting) on the concentration of oxytetracyclines (OTC) in poultry meat and organs. Muscle and liver tissues were harvested from birds that were treated with OTC either by intramuscular injection or orally in drinking water and analysed for residues using the three plate test (TPT) and enzyme-linked immunosorbent assay (ELISA). TPT at two different pH levels reduced the inhibition zones of raw muscles between 34-49%, 67-69.6% and 53-56% for microwaving, boiling and roasting respectively but the difference in the means were not statistically significant (P>0.05). TPT however, significantly (P < 0.05) reduced the inhibition zones of raw liver between 79- 80.9%, 57-60.29% and 88-89.71% for microwaving, boiling and roasting respectively, at both pH levels. ELISA determined a slight increase in mean OTC concentration in microwaved (1.2%) and roasted (0.3%) muscle tissues with a slight decrease by boiling (3.5%) but the differences were not statistically significant. A significant (P<0.05) decrease in OTC concentration was however noticed between raw microwaved and roasted liver samples by ELISA at microwaving (1.85%), boiling (2.83%) and roasting (3.17%) respectively. Roasting has a higher reduction effect on oxyteracycline residue.

75

EXPLORING THE EFFECTIVENESS OF AN EDUCATION INTERVENTION DELIVERED THROUGH LOCAL GROCERIES TO IMPROVE CHILD FEEDING PRACTICES IN RURAL COMMUNITIES. A CASE CONTROL STUDY

Halfan S. Ngowo[1], Elihaika G. Minja[1], Sarah G. Mtali[1], Ally Qassimu[2], Magreth Kagashe[1], Fredros O. Okumu[1]

[1]Ifakara Health Insititute, Ifakara, United Republic of Tanzania, [2]Ministry of Health and Social Welfare, Kilombero, United Republic of Tanzania

Malnutrition is one of the most life-threatening conditions in early childhood. It causes about 35% of all deaths in children below 5 years and 50% of child mortality in sub-Sahara Africa. The condition can be effectively addressed by local solutions such as using local food grocers who on daily basis make contacts with child caregivers. Using case-control design in 5 villages in rural south eastern Tanzania, we evaluated an intervention involving training local grocers to select and provide appropriate meals to caregivers with children below two years. The trained grocers also trained their client caregivers on appropriate feeding of children. Ten local food grocers (2 per village) were recruited and each grocer was assigned to 5 randomly selected households with children <2 years. The grocers were retrained monthly, and were asked to help select appropriate foods for and advice their clients on appropriate feeding practices. Monitoring was done monthly. Primary outcome was performance of caregivers on key feeding indicators: breastfeeding, caloric density, nutrients density and food safety and feeding style. Main secondary outcome was growth, measured by length and weight and Z-scores for weight-for-age and length-for-age. We enrolled 27 children from intervention and 27 from control groups and all caregivers in intervention group received recommended training from the assigned grocers. Children in intervention group had higher breastfeeding scores than controls (25 [93%] vs 20 [74%], p = 0.068), higher caloric density scores (22 [81%] vs 21 [78%], p=0.735), higher scores on nutrient density/diversity (22 [67%] vs 14 [52%], p = 0.268) and higher scores on recommended food safety and feeding style targets than control (23 [85%] vs 12 [44%], p = 0.002). Overall, intervention group performed better in all indicators of good feeding practices than controls (19 [70%] vs 8 [29%], p=0.003). Local food groceries in rural areas, if empowered with knowledge on proper feeding practices and malnutrition can improve child feeding practices and nutritional status of children. Since mothers and caregivers make more contact with food grocers in their communities than they do to health facilities, this approach could significantly improve coverage with appropriate nutritional education.

76

NUTRACEUTICALS: THE WAY FORWARD TO PREVENT ADVERSE HEALTH EFFECTS OF A CASSAVA-DOMINATED DIET

Nadege K. Ngombe

University of Kinshasa, Kinshasa, Democratic Republic of the Congo

More than 600 million people rely on drought-tolerant cassava as staple food, mostly under the tropics. Farming systems are dominated by harsh environmental conditions that mostly allow growing bitter cyanogen-containing cassava (manioc a.k.a tapioca or yuca) as staple crop. In times of war or agro-ecological crisis (and food shortages), populations are forced to adopt shortcuts in cassava processing , exposing themselves to cyanide poisoning and outbreaks of konzo, a permanent and irreversible paralytic disease. Cyanide is a mitochondrial toxin known to induce oxidative damage as evidenced by ongoing research that indicates high serum levels of isoprostanes F2 in proportion to motor and cognitive deficits in children relying on insufficiently processed cassava as staple

food. In anticipating interventional trials, we asked whether local food crops may be used as nutraceuticals (food with health benefits) to mitigate the neurotoxicity effects of cassava. We analyzed methanolic extracts of green vegetables (n=12), mushrooms (n=8), yams (n=2) and herbal teas (n=2) varieties that are consumed in a konzo-affected area using the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) free radical-scavenging assay. Scavenging actvities of methanolic extracts (2 - 40µg/ml) were compared using trolox and quercetin as references. The phytochemical screening was done by Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-UV/DAD) using Quercitrin, hyperoside, rutin and chlorogenic acid as references. Most extracts displayed efficient concentration-dependent inhibitory effects. The scavenging activity of extracts (mean ± SEM as reported) aligned as follows: herbal teas > green vegetables > yam > mushrooms. TLC and HPLC-UV/DAD analysis has demonstrated the presence of polyphenolic compounds, which may explain the observed free radical-scavenging effects. In conclusion**,** green vegetables contain rutin (cassava leaves and hibiscus), hyperoside (in hibiscus), and chlorogenic acid (in raphia) lookalike compounds, which may explain their antioxidant activities. Select food crops may be promoted as nutraceuticals in campaigns to mitigate the health effects of cassava neurotoxicity when adherence to modern interventional trials is not guaranteed.

77

THE BURDEN OF RABIES IN TANZANIA AND ITS IMPACT ON LOCAL COMMUNITIES

Maganga Sambo[1], Tiziana Lembo[2], Sarah Cleaveland[2], Heather Ferguson[2], Cleophas Simon[3], Honorati Urassa[1], Katie Hampson[2]

[1]Ifakara Health Institute, Ifakara, United Republic of Tanzania, [2]Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, Glasgow, United Kingdom, [3]Temeke Municipal Council, Dar Es Salaam, United Republic of Tanzania

Rabies remains a major public health threat in many parts of the world and is responsible for an estimated 55,000 human deaths annually. The burden of rabies is estimated to be around US$20 million in Africa, with the highest financial expenditure being the cost of post-exposure prophylaxis (PEP). However, these calculations may be substantial underestimates because the costs to households of coping with endemic rabies have not been investigated. We therefore aimed to estimate the household costs, health-seeking behaviour, coping strategies, and outcomes of exposure to rabies in rural and urban communities in Tanzania. Extensive investigative interviews were used to estimate the incidence of human deaths and bite exposures. Questionnaires with bite victims and their families were used to investigate health-seeking behaviour and costs (medical and non-medical costs) associated with exposure to rabies. We found that a large proportion of bite victims do not obtain PEP (28%) and that compliance is relatively poor (~10% drop out rate) amongst those who do obtain PEP. The average costs incurred by bite victims was ~US$40 but varied from US$0 (for ~16% of bite-victims who were provided PEP free-of-charge and the further 28% of victims who did not seek PEP) to over US$300 (for patients with complicated dog bites). We calculated that an average patient in rural Tanzania, where most people live on less than US$1 per day, would need to spend over US$100 to complete WHO recommended PEP schedules. High costs and frequent shortages of PEP led to poor compliance with PEP regimens, delays in presentation to health facilities, and increased risk of death. The true costs of obtaining PEP were twice as high as those previously reported from Africa and should be considered in re-evaluations of the burden of rabies.

78

PATHOGEN REDUCTION COMBINED WITH RAPID DIAGNOSTIC TESTS TO REDUCE THE RISK OF TRANSFUSION TRANSMITTED INFECTIONS IN SUB-SAHARAN AFRICA

Elissa K. Butler[1], Jeffrey McCullough[2]

[1]University of Minnesota Medical School, Minneapolis, MN, United States, [2]University of Minnesota, Department of Laboratory Medicine and Pathology, Minneapolis, MN, United States

A safe and adequate supply of blood is critical to improving health care systems in Sub-Saharan Africa. The region is burdened by a high prevalence of diseases transmissible by transfusion including HIV, HBV, HCV, and malaria. Current strategies to reduce transfusion-transmitted infections include screening with either more sensitive, but more expensive enzyme immunoassays or less expensive, but generally less sensitive rapid diagnostic tests (RDTs). Pathogen reduction, a developing strategy to improve blood safety, uses a nucleotide binding additive and ultraviolet light to irreversibly inactivate pathogen nucleic acids, effectively reducing pathogen load. It is possible that pathogen reduction in combination with RDTs could be both more effective and more cost-effective in reducing transfusion-transmitted infections compared to current practices. A proof of concept analysis was performed to determine the efficacy of pathogen reduction combined with RDTs compared to current screening methods used by the Uganda Blood Transfusion Service (enzyme immunoassays for HIV, HBV, and HCV; no testing for malaria). For HIV, HBV, HCV, and malaria, probability models were created to determine the risk of an infectious unit being released into the blood supply, accounting for RDT test performance, pathogen reduction capability, pathogen infectivity, and disease prevalence in the donor population. RDTs were chosen based on their availability in Uganda. Terumo, the producer of the Mirasol pathogen reduction system, provided data on the effectiveness of pathogen reduction for each pathogen. Probability models of RDT screening and pathogen reduction yielded a calculated risk of an infectious unit entering the blood supply of 0.006, 5, 0.6, and 150 per 10,000 units collected for HIV, HBV, HCV, and malaria, respectively. The relative risk reduction compared to current methods in Uganda is 88%, 20%, 95%, and 83% for HIV, HBV, HCV, and malaria, respectively. Proof of concept analysis reveals that screening with RDTs combined with pathogen reduction could be effective in reducing the risk of infectious units entering the blood supply. This could vastly reduce the incidence of transfusion-transmitted infections, decreasing economic and social costs of new infections.

79

UNDERSTANDING DISEASE AND ACCESS TO HEALTHCARE IN ISOLATED COMMUNITIES OF THE PERUVIAN AMAZON

Jonathan Williamson[1], Ronald Ramirez[2], **Tom Wingfield**[3]

[1]Bristol Medical School, Bristol, United Kingdom, [2]The Vine Trust and Union Bíblica, Iquitos, Peru, [3]Universidad Peruana Cayetano Heredia, Lima, Peru and Imperial College London, United Kingdom, Lima, Peru

Isolated Peruvian Amazon communities have limited healthcare access and infrequent visits from boat-clinics. Research concerning the health and health beliefs of such communities is scarce. This pilot project assessed disease understanding and pain burden, use of traditional and modern medicine, and healthcare access. Whilst accessing healthcare from a mobile boat-clinic, 85 participants from 13 Peruvian Amazon communities completed a locally-adapted questionnaire detailing socioeconomic position, medical history, understanding of diagnosed illnesses, pain, and use of traditional and modern medicine. 21% of respondents had completed secondary school, 87% lived in crowded houses, and 35% had gone to bed hungry in the past month. 73% had received a prior diagnosis from a healthcare professional: the most common being hypertension, malaria and urinary infections. 17%(13/78) felt they fully understood their diagnosed illness, 43%(34/78) had no understanding at all, and 80% (62/85) wanted further education. 98%(82/84) reported pain, mainly head or musculoskeletal. Most pain (69%) had not received formal diagnosis,

was experienced daily (53%) and treated successfully with modern rather than traditional medicine (72% [95%CI 63-82] vs 19% [95%CI 11-28], p<0.001). Respondents were more likely to have used (89% [95%CI 83-96] vs 71% [95%CI 61-80], p=0.002) and less likely to have refused (5% [95%CI 2.1-9.3] vs 27% [95%CI 17-36], p<0.001) modern rather than traditional medicine. 21% cited long waits and distance as reasons to avoid local healthcare posts. 95% had previously attended a medical boat. In conclusion, amongst pilot project respondents, there was a lack of understanding of diagnosed illnesses and a desire for further education. There was a high burden of undiagnosed pain, likely linked to agricultural work. While traditional and modern medicine were both used, more patients had taken modern medicine, especially analgesics. Individuals avoided using local healthcare posts due to limited access and almost all had had prior contact with mobile boat-clinics. Mobile boat-clinics may have a unique opportunity to target locally relevant healthcare issues (sun-exposure and headaches, self-physiotherapy for musculoskeletal pain) with simple educational interventions (pamphlets, community-advocate training) whilst healthcare infrastructure improves.

80

PAPER ANALYTICAL DEVICES FOR THE SCREENING OF SUBSTANDARD MEDICATIONS IN WESTERN KENYA: A COMPARISON OF TEST RESULTS

Mercy W. Maina[1], Abigael Weaver[2], Nick Myers[2], Celia Chema[1], Phelix Were[3], Rakhi Karwa[4], Sonak Pasakia[4], Marya Lieberman[2]

[1]Moi Teaching and Referral Hospital/Academic Model Providing Access To Healthcare, Eldoret, Kenya, [2]University of Notre Dame, South Bend, IN, United States, [3]Academic Model Providing Access To Healthcare (AMPATH), Eldoret, Kenya, [4]Purdue University, West Lafayette, IN, United States

Low quality medications are a global health challenge that has been associated with death and the development of drug resistance. The Paper Analytical Device (PAD) combines liquid chromatography and color reactions on a patterned paper to form an inexpensive method to screen for substandard drugs. The PAD overcomes challenges encountered, mostly in developed countries, in monitoring the quality of drugs, that is, cost of analysis and inadequate technical expertise. We aim to compare the interpretation of test results obtained in the optimization and validation of the PAD. The project's study site is Moi Teaching and Referral Hospital - Academic Model Providing applies Access To Healthcare (AMPATH). Samples of amoxicillin, ampicillin, amoxicillin-clavulanic acid tablets, azithromycin, ciprofloxacin and acetaminophen tablets were purchased using secret shoppers from registered and unregistered pharmacies in western Kenya. To determine the medication content, the user applies each sample onto the PAD and dips one edge of the PAD into water. After development, the user takes a picture of the utilized PAD and employs a color based key to interpret the results. The interpretation of the PAD test results is carried out by Kenyan pharmacy personnel and University of Notre Dame collaborators and the outcomes reported. The PAD outcomes are reported as "Pass" for samples which displayed similar color patterns to the standard or "Suspicious" for samples which did not. These drugs samples are also being analyzed using high performance liquid chromatography (HPLC) in order to validate the PAD. Of the 139 samples tested in Phase 1 and number of outcomes reported and compared, we found a reader agreement of > 90% in the interpretation of PAD test results. The high reader agreement indicates that the PAD test results can easily be interpreted and therefore may be used to address the challenge of inadequate technical expertise in resource limited settings. We also, anticipate having HPLC results for tested drug samples over the next few months.

81

DETERMINANTS OF COVERAGE AND AVAILABILITY OF ZINC AND ORAL REHYDRATION SALTS (ORS) IN THREE NIGERIAN STATES

Adewale Akinjeji

Clinton Health Access Initiative, Abuja, Nigeria

Globally, diarrhoea is responsible for 11% of all under-five deaths in 2010, and Africa accounts for over 40% of the global diarrhoea mortality burden. In Nigeria, it is the third largest cause of death in children under age five after malaria and pneumonia. While it is well established that WHO recommended treatments such as Oral Rehydration Salts (ORS) and Zinc can prevent the majority of diarrhoea associated deaths, the coverage of these treatments is not ubiquitous in Nigeria. It is estimated that only 25% of children with diarrhoea receive ORS and less than 1% receive zinc. In light of this, Clinton Health Access Initiative (CHAI) and Nigeria's Ministry of Health (MOH) established a 4-year program with the goal of scaling up the use of ORS and zinc for treatment of childhood diarrhoea in 8 Nigerian States. By increasing the use of Zinc and ORS to 80% in four years, it is estimated that the scale-up will save over 228,000 lives. The program aims to create a competitive market for ORS and Zinc by the end of 2015 by 1) securing conducive policy environment; 2) ensuring availability and affordability of zinc and ORS 3) increasing provider awareness about Zinc and ORS; and 4) generating awareness and demand among caregivers. The purpose of the study is to evaluate the efficacy of CHAI strategies and identify other potential predictors of Zinc and ORS uptake that occur during the period of the program. The evaluation employs a pre-post design using cross-sectional surveys of both households and health providers in the intervention areas. A multi-stage cluster sampling technique was employed to systematically sample households and healthcare providers (primary healthcare providers, private patent medicine stores, chemists and pharmacies) across 3 Nigerian states. Baseline data were collected between November 2013 and March 2014, with a total of 2,700 caregivers and 900 health providers surveyed. Questionnaires collected data on caregivers' care seeking attitudes and perspectives, provider knowledge and attitudes, medicine audits, and mystery patient information. Results from this baseline work will provide valuable insight on the determinates of coverage and availability of Zinc and ORS and can inform program implementation and policy at both the National and Global level.

82

ESTIMATING THE IMPACTS OF ROTAVIRUS VACCINATION ON GENDER DISPARITIES IN INDIA

Poulomy Chakraborty

University of Florida, Gainesville, FL, United States

This study aims to assess the gender disparities in rates of vaccination coverage amongst girls and boys in India over time,and estimate their potential impacts on the introduction of rotavirus vaccination, across various geographic and household economic settings. A Microsoft Excel based spreadsheet model is used to model the expected health and economic outcomes disaggregated by gender of the child, for one annual birth cohort of children during the first five years of life. Three data sets have been used in the model: National Family Health Survey-3 2005-6,District Level Health Survey-3 2007-8 and Coverage Evaluation Survey 2009 to estimate the changes over time, in 3 highest mortality states and 6 regions of India. There is an overall increase in vaccination coverage in India. The gap between boys and girls in vaccination coverage over time is estimated to have reduced. Increase in gender parity is estimated to increase in benefits and decrease in cost effectiveness ratio per DALY. It is of immense importance to sustain the efforts of bridging the gender gap in health care utilization and creating enabling conditions to introduce the proposed universal rotavirus vaccination. Preferential treatment to boys increases the risk of mortality amongst girls along with increasing the cost of implementation of vaccination programs.

83

QUANTIFYING THE IMPACT OF ACCESSIBILITY ON PREVENTATIVE HEALTHCARE IN SUB-SAHARAN AFRICA

Amy Wesolowski[1], Wendy Prudhomme O'Meara[2], Andrew J. Tatem[3], Nathan Eagle[1], Samson Ndege[4], Caroline Buckee[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Duke University, Durham, NC, United States, [3]University of Southampton, Highfield, United Kingdom, [4]Moi University, Eldoret, Kenya

Poor physical access to health facilities has been identified as an important contributor to reduced uptake of preventative health services, and is likely to be most critical in low-income settings. However, the relationship between physical access, travel behavior, and the uptake of healthcare remains difficult to quantify. Here we analyze individual travel patterns of 14,816,521 people across Kenya and show that long travel times to health facilities are strongly correlated with increased mobility in geographically isolated areas. We provide regional and localized estimates of the disparity between estimated travel times to facilities, as a standard measure of access, and observed mobility patterns, and compare these measures to data on the uptake of two preventative healthcare interventions in an area of western Kenya: childhood immunizations and antenatal care. We show that even in areas with equal physical access, mobile phone-derived measures of mobility predict which regions are lacking either type of care, highlighting the potential utility of this approach to map the uptake of preventative healthcare.

84

PERCEPTIONS ABOUT PROVIDERS AS DETERMINANTS OF APPROPRIATE TREATMENT SEEKING BEHAVIOR FOR SUSPECTED MALARIA IN CAMBODIA

Emily Carter[1], Megan Littrell[2], Henrietta Allen[3], Sochea Phok[3], Phou Mean[3], Kathryn O'Connell[4]

[1]Johns Hopkins School of Public Health, Baltimore, MD, United States, [2]PSI, Washington, DC, United States, [3]PSK, Phnom Penh, Cambodia, [4]Independent Consultant, Yangon, Myanmar

Seeking treatment for suspected malaria from an appropriate provider facilitates correct case management, contributing to malaria control and elimination and protection of antimalarial drug efficacy. Analysis of data from a nationally representative cross-sectional household survey in Cambodia was conducted to identify individual perceptions about providers associated with appropriate malaria treatment seeking behavior. 1012 individuals (334 age 0-15, 678 age 15+) were identified through screening members of randomly selected households for symptoms of malaria fever occurring in the past 2 weeks. Seeking treatment from an appropriate provider (public health facility or village malaria worker) was the main outcome. Nineteen percent of adults age 15+ and 18% of children <15 sought care at an appropriate provider. Potential covariates included individual preference for appropriate providers on measures of access and quality of care (e.g. cost and convenience of care, respectful and knowledgeable providers, effective medicines). Adjusting for age, gender, education, wealth, and artemisinin-resistance zone in a full logistic regression models, significant determinants of appropriate treatment seeking among adults at 15+ were viewing appropriate providers as: most convenient (AOR=2.9, 95% CI=1.0-4.6); most respectful (AOR=2.9, 95% CI=1.1-7.8), and most knowledgeable (AOR=4.0, 95% CI=1.2-13.6) relative to adults who did not view an appropriate provider as convenient, respectful, and knowledgeable. Relative to adults with fever only, people with additional symptoms were less likely to visit an appropriate provider (AOR=0.24, 95% CI=0.09-0.7). Determinants of appropriate treatment seeking for children <15 included viewing appropriate providers as possessing: lowest cost of transport (AOR=15.3, 95% CI=2.8-82.5); and lowest cost treatment (AOR=20.6, 95% CI=1.9-218.3) relative to caregivers who did not view an appropriate provider as having the lowest cost for treatment and transport. Interventions to improve access to appropriate providers that address cost of transport and treatment may improve appropriate malaria treatment seeking behavior for children. Promoting appropriate providers as knowledgeable and respectful, and encouraging provider practices that reflect these qualities are interventions that may improve appropriate treatment seeking behavior among adults.

85

PERFORMANCE AND RELIABILITY OF DYNEX M² MULTIPLEXED ASSAY SYSTEM FOR IMMUNITY ASSESSMENT FOR THE DEMOCRATIC REPUBLIC OF THE CONGO - DEMOGRAPHIC HEALTH AND SURVEY

Patrick Mukadi[1], Stephen Higgins[2], Anis Karmali[2], Melanie Poncheri[2], Andrew Fusellier[2], Jose Ngamboli[2], Nathalie Kavira[2], Reena H. Doshi[3], Nicole A. Hoff[3], Sue Gerber[4], Emile Okitolonda-Wemakoy[5], Jean-Jacques Muyembe-Tamfum[1], Anne W. Rimoin[3]

[1]Institut National de Recherche Biomedicale, Kinshasa, Democratic Republic of the Congo, [2]Dynex Technologies Inc., Chantilly, MD, United States, [3]University of California Los Angeles, Los Angeles, CA, United States, [4]Bill & Melinda Gates Foundation, Seattle, WA, United States, [5]Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo

Childhood vaccine- preventable diseases continue to be a major public health problem in terms of global morbidity and mortality. In endemic regions the only available tools of diagnosis are based on clinical and serological criteria. In support of the 2014 Democratic Republic of Congo -Demographic Health Survey (DRC-DHS) and in collaboration with University of California, Los Angeles, Fielding School of Public Health (UCLA-FSPH) an immunity assessment is being undertaken using dried blood spots (DBS). The Dynex Technologies M² ®multiplex immunoassay platform with a Measles, Mumps, Rubella, Varicella and Tetanus (MMRVT) panel is being used in which 10 polystyrene beads have been coated and immobilized within 54-well M² plates: separate assay beads with MMRVT antigens; positive controls with horseradish peroxidase, total human IgG, goat anti-human IgG; negative controls with MRC-5 and E6 cell lysate. Positive control DBS are 5-donor normal defibrinated serum and negative control DBS are pooled normal IgG-stripped serum. DBS are extracted into 1ml PBS, 0.5% tween20, 5.0% dried milk and processed on a modified Dynex DS2® automated ELISA system. DBS from 32 reference sera and 7-point dilutions of pooled sera were independently processed in DRC and USA. Assay cutoffs were set by reference to 13 singleplex and 1 multiplex FDA-approved ELISA kits. 423 DBS were collected as a pilot study from children visiting Kinshasa health centers and 9906 DBS during the principal DRC-DHS survey. Including optimization runs data from 60 plates comprising 32,400 data points have been collected including 423 pilot DBS and 1150 DHS samples, with the remaining to be completed by MMDDYY. Having been deployed to a substantially resource-limited environment the Dynex M² multiplex immunoassay system has proven to be a very robust assay platform with excellent sensitivity and specificity. This system in conjunction with DBS processing offers a very cost-effective reliable multiplexed immunoassay processing system for use in country-wide assessment of immunity status in challenging environments.

86

FACTORS ASSOCIATED WITH FREQUENT SICK VISITS FOR FEBRILE ILLNESS AMONGST CHILDREN ENROLLED IN A MALARIA VACCINE TRIAL IN SIAYA DISTRICT, WESTERN KENYA

Norbert A. Odero[1], Simon Kariuki[1], Catherine Saunders[2], Maquins Sewe[1], David Obor[1], Mary J. Hamel[3]

[1]Kenya Medical Research Institute and Center for Global Health and Research, Kisumu, Kenya, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

Prompt care-seeking is essential to prevent severe disease and death from malaria. We investigated whether social and demographic factors affected number of sick visits for febrile illness including malaria among

children participating in a malaria vaccine trial in Kenya. From January 2010 to January 2011, 800 infants aged 6-12 weeks were recruited into the vaccine trial. Of these 446 with complete social and demographic data were analyzed. The association between number of sick visits, dichotomized as high (>18) and low (<18), and child age, sex, maternal age, maternal education, wealth quintile, sex of household head, distance from health facility, and religion was measured using bivariate and multivariate models. Wealth index was created using a list of household assets and principal component analysis was used to divide households into quintiles, poorest, poor, middle, rich and richest. The number of sick visits per participant during the 12 month period of follow-up ranged from 0-45 (median = 12, mean = 12.9). In multivariate analysis, children from the poor and middle income quintiles were significantly more likely to have a high number of sick visits than those from the wealthiest quintile (OR=3.13, 95% CI 1.12-8.74; OR=2.80, 95% CI 1.02-7.71, respectively). Compared with households practicing Catholicism, Protestant and Indigenous religion households were significantly more likely to have a high number of sick visits (OR=4.36, 95% CI 1.34-14.19; OR=3.14, 95% CI 1.11-8.88, respectively). No significant associations were found between other variables and number of sick visits, including distance to health facility (OR=1.06, 95% CI 0.42-2.67). Even though prior studies have shown wealth to be associated with increased care-seeking for children (Taff 2005, Chuma 2007), we found in this trial setting, where transport reimbursement and free health care were provided, a high number of sick visits was associated with the lower income quintiles. Distance to health facility was not associated with number of sick visits. It might be that in this resource poor setting, removing the barrier of transport through reimbursement and providing free health care could increase health seeking.

87

EPIDEMIOLOGY OF LABORATORY CONFIRMED RUBELLA CASES IN ETHIOPIA, 2013

Mekonen Getahun Baynesagn, Mesfin Tefera, Berhane Beyene, Birke Teshome, Tassew Kassa, Almaz Abebe

Ethiopian Public Health Institute, Addis Ababa, Ethiopia

Rubella is a contagious rash illness. Trans-placental infection leads to serious fetal disorder called Congenital Rubella Syndrome (CRS). Worldwide, more than 110,000 infants are born with CRS each year; most of these occur in developing countries where information is limited on the epidemiology and vaccine not introduced. This study was conducted to see the annual prevalence, seasonal and geographic variation and age distribution of rubella infection. Serum/plasma samples and demographic data were collected from measles/rubella suspected cases from all the 9 regional states and 2 city administration of Ethiopia, January-December 2013. The samples were tested for rubella IgM by ELISA. The data was analysed by Epi Info software version 3.5.4. Among 3,587 patient samples tested, 858 (23.9%) were positive for rubella IgM. The positivity rate was higher among females (26%) than males (22.2%). The highest rubella positivity rate, 27.5% (287/1043), was seen among children 5-9 years. During March and August, the highest 33.6% (179/533) and lowest 10.7% (17/159) prevalence rates were seen respectively. The highest positivity rate was seen in Amhara 36.7% (301/821) followed by SNNPR 36.1% (227/628), 29.4% (35/119) of Tigray, 15.3% (64/417) of Addis Ababa and the lowest 13.4% (190/1416) in Oromiya. In conclusion, I\ in Ethiopia, rubella become an increasing non-reportable public health problem and the infection is seasonal mostly affecting children. The current prevalence of rubella cases calls for conducting CRS surveillance in infants, surveillance among pregnant mothers, reporting and necessitates the introduction of rubella vaccine into the national routine immunization services.

88

INTERNATIONAL AID AND NATURAL DISASTERS: A PRE AND POST-EARTHQUAKE LONGITUDINAL STUDY OF THE HEALTHCARE INFRASTRUCTURE IN LEOGANE, HAITI

Maxwell Kligerman

Stanford University, Menlo Park, CA, United States

Little is currently known about the interaction between international aid donors and local healthcare systems in the context of the healthcare recovery following natural disasters. Using data collected prior to and following the 2010 Haiti earthquake, we detail the response of aid agencies and their interaction with local healthcare providers in Leogane, the town closest to the earthquake's epicenter. We collected geocoded data in Leogane, Haiti during three time periods: five months prior to the earthquake, one year after the earthquake, and three and a half years after the earthquake. During each visit we collected the same census data, including healthcare facility services, funding source, and receipt of aid. Our findings demonstrate that both one and three years after the earthquake there were marked increases in the total number of healthcare facilities, inpatient beds, and surgical facilities as compared with the pre-earthquake period, and that international aid has been a driving force behind the recovery. Twelve out of thirteen new healthcare facilities that have opened since the earthquake have been aid-financed, and seven out of eight healthcare facilities that were rebuilt after the earthquake were aid-financed. Despite increases in free, aid-financed healthcare following the earthquake, private Haitian healthcare facilities have remained at a constant number. Reconstruction efforts have not been fully sustainable, as the planned phase-out of several aid-financed facilities will leave Leogane with fewer inpatient-beds and emergency medical services as compared with the pre-earthquake period. We hope that our assessment of the recovery effort thus far in Haiti will help frame policy decisions regarding how best to support local healthcare systems in both the acute and long-term phases of disaster recovery efforts.

89

SAMUEL LAPSLEY AND WILLIAM SHEPPARD: MISSIONARY MEDICINE, MEDICAL ENTOMOLOGY AND REALPOLITIK IN THE CONGO, 1890-1917

David Adams

AASU/Georgia Regents Medical University, Savannah, GA, United States

The dawn of the 20th century marked the height of European colonialism on the African continent. By 1900, the major European powers had carved the continent into sectors of social, cultural, political, and military control. Christian missionaries, Protestant and Catholic alike, soon followed by the thousands. Although many were European, some hailed from the United States. Among the best-known Americans were William Sheppard and Samuel Lapsley, Presbyterian missionaries who worked in the Belgian Congo from the early 1890s to the era of The Great War. As tales of Dr. David Livingstone spread widely in the late 1800s, it is likely that the Reverend Mr. Sheppard and the Reverend Mr. Lapsley would have expected the usual missionary tribulations. They would have been all too familiar with the experiences of the faithful who had preceded them: suspicious--and possibly warlike--natives, linguistic and cultural barriers, fierce animals, and sweltering tropical climates. As such, they arrived in the Belgian-controlled Congo in the early 1890s armed with hundreds of pounds of equipment and supplies, not least, Bibles. The work of Sheppard and Lapsley, however, soon took them not only into the changing world of late 19th-century tropical medicine but also into the grim underside of Congolese life under Belgian rule. This paper will examine only their medical and scientific efforts amidst the changing paradigms in tropical medicine but human-rights efforts in the Congo.

90

WOMEN'S PERCEPTION OF ANTENATAL CARE SERVICES IN PUBLIC AND PRIVATE CLINICS IN THE GAMBIA

Isatou K. Jallow

National Public Health Laboratory, Banjul, Gambia

The main objective of this study was to assess women's preferences and perception of antenatal healthcare services in public and private healthcare facilities. Descriptive cross-sectional study using a face-to-face interview based on the standardized World Health Organization questionnaire. Six public and six private health facilities in the Gambia. Five hundred and two pregnant women. Patient's perception of antenatal services received was main outcome variables and measured in three aspects: willingness to come back, willingness to recommend to others and level of satisfaction. The satisfaction rate with antenatal services was 79.9% for public facilities and 97.9% for private facilities. Pregnant women's poor perception with public facilities (after adjustment) included their unhappiness, with the following dimensions of antenatal care (ANC): inadequate privacy, inadequate space and neatness and inadequate communication with care providers. We found that although women tended to be highly satisfied with both private and public ANC facilities, those attending public clinics were significantly less satisfied than those attending private clinics. The main complaints were related to the physical environment, technical process and provision of information or reassurance. Because public facilities constitute the main care providers for the general population and particularly for disadvantaged women, better management of public clinics and better training in communication skills for public care providers may help to retain women patients and improve the quality of ANC in the public sector.

91

DEVELOPMENT OF ADVANCED SERO-ASSAYS TO BROADEN DIAGNOSTIC AND SURVEILLANCE CAPABILITY IN WEST AFRICA

Aileen O'Hearn[1], Matthew Voorhees[1], Abbe Ames[1], Joseph Fair[2], Randal Schoepp[1]

[1]United States Army Medical Research Institute of Infectious Diseases, Frederick, MD, United States, [2]Metabiota, Washington, DC, United States

West Africa is home to a wide variety of viral infectious diseases, many of which go untreated in the local populations. Lassa virus causes a hemorrhagic fever that occurs throughout the region, with fatality rates approaching 30%. It is endemic in Sierra Leone, where the Kenema Government Hospital (KGH) houses a Lassa fever ward and laboratory. Approximately 600 suspected Lassa fever cases are received here annually from throughout the country, however of these, only 30-40% of samples test positive for Lassa virus; therefore, there is a large number of patients presenting with diseases of unknown origin. Severely limited resources at KGH hinder treatment and diagnostic capacity of Lassa fever, as well as of the additional undiagnosed infections that present there. Here, we have approached these two issues - diagnostic capability for acute Lassa infection, and surveillance of additional circulating viruses in west Africa- by developing an array of diagnostic assays for detection of hemorrhagic fever and arthropod-borne viruses utilizing Magpix technology (Luminex, Austin, TX) and evaluating their clinical performance on site at KGH. Magpix assays detecting Lassa-specific IgM and Lassa antigen in serum were significantly faster and more sensitive compared to traditional ELISAs, which are the current standard at KGH. Identifying past exposures to possible circulating viruses using IgG detection was carried out in a multiplex format, detecting antibodies to Ebola, Marburg, Rift Valley fever, Crimean Congo hemorrhagic fever, Lassa, and an array of flaviviruses and alphaviruses in one well. These assays have shown to be a valuable asset in a clinical setting, significantly improving upon current acute Lassa fever diagnostics at KGH as well as generating a large pool of data that gives insight into the scope of viral diversity and prevalence in West Africa.

92

USING ITERATIVE PRACTICE FOR DEVELOPING PUBLIC HEALTH ICONOGRAPHY TO PREVENT SOIL-BORNE HELMINTHS

Sarah B. Paige, Tony L. Goldberg

University of Wisconsin Madison, Madison, WI, United States

Shoes are a proven method for disrupting the transmission of soil-borne helminths (STH). However, shoe-wearing practice is often uncommon in areas where STH are prevalent, largely because the health benefits of wearing shoes may not be apparent to local populations. This is especially true in non-literate populations where information dissemination through traditional means (e.g. posters, billboards) is difficult. We launched a public health intervention that combines an animated image (a "lenticular image") depicting the efficacy of shoes in preventing STH infection with "flip-flop"-style sandals. The image adheres directly onto the shoe, so that health information is linked to the primary means of intervention. Here, we describe the multi-phased, iterative design process used to develop the image and the process of its deployment and social uptake in rural communities in Uganda. We gathered standard iconographies that depicted specific elements of the image to show that shoes can interrupt STH. Elements included abstract constructs such as "healthy" and "don't," alongside concrete concepts such as "hookworm" and "sandals". Iconographies and color schemes were evaluated, deconstructed, and reconstructed over five focus group sessions. A resulting suite of "infection" and "prevention" messages were tested with 30 individuals across three study communities. The final versions of 'infection' and 'prevention' were selected based on the frequency of "correct" comprehension. Shoes with the image were then deployed to individuals in communities with high rates of hookworm infection. We found participants' overall shoe-wearing practices improved, but varied by activity and individual characteristics. This study demonstrates that holographic iconography can be an effective means of disseminating public health information to non-literate populations. It also demonstrates the importance of incorporating culturally appropriate images into pictorial depictions of public health messages. Interactive design processes are an efficient way to develop such messages.

93

FEASIBILITY OF A ONE-WEEK COURSE TO TEACH BASIC ULTRASOUND SKILLS TO PHYSICIANS IN RESOURCE-LIMITED SETTINGS

Zain Chagla[1], Robert Peck[2], Dana Sacco[3], Tiffany Alexander[4], Palmyra Immordino[4], Murtaza Rasheed[4], Shivani Datta[4], Esther Majaliwa[4], Sam Worley[4], Claire Webb[4], Nicolas Laing[4]

[1]McMaster University, Hamilton, ON, Canada, [2]Cornell University, Ithaca, NY, United States, [3]Columbia University, New York, NY, United States, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom

Portable ultrasound is an inexpensive and highly effective tool. Ultrasound provides real time data that can assist in diagnosis and therapeutic procedures, and may contribute to better outcomes in resource limited settings. However, the training required before a physician gains competency remains controversial. The optimal method of teaching, and which ultrasound views to teach are also questionable. This study aimed to assess whether a one-week intensive course would result in physicians ability to use ultrasound in daily clinical practice competently. The project was conducted at Bugando Medical Centre in Mwanza, Tanzania. The curriculum involved learning basic ultrasonography skills, and the ability to obtain and interpret views of the heart, perihepatic space and liver, perisplenic space, inferior vena cava, pelvis, and thorax. The curriculum involved daily lectures on theory, followed by practical sessions with healthy subjects and in-patients from medical wards. Informed consent was obtained from all participants. Candidates were required to complete a minimum of twenty-five total scans on different patients. Candidates submitted several still-images, which were evaluated for adequacy and

demonstration of normal or abnormal anatomy. Competency was also assessed by a practical examination at the end of the course involving an ultrasound examination of a standardized patient. All participants completed a minimum of twenty-five ultrasounds of each of the ten views. All candidates' passed the formative examination, with a median of 96%. Adequate images were submitted by all candidates, with a demonstration of a variety of pathology. Self-reported confidence was shown to be significantly greater after the completion of the course. The steep but rapid clinical learning curve demonstrates that an intensive training course to attain basic bedside ultrasound skills is feasible in a resource limited setting. Integration of a similar curriculum may be possible to enhance medical education and diagnostic accuracy in resource-limited settings.

94

ACCURACY OF ULTRASOUND AS A TELE-MEDICINE COMPONENT USING A COMMERCIALLY AVAILABLE TELE-CONFERENCE SYSTEM

Eric Zevallos, Matt Lyon

Georgia Regents University, Augusta, GA, United States

Point-of-care (POC) ultrasound has been shown to have many benefits in patient care. Many international providers are not trained to perform or interpret US results. As a component of the telemedicine (TM) system, POC US has the potential to provide diagnostic capability in locations with limited diagnostic services. Our hypothesis is that US transmission using a commercially available teleconferencing (TC) system will yield similar results to DICOM image transmission as well as comparable picture quality. Similarly, the accuracy of diagnosis will be unchanged. This was a prospective observational trial of transmitted US video using an internet based peer-to-peer communication system (Skype) compared to images transmitted via a DICOM system. US images were obtained from patients presenting to the ED. After consent, limited, POC US exams were performed using a SonoSite US. The images were stored using a DICOMM protocol and transmitted using a TC system. The TC system consisted of a USB to VGA image capture device (Epiphan) connected to the VGA output in the minidock of the US system. The image capture device connected to a Dell laptop and using Skype, images were transmitted to a Mac Pro. Video recordings were made of the transmitted image and stored as mp4 files. Images of the transmitted and non-transmitted images were of the same patient and were of the same US exam. Three ED physicians with hospital credentials for ED US reviewed the US exams using both methods in random order. 148 examinations (74 transmitted and 74 non-transmitted) were reviewed. The kappa statistic was calculated separately for each diagnostic test, and for all tests combined, to examine agreement between each of three reviewers and the gold standard. Kappa for Reviewer #1 was consistently in the "almost perfect agreement" range ($0.8 < \kappa < 1$). For the Gallstones and IUP diagnostic tests, all reviewers had κ values in the "almost perfect agreement" range ($0.8 < \kappa < 1$). There were statistically significant differences between the scores for the transmitted vs. non-transmitted for all three types (image detail, image resolution, and image quality) for Reviewers 1 and 3 only. In particular, non-transmitted scores were significantly higher than transmitted scores. POC US transmitted via commercially available systems may be a vital and accurate diagnostic option for international providers where other diagnostic radiological resources are unavailable.

95

TIME TRENDS FOR CHILD DIARRHEA PREVALENCE AND ORS USE IN A DOMINICAN REPUBLIC PERI-URBAN COMMUNITY

Jay Park, **John D. McLennan**

University of Calgary, Calgary, AB, Canada

Reducing child diarrhea prevalence and increasing oral rehydration solutions (ORS) use are important national policy aims for diarrhea control in low- and middle-income countries. Available national level data for some countries has identified gaps in attaining these aims. While informative, national level data may obscure variation across sub-national levels and may miss patterns within high-risk communities. Low-income peri-urban communities are particularly important given that they contain rapidly expanding populations and have conditions that may place children at high-risk for diarrhea (e.g., overcrowding, inadequate water and sanitation infrastructure). The aim of this study was to determine whether the prevalence of child diarrhea is decreasing and ORS use is increasing over time within a low-income peri-urban community of the Dominican Republic. The study community has received several health interventions initiated by non-governmental organizations over the last several years that included water, sanitation and health education projects. Information on child diarrhea and ORS use between 2009 and 2013 was extracted from a dataset derived from an ongoing child growth-monitoring program (GMP) located in the study community. All children in the community under four years of age were eligible to participate. The prevalence of child diarrhea among attendees at the GMP was determined within different time blocks over the 4 year study period. ORS rate was based on reported use within diarrhea cases over time. Data were available for 293 children from one or more appointments. Diarrhea prevalence remained relatively constant at 26 (SD: 2.6)% over the 4-year period with no reduction over time. The mean rate of ORS use over the 4-year period was 38 (SD: 3.4)% with no evidence of increase over time. Despite various health initiatives in this community, the burden of child diarrhea remained high and ORS use remained low. Examination of potential factors impeding progress on diarrhea control is still required, particularly in high-risk communities.

96

DEVELOPING SUSTAINABLE CAREER DEVELOPMENT SUPPORT FOR AFRICAN RESEARCHERS

Hazel E. McCullough, The Malaria Capacity Development Consortium Career Development Groups

London School of Hygiene & Tropical Medicine, London, United Kingdom

Developing global health researchers to proficiency is crucial to support the strengthening of health research capacity, yet researchers based in low and middle-income countries in Africa face a disadvantage in their career development to proficiency through limited access to research training, resources and appropriate support and mentorship. Currently much of this support comes from externally funded capacity development programs who invest in developing individuals, strengthening research training and enhancing research environments. However, continued support post program ends remain a challenge. Led by senior African researchers from each of the Malaria Capacity Development Consortium's (MCDC) African partner institutions, structured and supported Career Development Groups (CDGs) have been set up to advance the career development of researchers within their institutions, in addition to developing sustainable research training and systems of support for researchers within the institution. Oversight of the CDGs sits within the offices of the provost, deans of faculty and schools of postgraduate studies to ensure integration within institutional processes and policies, and sustainability beyond the MCDC program. The CDGs have undertaken a baseline assessment of existing institutional career development support and in wider consultation drawn up plans for development. Plans are specific to institutional need and context, aligned to strategic institutional objectives and incorporate the needs of other capacity development programs based at the institution. Activities and strategies of support will vary but with Personal Development Planning (PDP), formal mentoring and support in postgraduate supervision being key strategies that will be embedded within institutional career development support for researchers and research staff. An evaluation will be undertaken on the impact of the career development support as well as the process of using CDGs as a new way of working within African institutions to support the sustainable development of global health researchers in LMICs in Africa.

97

THE FIRST REPORTED CASE OF BABESIOSIS IN A RESIDENT OF OREGON

Jon E. Lutz[1], Emilio E. DeBess[2], Brad B. Bryan[3], Brenda Rose[1], Maniphet V. Xayavong[4], Leslie L. Henderson[4], Barbara L. Herwaldt[4]

[1]Bend Memorial Clinic, Bend, OR, United States, [2]Oregon Department of Human Services, Portland, OR, United States, [3]Central Oregon Pathology Associates, Bend, OR, United States, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States

Babesiosis is a tickborne infection caused by intraerythrocytic parasites of the genus *Babesia*. Most U.S. zoonotic cases have been caused by *B. microti*, which is endemic in the Northeast and upper Midwest. Sporadic cases caused by other *Babesia* species have been documented in various U.S. regions. The first reported case caused by *B. duncani* (the WA1-type parasite) occurred in Washington State in 1991. Here we describe the first known case of babesiosis diagnosed in Oregon—a parasitologically confirmed case of *B. duncani* infection, in a 69-year-old otherwise healthy asplenic man, who lived in a rural forested area of Deschutes County in central Oregon. He did not recall tick exposures or have a history of transfusions. On June 30, 2009, he was evaluated in an urgent-care center because of a several-day history of a febrile illness (maximum temperature, 40°C); his hematocrit was 37.3%, and his platelet count was 39 x10^9/L. The marked thrombocytopenia prompted manual review of a blood smear. Intraerythrocytic forms were noted, which were confirmed by a hospital pathologist as *Babesia* parasites; the parasitemia level was 3%. On July 1, he was hospitalized and began combination therapy with clindamycin plus quinine. When he was discharged on July 6, he had been afebrile for >48 hours and his parasitemia level was <1%. During a follow-up evaluation on July 9, no parasites were found on blood-smear examination and antimicrobial therapy was discontinued. In reference diagnostic testing at CDC, molecular and serologic analyses were negative for *B. microti* but were positive for *B. duncani* (the indirect fluorescent antibody titer was 4096); *B. duncani* also was isolated by inoculation of jirds (Mongolian gerbils). Clinicians and laboratorians should be aware that babesiosis can be caused by agents that are not detected by assays for *B. microti*. To date, parasitologically confirmed cases of *B. duncani* infection have been documented in Washington, California, and Oregon. The many unknowns about *B. duncani* include its geographic distribution, tick vector, and reservoir hosts.

98

DETECTION OF *ANAPLASMA PHAGOCYTOPHILUM*, *BABESIA MICROTI* AND *BORRELIA BURGDORFERI* IN *IXODES SCAPULARIS* COLLECTED FROM LOCATIONS SURROUNDING THE RESIDENCE OFA LOCALLY-ACQUIRED CASE OF HUMAN BABESIOSIS IN MARYLAND, USA

Ellen Y. Stromdahl[1], Katherine Feldman[2], Siok-Bi Wee[2], Maniphet Xayavong[3], Alexandre Dasilva[3]

[1]U.S. Army Public Health Command, Aberdeen Proving Ground, MD, United States, [2]Maryland Department of Health and Mental Hygiene, Baltimore, MD, United States, [3]Division of Parasitic Diseases and Malaria, Atlanta, GA, United States

The Delmarva Peninsula has long been recognized as home to populations of *Ixodes scapularis* infected with *Borrelia burgdorferi*, although reports of tick surveillance are few. *Babesia microti* has never been reported from *I. scapularis* there, although there is only one published investigation of *Ba. microti* in the area. In 2009, Maryland Department of Health and Mental Hygiene (DHMH) reported the investigation of an autochthonous case of human babesiosis on the Maryland Eastern Shore of the Delmarva Peninsula. The patient was also infected with *B. burgdorferi*. Subsequently, a team from DHMH and U.S. Army Public Health Command (USAPHC) performed a preliminary investigation of vector ticks in locations surrounding the patient's residence. Sixteen *I. scapularis* adults were collected by flagging at the patient's yard and a nearby site. PCR testing at the USAPHC detected *B. burgdorferi* in 8/16 of the ticks; 2 of these 8 were coinfected with *Ba. microti*, and 1 of the 8 was coinfected with *A. phagocytophilum*. PCR positives were reconfirmed with second PCR at USAPHC, and *Ba. microti* positive tick samples were sent to Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, Georgia, for reconfirmation with nested primers targeting the ss-rDNA gene. To our knowledge, these are the first *Ba. microti* positive *I. scapularis* collected in Maryland, and the human case is the first locally acquired babesiosis infection reported from Maryland. *B. burgdorferi* infection can be quite robust in the mid-Atlantic region, so finding 50% of these ticks positive for *B. burgdorferi* was not completely unexpected. However, the regional prevalence of infection with *A. phagocytophilum* is low (2-3%), and *Ba. microti* has never been reported from *I. scapularis* in Maryland, so detection of these pathogens in a small sample of ticks was remarkable. Further investigation at this location of nymphal *I. scapularis,* the stage most likely to transmit human diseases, is warranted.

99

IMPACT OF CLIMATE CHANGE ON TICK-BORNE RELAPSING FEVER BORRELIOSIS DISTRIBUTION IN WEST AFRICA

Georges Diatta[1], Patrick Durand[2], Jean-Marc Duplantier[2], Laurent Granjon[1], Gil Mahe[2], François Renaud[2], Jean-François Trape[1]

[1]Institut de recherche pour le developpement BP 1386, Dakar, Senegal, [2]Institut de recherche pour le developpement BP 1386, Montpellier, France

Tick-borne relapsing fever (TBRF) borreliosis due to *Borrelia crocidurae*, is a major cause of morbidity in most rural areas of Senegal where its distribution, previously limited to the Sahel, spread to Sudan savannah areas during the 1970s. We report, here, studies conducted in 12 West African countries to investigate the distribution of ticks *Ornithodoros sonrai*, the occurrence of *B. crocidurae* infections in vectors and small mammals and their relationship with climatic change. From 2002 to 2012, we investigated the occurrence of *O. sonrai* in rodent burrows in 210 study sites from West African countries. Ticks collected in each site and their Borrelia infections were genetically characterized by sequencing. We collected small mammals in Mauritania, Senegal, Mali, Niger and Benin. They were tested for Borrelia infections by thick blood film, blood or brain inoculation into white mice and/or by PCR. Out of 8,716 burrows examined, 973 (11.1%) were found colonized by Ornithodoros ticks identified as *O. sonrai.* Only sites in Senegal, Mali, the Gambia and Mauritania were found positive for this vector. B. crocidurae infections were highlighted in 245/1,121 (21.8%) of ticks tested by nested PCR and 70/842 (8.3%) of rodents and insectivores. All infected mammals were collected in areas where we found the vector. The southern limits of *O. sonrai* and *B. crocidurae* corresponded approximately to the 750-mm isohyet. *O. sonrai* ticks and *B. crocidurae* are massively distributed north of latitude 13°30'N. Although climatic factors are clearly associated with the distribution and the recent spread of TBRF in the westernmost part of West Africa, the vector appears absent from climatically suitable areas of Burkina Faso and Niger. It is only in central and eastern Mali that the distribution of the vectors was associated with the principal riverbed of Niger River and its mains inflows.

100

SPILLOVER AND GENETIC DIVERSITY OF *RICKETTSIA PARKERI* IN NORTHERN VIRGINIA

Maureen K. Kessler[1], Tyler C. Henning[1], John M. Orr[2], Joshua D. Smith[2], Jorge R. Arias[2], Douglas E. Norris[1]

[1]The W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Fairfax County Health Department, Fairfax, VA, United States

Spotted Fever group *Rickettsia* (SFGR) cause a spectrum of disease worldwide, with symptoms ranging from mild to severe febrile illness. In the United States the most notable is *R. rickettsii*, the causative agent

of Rocky Mountain Spotted Fever (RMSF). Several other SFGR have been linked to human infection, many of which cause similar clinical symptoms and contribute to misdiagnosis. Previously thought to cause only zoonotic infections, *R. parkeri* was recognized as a human pathogen in 2002, and has subsequently been linked to twenty cases of spotted fever rickettsiosis in the U.S. and Argentina. The primary vector of *R. parkeri* is *Amblyomma maculatum*, which has spread beyond its historic range to occupy foci across the central and southeastern U.S. In 2010, *R. parkeri* -infected *A. maculatum* was discovered at a landfill in Fairfax County, Virginia, where sympatric populations of many tick species exist. Initial findings discovered *R. parkeri* infection of other ticks near the landfill, including *Dermacentor* variabilis, *Rhipicephalus sanguineus*, and *Haemaphysalis leporispalustris*, which are documented to bite humans. This is significant, and highlights the potential for spillover into vectors not typically associated with *R. parkeri*. Preliminary analysis of a relatively conserved outer membrane protein (gene *rompB)* indicates 97-99% sequence homology among *R. parkeri* samples. Low diversity implies that infection of novel vectors may be attributable to spillover from sympatric populations co-feeding on infected hosts. Further sequence analysis of more variable gene targets is ongoing; preliminary findings indicate that genetic variation may exist, which, if confirmed, will provide evidence for the introduction of multiple *R. parkeri* variants. This work represents a novel approach to understanding the ecology of *R. parkeri* in North America. Pathogen spillover of multiple genetic variants into novel vectors could lead to increased transmission and propagation of the disease cycle, resulting in an increase in spotted fever rickettsiosis cases.

101

CONTROL OF TUNGIASIS (SAND FLEA DISEASE) IN EAST AFRICA: SELF-DIAGNOSIS AS A MEANS TO TARGET TREATMENT IN ENDEMIC AREAS

Hermann Feldmeier[1], Marlene Thielecke[1], George Mukone Mudanga[2], Mark Corbett[3], Per Nordin[4]

[1]Department of Microbiology and Hygiene, Charité Un, Berlin, Germany, [2]Ministry of Health, Kampala, Uganda, [3]The Kenyan Children's Project, Kakamega, Kenya, [4]Skaraborg Institute for Research and Development, Skövde, Sweden

In East Africa, tungiasis (sand flea disease) has re-emerged in epidemic proportions. This has prompted Ministries of Health and non-governmental organizations to conceive control measures. To target treatment in a cost-effective manner, diagnostic tools are required allowing a rapid assessment of the prevalence and severity of tungiasis at the community level.
We investigated the sensitivity, specificity and predictive values of self-diagnosis in rural Uganda and Kenya and determined factors which might have an impact on the validity of this diagnostic approach. The study was carried out in the Makoma Primary School, Bugiri County, Uganda, and in Shivakala village, Kakamega County, Kenya. All the primary school pupils (n = 589) and all the inhabitants of Shivakala village (n = 386) present on two consecutive days were individually asked whether they thought they had tungiasis on at least one foot. Immediately after the self-diagnosis, the individual was carefully examined for the presence of embedded sand fleas, and tungiasis-associated morbidity was assessed semi-quantitatively. In Makoma Primary School, the prevalence of tungiasis was 71.9% (95% CI 66.6 - 76.6). In Shivakala village, the prevalence was 70.3% (95% CI 62.2 - 77.3) in school-age children and 56.5% (95% CI 51.5 - 61.3) in the general population. At both locations, the ratio of infected males to females was 1.2. Whereas in the Ugandan primary school the sensitivity of self-diagnosis was very high (85.1%; 95% CI 80.0 - 89.1), the specificity was rather low (69.6%; 95% CI 59.5 - 78.0). In contrast, in the Kenyan village the specificity of self-diagnosis was high (97.1%; 95% CI 92.8 - 98.9), but the sensitivity was low (66.5%; 95% CI 59.3 - 73.0). In school-aged children the positive predictive value was 98.5 (95% CI 92.0 - 99.7), and the negative predictive value was 56.3 (95% CI 44.8 - 67.3). It was concluded that self-diagnosis can be relied upon to enable targeted community based control measures against tungiasis, but that the validity of this diagnostic approach has to be determined in different settings.

102

EFFECTIVE TREATMENT OF TUNGIASIS WITH DIMETICONE: A TARGETED TOPICAL APPLICATION IS SUPERIOR TO WETTING THE SKIN OF THE WHOLE FOOT

Hermann Feldmeier[1], Nicholas Ngomi[2], George Mukone Mudanga[3], Marlene Thielecke[1]

[1]Department of Microbiology and Hygiene, Charité Un, Berlin, Germany, [2]African Population and Health Research Institute, Nairobi, Kenya, [3]Ministry of Health, Kampala, Uganda

Tungiasis (sand flea disease) is a neglected tropical disease associated with debilitating acute and mutilating chronic morbidity. In the endemic areas patients attempt to remove embedded sand fleas with inappropriate sharp instruments, such as safety pins, thorns or sharpened pieces of wood - a hazardous procedure by itself. Recently, we demonstrated that a single topical application of a mixture of two dimeticones with low viscosity onto the skin of the feet effectively killed embedded sand fleas and significantly reduced local inflammation within seven days. We attempted to increase the efficacy of the new therapeutic approach by targeting the dimeticone to the abdominal rear cone of the parasite which protrudes over the corneal layer of the epidermis. 59 children from Bulidha sub-county, Bugiri District, Eastern Uganda, aged from 5 to 12 years, with a total of 311 embedded sand fleas were included in the study. The left and the right foot were randomized to either receive the "simple" or the targeted application with the dimeticone, respectively. The viability of the embedded parasites was assessed by a handheld digital video microscope and the degree of inflammation was determined by means of an inflammation score. The lesions were observed daily during 7 days. After the "simple" treatment 87.3% (95% CI 81-92%) of the embedded fleas lost all viability signs within seven days, and after the targeted treatment 97.5% (95% CI 94-99%; p = 0.008) giving a difference of 10.2% (95% CI 7-14%) in efficacy. We conclude that the targeted application is superior to wetting the skin of the whole foot with dimeticone. The targeted treatment requires less dimeticone, can be performed by the patient himself and is a promising tool for control measures aiming at the reduction of tungiasis-associated morbidity. In view of the high efficacy and safety of the topical application of dimeticone, the hazardous extraction of embedded sand fleas with sharp instruments is no longer warrantable.

103

A MODEL OF INVESTIGATION OF HOST IMMUNITY DURING *ORIENTIA TSUTSUGAMUSHI* INFECTION

Guang Xu, Thomas R. Shelite, Nicole L. Mendell, Yenny Goez-Rivillas, Lynn Soong, Bin Gong, Donald H. Bouyer, David H. Walker

University of Texas Medical Branch, Galveston, TX, United States

Scrub typhus, a long neglected but important tropical disease, is caused by a Gram-negative obligately intracellular coccobacillus, *Orientia tsutsugamushi*. Scrub typhus is a serious global public health problem that causes illness in one million people each year, the majority in the Asia-Pacific region. Without appropriate diagnosis and treatment, the disease can cause severe multiorgan failure with a mortality rate of 7-15%. However, the mechanisms behind the interactions between *O. tsutsugamushi* and host immunity are largely neglected and unknown. With our newly developed intravenous (i.v.) mouse model, we demonstrated that there were significant changes in the host immune responses after *Orientia* infection. We discovered that host immunity leaned towards T_h1 responses from 12 days post infection (dpi) until 3 months post infection. Our flow cytometry data determined that more $CD8^+$ T cells than $CD4^+$ T cells appeared in the spleen of infected mice after 12 dpi. We also found that $CD4^+CD25^+Foxp3^+$ T_{reg} cells and IL-10 levels significantly increased from 6 dpi, which was in parallel with the body weight changes and bacterial loads. The mice that received a lethal dose (4.5×10^6 PFU/mL) began losing weight as early as 3 dpi. These mice died on 12 dpi. The mice inoculated with a sublethal dose (4.5×10^5 PFU/

mL) began losing weight at 7 dpi, reached a nadir on 9 dpi, and regained weight from 13 dpi. The spleen, lung and liver from both groups, and the kidney from lethal dose group had highest bacterial loads on 6 dpi, except the kidney in the sublethal dose group which peaked on 12 dpi. Further studies, especially study of human immune responses after *Orientia* positive mite bites in endemic areas, will benefit the understanding and control of scrub typhus well as development of vaccine and other prevention measures.

104

THE EFFECT OF *AMBLYOMMA MACULATUM* FEEDING ON *RICKETTSIA PARKERI* INFECTION IN RHESUS MACAQUES

Kaikhushroo H. Banajee[1], Monica E. Embers[2], Kevin R. Macaluso[1]

[1]Vector-Borne Disease Laboratories, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA, United States, [2]Division of Bacteriology and Parasitology, Tulane National Primate Research Center, Covington, LA, United States

Rickettsia parkeri is an emerging pathogen in the spotted fever group of *Rickettsia* that is transmitted by the bite of the Gulf coast tick, *Amblyomma maculatum*. The immune response to spotted fever group *Rickettsia* has been studied extensively in mice via intradermal, intraperitoneal, and subcutaneous routes of infection. However, these studies did not evaluate the effect of tick infestation on the immune response and rickettsial transmission. Since hard ticks feed on vertebrate hosts for several days, they must be able to counteract the host immune response. Several studies have shown that tick saliva alters several aspects of both the innate and adaptive arms of the immune system. However, the effect of this immunomodulation on *Rickettsia* transmission and pathology in the vertebrate host has not been examined. We hypothesize that by modulating the host immune response, tick feeding enhances infection and pathology of pathogenic SFG *Rickettsia* sp. in non-human primates. In order to assess this interaction *in vivo*, we will use rhesus macaques to compare intradermal needle inoculation of *R. parkeri* alone to inoculation during *Rickettsia*-free *A. maculatum* feeding and *A. maculatum* feeding without *Rickettsia* administration. For up to one month post inoculation, skin and lymph node biopsies, and blood will be collected to evaluate pathology, quantification of rickettsial load, and assess the acute phase response and cytokine concentrations. Comparison of the disease course, pathology and immune responses to infection in the presence or absence of tick feeding, will help define the role of the tick in spotted fever group *Rickettsia* transmission and develop a primate model of *Rickettsia parkeri* rickettsiosis.

105

CHARACTERIZATION OF THE BACTERIAL COMMUNITY OF SPECIES AND POPULATIONS OF *DERMACENTOR* TICKS

Gregory A. Dasch[1], Maria L. Zambrano[1], Lauren M. Shanker[1], Jusun Hwang[2], Nicole L. Gottdenker[2], Renjie Hu[3], Sarah A. Billeter[3], Marco E. Metzger[3], Alece Powery[4], Amanda J. Williams-Newkirk[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]University of Georgia, Athens, GA, United States, [3]California Department of Public Health, Ontario, CA, United States, [4]Muskingum College, New Concord, OH, United States

Our metagenomic analysis (*rrs* fragment pyrosequencing) of the bacterial communities present in 23 adult *Dermacentor variabilis* (Dv) ticks from Atlanta, Georgia and southeastern Ohio identified 27 taxonomic groups; however, only 6 were present in at least 3 ticks - 2 types of *Rickettsia*, 3 types of *Francisella* endosymbiont (FE) and a *Rickettsiella*. *Dermacentor* ticks are classic vectors for *Rickettsia* (7 ticks) while the FE was found in higher abundance and in all ticks; 15 (both sites) had two types of FE. In order to select other tick samples for further metagenomic analyses and to determine whether there was any relationship between the presence of *Rickettsia* and FE agents, additional Dv adults from 8 sites on a coastal barrier island in Georgia and 6 ecologically variant sites in Ohio, and *D. occidentalis* (Do) from 6 sites in southern California were collected. Their DNA was analyzed by semiquantitative EVAGreen assays (qPCR) for fragments of *omp*A (*Rickettsia*) or *rrs* (FE). The *Rickettsia* species were identified by sequencing *omp*A, *omp*B, and/or 17 Kd antigen gene amplicons. Nearly all samples again contained FE while *Rickettsia* was found in lower prevalence and abundance irrespective of species. Primers for 11 genes commonly used in multi-locus sequence typing (MLST) for pathogenic *Francisella* (PF) were tested by PCR with the Dv FE but products were obtained for only 4 of them. Three MLST targets exhibited no genetic variation between Dv populations but they had <90% homology to PF; however, a small number of 16S genotypes were detected at each site including some new variants. While the quantity of FE detected by qPCR varied significantly between ticks and tick locations for both Dv and Do, it did not correlate with either tick sex or the presence of a *Rickettsia*. The physiological importance of the widespread dominant prevalence of different *Francisella* endosymbionts to *Dermacentor* ticks is unknown; if (and how) their presence may alter these ticks' responses to acquisition, growth and transmission of *Rickettsia* also needs experimental investigation.

106

CHARACTERIZATION OF VERTICAL AND HORIZONTAL TRANSMISSION OF PATHOGENIC AND NONPATHOGENIC RICKETTSIA WITHIN THE TWO TICK HOSTS *DERMACENTOR VARIABILIS* AND *AMBLYOMMA MACULATUM*

Emma Harris[1], J. Macaluso[2], V. Verhoeve[1], K. Banajee[1], A. Azad[2], K. Macaluso[1]

[1]Vector-Borne Disease Laboratories, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, United States, [2]Department of Microbiology and Immunology, School of Medicine, University of Maryland Baltimore, Baltimore, MD, United States

Ticks act as vectors for an array of rickettsial species with primary pairings existing between specific tick and rickettsial species. However, transmission of pathogenic *Rickettsia* species by newly recognized vectors, coinciding with a rise in tick-borne rickettsial diseases has raised the need to consider variations in known tick/*Rickettsia* relationships. To fully understand the infection potential between several tick and rickettsial species, two sympatric species of ticks, *D. variabilis* and *A. maculatum,* were exposed to the rickettsial pathogens *Rickettsia rickettsii* or *Rickettsia parkeri*; non-pathogenic *Rickettsia montanensis* or *Rickettsia amblyommii;* or, flea-borne *Rickettsia felis* Adult, female *D. variabilis* and *A. maculatum* were capillary fed with either *Rickettsia* or delivery control and allowed to feed to repletion. Fitness-related metrics such as engorgement weight, egg production index, nutrient index, and egg hatch percentage were then assessed. Subsamples of egg clutches for each treatment group were assessed for transovarial transmission (TOT) of rickettsiae by qPCR. Results show that fitness of *D. variabilis* was not influenced by rickettsial exposure, excepting a decrease in egg production for *R. montanensis*-exposed ticks. TOT was observed for all groups except *R. rickettsii*. In contrast, engorgement weight for *A. maculatum* was reduced for all groups except *R. rickettsii*. Egg production was decreased in *R. montanensis*-exposed *A. maculatum*, which was also shown in nutrient index measurements. TOT was demonstrated only for *R. amblyommii* and *R. parkeri*-exposed *A. maculatum*, with the nutrient index of *R. parkeri*-exposed ticks significantly decreased. Results of this study will lead to a better understanding of the ecology and epidemiology of rickettsial diseases.

107

CLINICAL VALIDATION OF NEW AND EXISTING *ANAPLASMA PHAGOCYTOPHILUM* REAL-TIME PCR ASSAYS

Ida H. Chung, Amy L. Austin, Robert F. Massung, Cecilia Y. Kato

Centers for Disease Control and Prevention, Atlanta, GA, United States

Anaplasma phagocytophilum, an obligate intracellular bacterium, is the causative agent of human granulocytic anaplasmosis (HGA). Characterized by fever, headache, myalgia, leukopenia, and thrombocytopenia, HGA is a vector-borne disease carried by *Ixodes* tick species. While HGA has a low fatality rate, the risk increases with delayed treatment or when the elderly or immunocompromised are infected. Currently, our laboratory uses a SYBR Green assay that targets the 16S gene of *Anaplasma* and *Ehrlichia*, which requires sequencing confirmation to distinguish the species. The msp2 gene encodes an outer membrane protein unique to *Anaplasma*. Depending on the isolate, *A. phagocytophilum* may contain >80 copies of msp2 variants. An existing real-time PCR multiplex assay targets the msp2 gene of *A. phagocytophilum* and the 23S gene of *Borrelia burgdorferi* (Courtney JW et al., 2004). In this study, we validate the *A. phagocytophilum* msp2 primer/probe set as a singleplex real-time PCR assay for testing clinical specimens in the CDC Rickettsial Diagnostic Laboratory. A second set of primers were designed to flank the original amplicon (77-bp) extending the product to provide a template (213-bp) suitable for DNA sequencing. Primer/probe concentrations were optimized and analytical specificity was tested with exclusivity panels of environmental DNAs (33) and bacterial near neighbor DNAs (41). Sensitivity of the assays was determined by spiking known concentrations of *A. phagocytophilum* into PBS and blood. The assays have a limit of detection of 10 copies of the msp2 gene. Assay verification was performed by testing a blind panel of DNA extracted from blood with high, medium, and low concentrations of the organism. Results from the blinded panel correlated with expected values. Assay reproducibility (inter-assay and intra-assay) was also evaluated, generating consistent results. In conclusion, two sensitive and specific real-time PCR assays have been validated and can be used as effective diagnostic tools for the detection of *A. phagocytophilum* in clinical blood specimens.

108

SEROLOGICAL SURVEILLANCE OF RICKETTSIAL DISEASE IN NORTHEASTERN AND CENTRAL CAMBODIA

Michael Prouty[1], Chonthida Supaprom[1], Phireak Hip[1], Pidor Ouch[1], Kim Ly[1], Agus Rachmat[1], Chenda Yi[1], Allen L. Richards[2], Ju Jiang[2], Rekol Huy[3]

[1]U.S. Naval Medical Research Unit - 2, Phnom Penh, Cambodia, [2]Naval Medical Research Center, Silver Spring, MD, United States, [3]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia

Rickettsiosis are arthropod-borne diseases caused by intracellular bacteria of the genera *Rickettsia* and *Orientia* and are classified into 3 groups: spotted fever group (SFG), typhus group (TG) and scrub typhus group (STG). Rickettsial diseases generally present as a febrile illness that can range from moderate to severe symptoms. Limited data has been generated on the prevalence of rickettsial diseases in Cambodia. To investigate further, we have conducted febrile syndromic surveillance in the Northeastern provinces of Cambodia near the Lao PDR border and a second region within central Cambodia from July 2010-April 2011. Subjects who reported to local clinics with an acute fever greater than 37.5°C were enrolled. Data thus far includes a total of 390 enrolled patients who tested negative for influenza, dengue and malaria were checked for the presence of specific antibodies against SFG (*R. rickettsii*), TG (*R. typhi*), and STG (*O. tsutsugamushi*) by ELISA. Convalescent sera positive for antibodies specific to one of the three rickettsial groups were detected in 116 of 390 (29.7%) samples and positive in 77 (19.7%), 23 (5.9%) and 48 (12.3%) of cases for SFG, TG and STG, respectively. Titrations were performed on positive convalescent specimens and corresponding acute specimens. Recent infections, defined by a fourfold rise in antibody titers, or seroconversion were determined in 13 (11.2%) of SFG, 3 (3.9%) of TG and 4 (8.3%) of STG rickettsiosis. These results suggest that rickettsial infections may be a common etiology of fever for this area. Continued monitoring of this population and molecular characterization of these specimens will provide further insight to the epidemiology of rickettsiosis in this area.

109

RICKETTSIA SPECIES DETECTED IN *DERMACENTOR VARIABILIS* FROM NORTH CAROLINA

Madhavi Kakumanu[1], Loganathan Ponnusamy[1], Haley Thornton[1], Marcee Tolliver[2], Charles S. Apperson[1]

[1]North Carolina State University, Raleigh, NC, United States, [2]North Carolina Department of Health and Human Services, Raleigh, NC, United States

We screened *Dermacentor variabilis* (American dog tick) for the genus *Rickettsia* under the hypothesis that less <10% of the ticks would harbor rickettsiae. To test our hypothesis, we extracted genomic DNA from 532 *Dermacentor* variabilis that were collected from several NC counties over years 2009 and 2010. *Rickettsia* species were identified by PCR amplification of 23S-5S intergenic spacer fragments combined with reverse line blot (RLB) hybridization with species-specific probes. Thus far, amplification of 100 genomic DNA samples has produced 61 (61%) samples, which showed a band of the correct size on agarose gels, and are considered to contain rickettsiae. PCR-RLB of 33 ticks showed that 96% (32/33) of the samples hybridized with a *Rickettsia* genus-specific probe. With species-specific probes, *R. amblyommii* was found in the majority of the ticks (21/33); other *Rickettsia* species detected included *R. massiliae* (3/33), *R. canadensis* (2/33), *R. montanensis* (2/33), *R.belli* (1/33) and *R.conorii* (4/33). Of the 33 samples, 10 samples showed hybridization with more than 1 species of *Rickettsia* and 3 samples contained unknown *Rickettsia* species. DNA sequencing to confirm the detection of *Rickettsia* and to verify species identifications obtained through RLB hybridization assays is in progress. Based on the ticks examined thus far, the presence of *Rickettsia* in D. variabilis is more common than we hypothesized. Our analysis of D. variabilis is ongoing and should provide informative results about the prevalence and species of *Rickettsia* harbored by this tick species.

110

STUDY OF SEROPREVALENCE AND VECTORS OF *RICKETTSIA* IN THE COLOMBIAN CARIBBEAN

Margaret Paternina Gómez, Luis E. Paternina, Daniel Verbel, Lily Martínez, Arnol Contreras, Eduar E. Bejarano

Universidad de Sucre, Sincelejo, Colombia

The first clinical signs and symptoms of spotted fever caused by *Rickettsia* are nonspecific, so can be easily confused with other febrile infections. The medical diagnosis is even more difficult because there are no diagnostic tests during the acute phase of the disease. Thus, clinical suspicion based on epidemiology, ecology and distribution of vectors, followed by subsequent clinical manifestations of the disease becomes the best strategy for a timely diagnosis. Conduct studies to establish risk areas is necessary to prevent deaths by rickettsiosis, therefore the objective of this work was to determine the seroprevalence in human populations and natural infection with *Rickettsia* in ticks in the department of Sucre, located in the Colombian Caribbean. A blood sample was taken from people, in the rural area of five municipalities of this area, who signed an informed consent and underwent an interview to establish risk factors. An indirect immunofluorescence test was performed to detect anti-*Rickettsia* IgG antibodies. The independence between the seroprevalence and risk factors was tested by a Chi-square test at a 5% level of significance. Additionally, ticks were captured from animals and drag sampling on vegetation, which were taxonomically identified and processed in order

to detect *Rickettsia* DNA by PCR. 320 human samples were taken, where 20 of them were seropositive (6.2%). Seropositive individuals were mainly elderly men ($p<0.05$), who were engaged in agriculture. 10,217 ticks were captured, D. nitens (7922), larvae of *Amblyomma* sp. (909), *R. sanguineus* (882), *R. (B.) microplus* (372), *A. cajennense* (96), *A. dissimile* (23), *H. leporispalustris* (6), *A. ovale* (5) and *A. auricularium* (2). *Rickettsia felis* DNA was detected in *R. sanguineus* and *D. nitens*, with minimum infection rates of 7,4 and 1,9 respectively. This study provides evidence of the presence of bacteria from the genus *Rickettsia* in ticks, and its circulation in human population from rural areas of the department of Sucre.

111

Q FEVER AND RICKETTSIOSIS IN U.S. MARINES DEPLOYED TO AFGHANISTAN

Christina M. Farris, Allen L. Richards, Todd E. Myers

Naval Medical Research Center, Silver Spring, MD, United States

Many dangers face military personnel when deployed in combat zones, but one of the most formidable opponents is infectious diseases. Operation Iraqi Freedom and Operation Enduring Freedom have brought more than 100,000 US troops to Central Asia exposing them to a multitude of pathogens that they may not have been exposed to otherwise. Several of these include the causative agents of the operationally relevant arthropod borne diseases Q fever (*Coxiella burnetii*) scrub typhus (*Orientia tsutsugamushi),* murine typhus (*Rickettsia typhi*), and spotted fever group rickettsioses (*Rickettsia sp.)*. Since 2003, more than 150 cases of Q fever have been confirmed among troops deployed to Iraq and Afghanistan, though the symptoms of infection often mimic a multitude of other infections and can often be misdiagnosed. To assess the risk of these pathogens among military personnel in Afghanistan we initiated a survey of close to 1000 Marines deployed to Afghanistan for at least one continuous year. The DOD Serum Repository provided serum from pre-deployment and post-deployment blood draws, paired for each Marine. Analysis of these sera revealed sero-conversion of approximately 6% for Q fever, and 0.5% for spotted fever, but no sero-conversions for typhus group or scrub typhus. This study leads to a greater understanding of the infectious disease risk in Central Asia and provides valuable information for diagnosis and treatment of acute febrile illness in the area.

112

SCRUB TYPHUS IN AFRICA

Allen L. Richards[1], Ju Jiang[1], Alice Maina[1], Katherine Horton[2], Erica Dueger[2], Guillermo Pimentel[2], Jacqueline Thiga[3], Beth Mutai[3], John Waitumbi[3]

[1]Naval Medical Research Center, Silver Spring, MD, United States, [2]U.S. Naval Medical Research Unit - 3, Cairo, Egypt, [3]Walter Reed Project Kenya Medical Research Institute, Kisumu, Kenya

Since a report in 1951 evidence has been slowly increasing that scrub typhus exists in sub-Saharan Africa. The previous evidence included two cases (Cameroon and Congo) and evidence of O. tsutsugamushi-specific antibodies in humans (Ruanda-Urundi). With the recent reports of scrub typhus in Dubai and Chile, outside the previously accepted scrub typhus endemic triangle area of Asia, northern Australia and the western Pacific, we decided to investigate whether scrub typhus exists in Africa. Utilizing orientia-specific ELISA-IgG assays and two recombinant protein Western Blot assays (Kp r56 and Kp r47) we assessed human sera collected from abattoir workers in Djibouti and fever patients in Kenya for presence of antibodies-specific to Orientia spp. Antibody prevalence among fever patients in Kenya was 5% (67/1401) and abattoir workers was 4% (2/49). In addition one of the two abattoir workers positive for antibodies against Orientia had a baseline serum sample at the beginning of the study that was non-reactive to orientia-specific ELISA and WB antigens. These results add to the evidence of the presence of scrub typhus in Africa. However, it is unknown whether the scrub typhus in Africa is due to infection with O. tsutsugamushi, O. chuto, both, or another Orientia sp. Also unknown is what the vector(s) is/are. Thus, much more research needs to be performed to characterize African scrub typhus.

113

IDENTIFICATION OFA FULL LENGTH TRANSCRIPT ENCODINGA PUTATIVE RELISH-TYPE NF-KB PROTEIN, DVRELISH, IN *DERMACENTOR VARIABILIS*

Victoria I. Verhoeve, Natthida Petchampai, Emma K. Harris, Kaikhushroo H. Banajee, Kevin R. Macaluso

Louisiana State University, Baton Rouge, LA, United States

As a vector for *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever, *Dermacentor variabilis* are known to upregulate antimicrobial peptides (AMP) in response to rickettsial infection. The relationship between AMP production and vector competence is unknown and the mechanism required for the regulation of AMPs has yet to be characterized in *D. variabilis*. In the model arthropod *Drosophila melanogaster*, the Rel/NF-kB transcription factors Dorsal, Dif, and Relish control AMP regulation. Upon immune challenge in *D. melanogaster*, the Rel/NF-kB proteins localize to the nucleus to regulate the transcription of immune responsive genes. The overall goal of the project is to identify and functionally characterize Rel/NF-kB proteins in *D. variabilis* and examine the importance of these proteins in the regulation of immune responsive genes in *Rickettsia*-infected *D. variabilis*. The broad hypothesis is that differential regulation of Rel/NF-kB proteins occurs in a *Rickettsia*-specific manner, and that this response drives vector competence. Using traditional PCR, we have isolated a 3,183 base pair transcript encoding a 873 amino acid Relish-type NF-kB transcription factor in *D. variabilis*, containing a canonical Rel-homology domain, immunoglobulin/plexin/transcription factor fold domain, nuclear localization sequence and inhibitory ankyrin repeat domains. In *D. variabilis,* after capillary feeding challenge with $2.5x10^8$/ml intracellular *R. rickettsii* in Vero host cells for 1 hour, transcript encoding DvRelish increases significantly as compared to ticks fed only Vero host cells. In contrast, expression of DvRelish transcript decreases significantly in ticks capillary fed for 3 hours with the same number of *Rickettsia*. Further projects will explore the temporal changes in DvRelish transcription and protein expression in response to increasing concentrations of *R. rickettsii* compared to non-pathogenic *Rickettsia montanensis*. These studies will increase our understanding of the molecular regulation of the immunological response to rickettsial infection in ticks, the mechanisms of which may define vector competence.

114

A STUDY OF RICKETTSIAL AND ORIENTIA INFECTION AMONG ABATTOIR WORKERS AND THE DETECTION OF *RICKETTSIA* IN TICKS, DJIBOUTI

Ju Jiang[1], Katherine C. Horton[2], Alice N. Maina[1], Erica Dueger[3], Alia Zayed[2], Ammar A. Ahmed[4], Guillermo Pimentel[1], Allen L. Richards[1]

[1]Naval Medical Research Center, Silver Spring, MD, United States, [2]U.S. Naval Medical Research Unit - 3, Cairo, Egypt, [3]U.S. Naval Medical Research Unit - 3, Cairo, Egypt and Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]The National Institute of High Health Sciences, Djibouti, Djibouti

Little is known about rickettsial infection in Djibouti located in the Horn of Africa. A study was conducted to examine the exposure to vector-borne and zoonotic pathogens in a high-risk environment, an abattoir in Djibouti. Human serum samples were collected from the abattoir workers in September 2010 and followed up every 4 weeks during a 20 week long period. Ticks were collected from the freshly-slaughtered cattle. 49 baseline and 11 follow-up human serum samples were tested for IgG antibodies against whole-cell antigens of SFGR, TGR and orientiae by enzyme-linked immunosorbent assays (ELISAs). Eight (16%), two (4%), and three (6%) were found seropositive for IgG antibodies against SFGR,

TGR and orientiae, respectively. Analysis of paired sera for 11 workers showed one was seroconversion for antibodies against orientiae during the study period, and the positive reactions were confirmed by Western blot assays using recombinant protein Kpr56 and Kpr47b. No seroconversions were observed for antibodies against SFGR or TGR. DNA extracted from 189 tick pools (contain 1-3 ticks) including 6 tick species from 72 cattle imported from Ethiopia were tested for the infection of *Rickettsia* using a *Rickettsia* genus-specific quantitative real-time PCR (qPCR) assay (Rick17b) and the positives were further tested by a qPCR assay specific for *Rickettsia africae* (RafriG). Overall, 32 (17%) pools of ticks from 26 (36%) cattle were infected with *Rickettsia*, of which 25 (47%) were *R. africae*, the causative agent of African tick-bite fever. Three types of *R. africae* variants were identified from *Ambylomma variegatum* ticks (but none from *A. lepidum*) by PCR and sequencing of a fragment of *ompB*. In addition, *R. sp.* S strain was detected in 2 (6%) pools of *Hyalomma marginatum* ticks. Although future studies are needed to determine the extent and impact of rickettsial infections in Djibouti, healthcare providers should be aware of possibility of these infections among their patients.

115

RICKETTSIA AFRICAE VARIANTS

Alice N. Maina, Ju Jiang, Allen L. Richards

Naval Medical Research Institute, Silver Spring, MD, United States

Rickettsia africae causes African tick-bite fever (ATBF) and is one of the main causes of fever and illness in travelers returning from sub-Saharan Africa. *R. africae* belongs to the spotted fever group rickettsiae (SFGR) and is transmitted to humans by ticks of the genus *Amblyomma* found throughout sub-Saharan Africa. An infection rate in these ticks in endemic areas is high, and may reach 100%. Recent studies have shown existence of *R. africae* variants in ticks from several countries in Africa and New Caledonia. The genetic variants have mainly been detected in *Ambylomma variegatum* ticks and are closely related to wild type *R. africae*, but have not been associated with ATBF. The variability observed is mainly due to nucleotide substitution but some may have deletions and/ or insertions leading to premature stop codons in the amino acid sequence. The impact of these mutations to the infectivity of the *R. africae* variants is currently unknown but we hypothesize that it may result in nonfunctional proteins and preclude human infection. The variability observed among *R. africae* genotype underscores the need to undertake comparative assessment of variants associated with clinical ATBF and those circulating within the tick population to understand the role that these variants play in the epidemiology of ATBF.

116

AN ONLINE TOOL FOR MAPPING INSECTICIDE RESISTANCE IN MAJOR *ANOPHELES* VECTORS OF HUMAN MALARIA PARASITES AND A REVIEW OF INSECTICIDE RESISTANCE STATUS FOR THE AFROTROPICAL REGION

Elijah O. Juma[1], Melinda Hadi[1], Helen P. Jamet[2]

[1]Vestergaard Frandsen (EA) Ltd, Nairobi, Kenya, [2]Vestergaard Frandsen SA, Lausanne, Switzerland

Rapid evolution of insecticide resistance in malaria vectors threatens to erode gains made in malaria control unless action is urgently taken. Upto-date insecticide resistance data are urgently needed for inform vector control policy and programmes. A new online geospatial mapping tool, IR Mapper, is introduced herein. A systematic search of peer-reviewed literature was performed and *Anopheles* phenotypic and resistance mechanisms data extracted to Microsoft Excel 2010 database. IR Mapper (www.irmapper.com) was built on ArcGIS for JavaScript. Additional integrated functionalities allowing triangulation of entomological monitoring data with epidemiological data include *Plasmodium falciparum* and *P. vivax* endemicity layers, entomological inoculation rate layers, and an "Add Own Data" functionality enabling users to add and temporarily view their own data on the application, and print user-generated maps. IR Mapper contains 8, 596 data points from 1, 587 georeferenced localities in 55 countries for data collected between 1954-2013. Susceptibility reports were available for 53 countries; 43 reported resistance confirmed to at least one insecticide. One or more types of resistance mechanisms were detected in 37 countries. In the Afrotropical region pyrethroids and DDT were commonly tested in susceptibility assays than carbamates or organophosphates. Between 2001 and 2012, there was an increase in cases of confirmed resistance of *An. gambiae s.l.* to pyrethroids (from 41 to 87%) and DDT (from 64 to 91%). Resistance mechanism assays in *An. gambiae* mainly focused on target site mutations with very few reports on elevated metabolic enzyme assays. Mapper is a dynamic tool that collates resistance information from various sources and in various formats into a single platform. Mapper is useful for investigating temporal and spatial trends in *Anopheles* insecticide resistance, informing rational deployment of vector control interventions based on the evidence, as well as for pointing out gaps in resistance monitoring for appropriate action.

117

INSECTICIDAL PROPERTIES OF LOCAL PLANTS USED AGAINST *ANOPHELES GAMBIAE*, MALARIA VECTOR IN BURKINA FASO, WEST AFRICA

Dimitri W. Wangrawa[1], Badolo Athanase[1], Wamdaogo Moussa Guelbeogo[2], Roger Nebie[3], Sagnon N'falé[2], Sanon Antoine[4]

[1]University of Ouagadougou/Centre National de Recherche et de Formation sur le paludisme, Ouagadougou, Burkina Faso, [2]Centre National de Recherche et de Formation sur le paludisme, Ouagadougou, Burkina Faso, [3]Institut de recherche en sciences appliquées et technologie (IRSAT), Ouagadougou, Burkina Faso, [4]University of Ouagadougou, Ouagadougou, Burkina Faso

Malaria remains a serious public health problem killing mostly in Africa. It is estimated to 207 millions of cases and 627000 deaths the burden of malaria in 2012. Malaria control is still heavily dependent of insecticides for vector control but the emergence of resistance to insecticides in Malaria vector population can jeopardize this control effort. The main objective of this study was to evaluate, larvicidal and adulticidal properties of essential oil of local plants, namely, *Ocimum canum, Hyptis suaveolens, Hyptis spicigera* and *Lantana camara* extracts on *Anopheles gambiae* and their inhibiting activity of acytylcholinesterase (AChE). Young branches with leaves were collected from local plants and the extraction of essential oil was processed. Adults of 3-5 old days and third-fourth stage larvae were used for bioassay tests based on WHO protocols. Different concentrations have been used for each plant essential oil and results have been assessed as mortality and Knock down rate of adults after 1 hour exposure. Larvae mortality after 24 hours and the lethal concentration were calculated for each essential oil. The inhibition effect of essential oils on acetylcholinesterase activity was assessed using Ellman's spectrophotometric method. All tested essential oils exhibited adulticidal and larvicidal activities. The LD50 and LD90 lethal doses value observed were 0.82% and 1.55% respectively on adults for L. camara. On larvae, the LC50 and LC90 values of this oil were 61 and 125 ppm respectively. The high inhibitory activity on acetylcholinesterase was observed with O. americanum and H. suaveolens essential oil with 50% Inhibitory concentration (IC50) of 0.21 and 0.55 µg/ml respectively. Our results highlighted that essential oils of these plants have a potential as insecticides for malaria vector control and can be considered as an interesting source of natural and ecofriendly substances for vector control.

118

FIELD EFFICACY OF VECTOBAC GR AS A MOSQUITO LARVICIDE FOR THE CONTROL OF ANOPHELINE AND CULICINE MOSQUITOES IN NATURAL HABITATS IN BENIN, WEST AFRICA

Armel Djenontin[1], Cedric Pennetier[2], Barnabas Zogo[2], Koffi Bhonna Soukou[2], Marina Ole-Sangba[2], Martin Akogbeto[3], Fabrice Chandre[4], Rajpal Yadav[5], Vincent Corbel[6]

[1]FAST-UAC and CREC/IRD, Cotonou, Benin, [2]CREC/IRD, Cotonou, Benin, [3]FAST-UAC and CREC, Cotonou, Benin, [4]LIN-IRD, Montpellier, France, [5]Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland, [6]MIVEGEC (IRD 224-CNRS 5290-UM1-UM2)/Department of Entomology, Kasetsart University, Ladyaow Chatuchak Bangkok, Bangkok, Thailand

The efficacy of Vectobac GR (potency 200 ITU/mg), a new formulation of bacterial larvicide Bacillus thuringiensis var. israelensis Strain AM65-52, was evaluated against *Anopheles gambiae* and *Culex quinquefasciatus* in simulated field and natural habitats in Benin. In simulated field conditions, Vectobac GR formulation was tested at 3 dosages (0.6, 0.9, 1.2 g granules/m² against *An. gambiae* and 1, 1.5, 2 g granules/m² against Cx. quinquefasciatus) according to manufacturer's product label recommendations. The dosage giving optimum efficacy under simulated field conditions were evaluated in the field. The efficacy of Vectobac GR in terms of emergence inhibition in simulated field conditions and of reduction of larval and pupal densities in rice fields and urban cesspits was measured following WHO guidelines for testing and evaluation of mosquito larvicides. Vectobac GR caused emergence inhibition of ≥80% until 21 [20-22] days for *An. gambiae* at 1.2 g/m² dose and 28 [27-29] days for *Cx. quinquefasciatus* at 2 g/m² in simulated field habitats. The efficacy of Vectobac GR in natural habitats was for 2 to 3 days against larvae and up to 10 days against pupae. In conclusion, treatment with Vectobac GR caused complete control of immature mosquito within 2-3 days but did not show prolonged residual action. Larviciding can be an option for malaria and filariasis vector control particularly in managing pyrethroid-resistance in African malaria vectors. Since use of larvicides among several African countries is being emphasized through Economic Community of West Africa States, their epidemiological impact should be carefully investigated.

119

EFFECTS OF COMBINATIONS OF A SUNLIGHT-ACTIVATABLE PORPHYRIN AND NEEM TREE PARTS AGAINST LARVAE OF *ANOPHELES GAMBIAE* S.L., POTENTIAL COMPLEMENTING TOOLS FOR INTEGRATED STRATEGIES OF INSECTICIDE RESISTANCE MANAGEMENT

Robert Kossivi Ouédraogo[1], Serge R. Yerbanga[1], Silvia Damiano[2], Simon P. Sawadogo[1], Clara Fabris[3], Leonardo Lucantoni[2], Abdoulaye Diabaté[1], Olimpia Coppellotti[3], Giulio Lupidi[2], Giulio Jori[3], Jean-Bosco Ouédraogo[1], Annette Habluetzel[2], Roch K. Dabiré[1]

[1]Institut de Recherche en Sciences de la Santé, Bobo Dioulasso, Burkina Faso, [2]School of Pharmacy, University of Camerino, Camerino, Italy, [3]Department of Biology, University of Padova, Padova, Italy

Meso-tri(N-methyl-pyridyl),mono(N-dodecyl-pyrydyl)porphine is a porphyrin known as C12. Loaded on ground cat food pellets (CF), the C12 has rapid killing effect on larvae of *Anopheles gambiae* s.l. The present study aimed at screening combinations of C12 and neem portions with the ability of both rapid killing and delayed-acting effects (additive effect) and subsequent residual efficacy (synergistic effect) on larvae of the malaria vectors using outdoor tray experiments. C12-neem combinations were obtained by incubating finely ground neem leaves (NL), bark (NB), fruit (NF) and 10% NeemAzal-loaded cat food (CFNA) in 500-µM C12 solutions. Batches of 60 larvae of wild or laboratory colonies (2nd - 4th instars) were exposed outdoors with 20 - 30 mg of C12-neem products in water samples from typical water bodies. Larvae treated with CF and C12-CF free of neem served for negative and positive controls respectively. Three consecutive experiments were run allowing to determine respectively larval mortality efficacy after i) a short time of 48 h post-treatment, ii) a prolonged-exposure up to 9 days post-treatment and iii) residual efficacy as larval mortality and subsequent inhibition of pupation up to 9 days post-treatment. Alike the C12-CF, C12-neem combinations particularly C12-NF and C12-NL yielded rapid killing efficacy of ~100 % larval mortality after 48 h post-treatment in spring water. This effect decreased in turbid water types. However, a prolonged-exposure allowed to achieve a delayed-effect of 70 - 100% mortalities in turbid water type after 4 - 5 days post-treatment. Upon a prolonged-exposure time of 9 days in spring water, C12-NF and C12-CFNA yielded residual effects of 70 - 100% larval mortalities. However, C12-CF yielded such residual activity for only 21% mortality. C12-NF possesses at least an additive efficacy on larvae of *An. gambiae* s.l. in this preliminary study. Further investigations should help to reinforce efforts towards the development and validation of such larvicidal tools in the context of integrated management of insecticide resistance.

120

EVALUATION OF THE FACTORS AFFECTING THE FUNCTIONALITY OF INDOOR RESIDUAL SPRAYING AND LONG LASTING INSECTICIDE NETS PROGRAMS USED FOR MALARIA CONTROL IN WESTERN KENYA

Christine L. Wanjala[1], Yaw A. Afrane[1], Edna N. Ototo[1], Andrew K. Githeko[1], Guiyan Yan[2]

[1]Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya, [2]University of California Irvine, CA, United States

Indoor Residual Spraying and long-lasting insecticidal nets have been extensively used for malaria prevention and control in Kenya. However there are concerns that certain factors greatly affect the efficacy of these programs. We evaluated the persistence of ICON and deltamethrin on the sprayed mud walls and the bio efficacy of Long Lasting Insecticide nets. Wall bioassays were performed on artificial mud walls and filter papers sprayed with ICON and Deltamethrin using mosquitoes collected from seven sites in western Kenya and Kisumu strain as a control. Net cone bioassays was performed on nets collected from the fields using wild mosquitoes from Emutete and Bungoma and Kisumu susceptible strain was used as a control. Chemical analysis of the netting material was done using gas chromatography. Kisumu strain was susceptible to the insecticides with 100% mortality. ICON persisted on the mud wall for Six months where as Deltamethrin Persisted on the Mud walls for four months. Sprayed artificial walls showed lower mortality rates compared to sprayed filter papers. ICON had high mortality rates on the mosquitoes compared to Deltamethrin. Mosquitoes from Chulaimbo, Ahero, Emakakha and Kisian showed susceptibility to both deltamethrin and ICON with the mortality rates ranging between 80% - 85% but mosquitoes from Bungoma and Emutete had lower mortality rates to both ICON and Deltamethrin with mortality rates ranging between 69%-74%. Wild mosquitoes showed low mortality rates to LLINs nets collected from the field (60%-75%), compared to the control strain (100%). Net chemical analysis results indicated that there was no difference in the net chemical content in the nets between 6months - 2yrs old, with the net chemical content ranging between 0.06 wt% - 0.13 wt%. In conclusion, insecticide resistance, persistent of the insecticide on the sprayed surfaces, bioavailability of the insecticide and the physical condition of long-lasting insecticide nets affect the efficacy of indoor residual spraying and Long Lasting Insecticide nets, therefore these factors should be considered in malaria control programs.

121

NEAR-INFRARED CAN DETECT PRESENCE OF *WOLBACHIA* IN FEMALE AND MALE *AEDES AEGYPTI*

Maggy Sikulu[1], Peter Masabho[2], Nikita Lysenko[3], Michael Henry[4], Greg Devine[5], Floyd Dowell[6]

[1]QIMR Berghofer Medical Research Institute/Ifakara Health Institute, Brisbane, Australia, [2]Ifakara Health Institute, Ifakara, United Republic of Tanzania, [3]University of Basel, Basel, Switzerland, [4]Pennysylvania State University, State College, PA, United States, [5]QIMR Berghofer Medical Research Institute, Brisbane, Australia, [6]United States Department of Agriculture, Manhattan, KS, United States

Vector control strategy utilizing endosymbiotic bacterium; *Wolbachia* has been reported successful in Australia. The ability of *Wolbachia pipientis* to shorten the lifespan of mosquitoes and block dengue transmission simultaneously makes it a very effective vector control intervention. The success of such an intervention is determined by its ability to rapidly spread in a given mosquito population. Currently, *Wolbachia* infected mosquitoes can only be differentiated from non-infected population by use of Polymerase Chain Reaction (PCR) assay. However, PCR is costly and time consuming. In this study, we applied the Near-infrared spectroscopy to detect the presence of two pathogenic strains of *Wolbachia pipientis*; wMel and wMelPop in male and female *Aedes aegypti* reared in the laboratory. Using cross validation technique, the accuracy of differentiating infected from non-infected females of the wMel and wMelpop *Wolbachia* was 86.5% and 85%, respectively. wMel and wMelP*op* infected males were predicted with an accuracy of 86% and 96% respectively. Furthermore, independent female sets of wMel and wMelPop were predicted with an accuracy of 84% and 97%, respectively whereas independent male sets were predicted with an overall accuracy of 90%. Where processing of large field samples are required to evaluate the efficacy of strategies involving *Wolbachia*, NIRS could be a complementary diagnostic tool as thousands of samples can be scanned daily. However, we recommend a further assessment of the accuracy of this tool to detect *Wolbachia* infected wild mosquitoes relative to PCR.

122

OVERDOMINANCE OF KDR MUTATION IN *ANOPHELES GAMBIAE* ON A BEHAVIORAL TRAIT UNDER INSECTICIDE SELECTIVE PRESSURE

Malal Diop[1], Hadrien Martin-Herrou[2], Olayidé Boussari[1], Nicolas Moiroux[1], Angélique Porciani[1], Stéphane Duchon[2], Fabrice Chandre[2], Cédric Pennetier[1]

[1]IRD/CREC, MIVEGEC UMR224, Cotonou, Benin, [2]LIN-IRD, MIVEGEC UMR224, Montpellier, France

The anthropophilic *Anopheles gambiae* malaria mosquito females need a blood meal to mature eggs. Faced to the new physical and chemical barrier (i.e. the pyrethroid insecticide treated bed net), *Anopheles* mosquitoes exhibit evolutionary responses such as the Kdr mutation that provides physiological resistance to the pyrethroid insecticides family. Nevertheless, the impact of physiological resistance specifically Kdr mutation on the short-range of host seeking behaviour, when the host is under treated net remains poorly studied. In the present study, we investigated the ability of *An. gambiae* s.s. of the three genotypes of the Kdr mutation to find a hole in a piece of net (either untreated or insecticide treated) to reach a bait in a wind tunnel. Homozygous resistant mosquitoes were the less efficient to pass through an untreated holed net indicating for the first time a cost for the Kdr mutation in *An. gambiae*. This reduced performance was likely to be due to a reduced host-seeking activity as revealed by video-tracks analysis of the mosquitoes. When faced to insecticide treated net, heterozygous mosquitoes were the most efficient evidencing overdominance of the Kdr mutation under pyrethroid selective pressure. Our findings brought valuable new insights in the study of heterosis with an evidence of overdominance of a single gene through behavioral traits. These striking results raise a lot of questions the evolution of such insecticide resistance mutations in natural conditions.

123

ENTOMOLOGICAL MONITORING OF INDOOR RESIDUAL SPRAYING INTERVENTION IN LAKE VICTORIA BASIN REGIONS, TANZANIA

Jackline L. Martine, Safari M. Kinung'hi, Fabian Mashauri, Alphaxard Manjurano, John Changalucha

National Institute for Medical Research Mwanza, Mwanza, United Republic of Tanzania

In response to the success of indoor residual spraying (IRS) intervention in Kagera region, which resulted to the decline of entomological indices. The Government of United Republic of Tanzania through financial support of the US President's Malaria Initiatives decided to scale-up the IRS programme in all regions of the Lake Victoria basin, Tanzania. Entomological monitoring activities were carried out at seven representative sentinel districts in Mwanza, Mara, Geita and Kagera regions where IRS intervention have been implemented from July 2011 - August 2012. Mosquitoes were collected using CDC light traps, Pyrethrum spray catch (PSC) and pit trap (PT) method to determine malaria vector species, abundance and sporozoites rates. Vector species were identified by PCR and sporozoite rates were determined by ELISA technique. To determine residual effect of insecticide on various sprayed surfaces, eight houses were selected in each sentinel site. Two to five days old 20 laboratory susceptible female *Anopheles gambiae* s.s. (Kisumu strain) were used. A total of 1713 anopheline mosquitoes were collected. Out of these, CDC light traps collected 879(513%), PT 755(44.5%) and PSC 79(4.7%). A total of 637 anopheline mosquitoes were identified to species. Predominant malaria species were An. *arabiensis* accounted 52.4%, *An. funestus* 24.8%, *An. gambiae* s.s 17.9% and *An. parensis* 4.9%. A total of 972 mosquitoes were tested for sporozoites. The prevalence of sporozoite rates was 1.01% for *P. falciparum*. Bioassays results indicate that wood and cement surfaces have long retention rate. In conclusion, mosquito abundance indicates that high numbers of mosquitoes were collected indoors using CDC light traps. Generally, anopheline mosquitoes were collected in high number in the period of May and June in all sentinel sites. The IRS intervention may have contributed to the decline of malaria in the area.

124

PYRETHROID KNOCKDOWN RESISTANCE (KDR) HAPLOTYPES IN *ANOPHELES GAMBIAE* POPULATIONS FROM MALARIA SURVEILLANCE SITES IN THE GAMBIA

Majidah Hami-Adiamoh[1], Alfred Amambua-Ngwa[1], Musa Jawara[1], Martha Betson[2], Taiwo S. Awolola[3], Davis Nwakanma[1]

[1]Medical Research Council Unit, Banjul, Gambia, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Nigerian Institute of Medical Research, Lagos, Nigeria

Knockdown resistance (kdr) is a target-site resistance mechanism implicated for DDT and pyrethroid insecticides resulting from point mutations at the domain II of the voltage-gated sodium channel gene of *Anopheles gambiae* s.s. Indications of DDT and pyrethroid resistance among *An. gambiae* s.l. in The Gambia were reported in a recent bioassay study. The aim of the present study was to investigate the underlying target-site resistance mutation and associated kdr haplotypes in the same mosquito population. A combination of allele-specific polymerase chain reaction (AS-PCR), sequencing and a high resolution melting (HRM) assay modified by use of primers further away from kdr point mutation on the sodium channel gene was employed to describe the kdr genotypes in over 1000 Gambian *An. gambiae* specimens from four sites analysed in previous bioassays. Five haplotypes of kdr mutations to pyrethroids describing, L1014S, kdr east and L1014F, kdr west mechanisms

were identified with a total frequency of 15% mainly in *Anopheles* arabiensis. With the modified HRM assay, four unique melt profiles could be distinguished for kdr mutant genotypes representing six mutant haplotypes from polymorphisms at positions 1013 (T/C) and 1014 (T/A). Four kdr wild-type haplotypes resulting from 1013 (T/C), 1014 (T/A), 1033 (G/T), 1042 (C/G) and 1059 (A/T) substitutions could also be identified by different clusters of melting profiles and sequenced. The contribution of these different haplotypes to vector survival and impact on vector control remains undetermined. These results provide evidence of different pyrethroid kdr haplotypes in The Gambia and present HRM as a useful assay for rapid scanning of kdr-based insecticide resistance in a mosquito population.

125

ELECTROPHYSIOLOGICAL RESPONSES OF DIFFERENT *ANOPHELES GAMBIAE* RESISTANT STRAINS TO INSECTICIDE AND HOST ODORS

Angélique Porciani, Cédric Pennetier

Institut de recherche pour le Développement, Cotonou, Benin

Major mean of malaria vector control are based on use of insecticides. Their efficiency is threatened by widespread resistance mechanisms. In addition to the physiological resistance mechanisms already well studied, the issue of the behavioral modulation as cause or consequence of the resistance is largely overlooked. Nevertheless there are evidences that insecticide-based control tools alter mosquito behavior before any contact, suggesting that the mosquitoes can detect the presence of the insecticide. In the present study, we tested this hypothesis by investigating the olfactory responses of different resistant strains of *Anopheles gambiae* to host odors and volatile insecticide chemicals. We used *An. gambiae* females aged of 5-8 days old from different strains sharing the same genetic background but insecticide resistance mutations *Kdr* and *Ace1*^{R}. We run electroantenograph (EAG) experiments with different genotypes for these loci (SS, RS, RR) and compared the electrical responses of the antennae. We investigated the responses to the following odours: Octenol, human sweat odour, permethrin, deltamethrin, bendiocarb, propoxur. We described the dose-response relationship to all of these chemicals (except human sweat odour) and run a comparative analysis of the responses of each genotype for the two insecticide resistance loci. Genotypes responded differentially to the odour suggesting that resistance mutations impact olfactory sensitivity of mosquitoes to their environment. Moreover, sensitive strain, Kisumu, responded to permethrin, evidencing that mosquitoes are able to smell insecticide molecule.

126

DETECTION OF G119S ACE-1R MUTATION IN FIELD-COLLECTED MOSQUITOES USING ALLELE SPECIFIC LOOP MEDIATED ISOTHERMAL AMPLIFICATION METHOD (AS-LAMP)

Athanase Badolo[1], Shinya Fukumoto[2], Kiyoshi Okado[3], Wamdaogo M. Guelbeogo[4], Hiroka Aonuma[3], Hironori Bando[2], N'Fale Sagnon[4], Hirotaka Kanuka[3]

[1]University of Ouagadougou, Ouagadougou, Burkina Faso, [2]Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan, [3]Department of Tropical Medicine, The Jikei University School of Medicine, Tokyo, Japan, [4]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso

Malaria vectors have developed resistance to the four families of insecticides available for public health purpose. The resistance to organochlorines, pyrethroids, organophosphates and carbamates has increased along with the increasing of the *1014F kdr* and the *G119S ace-1*^{R} mutations frequency in Burkina Faso. The spread of *kdr* and *ace-1*^{R} mutations in malaria vectors is the main constraint for effective insecticide based control of malaria. There is an urgent need to improve resistance management using existing insecticides and new tools to assess resistance level on time for quick decision-making. With the aim of developing a fast and reliable tool to detect *ace-1*^{R} mutation, we report an allele specific LAMP (AS-LAMP) method to detect the *ace-1*^{R} allele. We designed specific primers with the mutation on the 5' end of the inner primers (BIP and FIP) and additional mismatch nucleotides to increase the specificity and distinguishing between the resistant and wild type of *ace-1*^{R} alleles. Plasmids inserted with targeted DNA sequence as well as genomic DNA were used as DNA template to set up the detection method. The reaction was processed in a real time turbidimeter at 63C for 75 min and detected by the turbidity values as well as by naked eye. The results have been confirmed by direct sequencing and the specificity and sensitivity were compared to RT-PCR using more than 100 mosquitoes. The primers designed for LAMP can distinguish between the wild type allele (*ace-1*^{S}) and the mutated type allele (*ace-1*^{R}). The detection time for the wild type homozygous is 50 min and 64 min for the heterozygous using the wild type specific primers. Using these primers, there is no amplification for the resistant type (*ace-1*^{R}) until 75 min. For the *ace-1*^{R} resistant type, the detection time is 51 min for the resistant homozygous and 55 min for the heterozygous, there is no amplification for the wild type (*ace-1*^{S}) until 75 min when using the resistant type primers. The gel electrophoresis of the LAMP products confirmed that the amplification is primer-DNA specific, the primers can only amplify their target specific DNA. Comparing the AS-LAMP to RT-PCR for *ace-1*^{R} detection, the sensitivity and specificity of the of methods were globally similar This fast and reliable detection method which can be performed with just a water bath at 63°C and yields a result detectable by the naked eye, can be used for resistance monitoring at the site of transmission for quick decision-making.

128

LABORATORY SELECTION FOR PYRETHRIOD RESISTANCE IN FIELD *ANOPHELES SINENSIS*, THE MAJOR ASIAN MALARIA VECTOR

Guoding Zhu, Huayun Zhou, Julin Li, Jianxia Tang, Weiming Wang, Yaping Gu, Yaobao Liu, Feng Lu, Yuanyuan Cao, Chao Zhang, Sui Xu, Jun Cao, Qi Gao

Jiangsu Institute of Parasitic Diseases, Wuxi, China

Anopheles sinensis (Diptera: Culicidae) is one of the major malaria vectors in China and other Asian countries. Increased attention has been shifted to this species due to its wide distribution, high abundance, and modest susceptibility to malaria parasites. The rapid spread of insecticide resistance becomes the major obstacle for insecticide-based strategy of vector control, which has placed current national efforts in malaria elimination at risk. Since we already have colonized the insecticide-susceptible in *An. sinensis*, the establishing of insecticide-resistant population is essential for the genetic approaches to further understand the molecular mechanism of insecticide resistance and to enhance resistance surveillance in this species. In 2013, the natural *An. sinensis* mosquitoes (F0) were collected from regions, where pyrethriod resistance had been demonstrated in central China, and identified by the morphological characters on both adults and eggs to avoid the species contamination. The adults (F1) 3 days post emergence were treated by standard WHO tube bioassay with 0.05% deltamethrin, and the survivors 24 h post exposure were left for blood-feeding, followed by the forced mating method to overcome the low-mating rate. Eggs from each generation were reared through to adults and selected for resistance to deltamethrin in the same way. After the continuous pyrethriod selection, the survival rate in F3 generation significantly increased to 99.16% and 99.73% in male and female populations, respectively. Two types of kdr mutations (L1014F: TTG ◊ TTT; L1014C: TTG to TGT) at codon position 1014 of the para-type sodium channel gene were detected during the selection process, and the kdr mutation frequency reached to 100% in F2 and F3 generations, with a predominant kdr mutation L1014F. As a conclusion, we have selected and colonized the deltamethrin-resistant population from field *An. sinensis*, which would provide the fundamental material for genetic studies in this important malaria vector.

129

UNDERSTANDING THE VARIATION OF INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE* S.S FROM CÔTE D'IVOIRE: TOWARD ALTERNATIVE OPERATIONAL STRATEGIES FOR MALARIA ELIMINATION

Ako Victorien C. Edi[1], Benjamin Koudou[1], Weetman David[2], Hilary Ranson[2]

[1]Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Côte D'Ivoire, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Malaria control is heavily dependent on the use of insecticides that target adult mosquito vectors via insecticide treated nets (ITNs) or indoor residual spraying (IRS). In Côte d'Ivoire, where IRS is not implemented, ITNs and more recently LLINs remain the main control measure. We used different approaches based on literature review, field collections, Geographical Information System (GIS) and IR mapper tool to investigate the report the spatial and temporal variation of resistance to WHO-approved insecticide for LLINs and IRS. Overall, mosquitoes collection covered the period from 1993 to 2013, with 60 % of records from 1993-2003 and 40 % between 2004 and 2013. Data were stratified according to the four ecological zones of the country with pyrethroids (OP) and organochlorines (OC) largely tested compared to carbamates and organophosphates ($X2 = 10.8$, $p<0.05$). Organophosphate (52.2%) and carbamates (47.8%) were tested in 23 sites mainly, with no fenitrothion and pirimiphos methyl resistance detected in ecological zone 2, but multiple insecticide resistance detected in only four sites mainly located in zone 1. Within OP and OC, permethrin, deltamethrin and DDT were largely tested between both decades. Temporal trend based on three clusters gathering three yearly mean mortality data per insecticide (exception for DDT in cluster 1, no available data for 1993) showed non-significant increase of susceptibility to deltamethrin in cluster 2 ($X2= 3.879$, $p= 0.144$). We then checked the trend between clusters for each insecticide across zones. Overall, no significant difference was observed in ecological zone 1 between cluster 1 and 2 these insecticides ($P> 0.05$). Mortality to DDT has significant increase in in zone 3 ($X2= 49.94$, $p= 0$), while non-significant variation was detected in 4 ($X2= 1.07$ $p= 0.300$). Data of this study provide valuable information to National malaria control programme for vector control intervention. Deltamethrin could be used for vector control in several areas of ecological zones 3 and 4. In ecological zone 2 where no resistance to fenitrothion and pirimiphos methyl have been detected for most investigated sites, OP could be a potential alternative for IRS. Finally, in ecological zone 1, where multiple insecticide resistance was detected in four sites, new strategies and tools for vector control are needed for vectors control.

130

SUSCEPTIBILITY TO PYRETHROIDS OF *CULEX* SPP AND *ANOPHELES* SPP FROM TWO DIFFERENT TYPES OF ENVIRONMENTS IN A CONTEXT OF MASSIVE USE OF INSECTICIDES IN AGRICULTURE FROM TIASSALE, SOUTHERN COTE D'IVOIRE

Charlene A. Yoboue[1], Chouaibou S. Mouhamadou[1], Benjamin G. Koudou[1], Hilary Ranson[2]

[1]Centre Suisse de Recherches Scientifiques, Abidjan, Côte D'Ivoire, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

The use of insecticides in agriculture and public health has led the emergence of resistance in mosquitoes. Previous studies in the locality of Tiassalekro have shown that the malaria vectors, *Anopheles*, from rice field are resistant to various insecticides. Given that *Culex* mosquitoes also breed in the same rice field and subject to the same selection pressure, it would be interesting to look at their resistance profile, and compare this to *Anopheles*. The current study was undertaken to determine the resistance status of larvae and adult of both *Culex* and *Anopheles* from rice field. The investigation was extended to neighboring houses in order to find out whether the resistance level was the same in the two environments. *Culex* and *Anopheles* larvae were collected in rice fields and reared until adult stage. Blood fed *Culex* and *Anopheles* were collected in sleeping rooms, reared until oviposition. Larvae from eggs were reared to adult stage. Late third instar larvae were exposed to a range of deltamethrin concentrations to determine the LC_{50}. Beside this, *Culex* and *Anopheles* adult females 2-5 days old were exposed to 0.05% deltamethrin treated papers. Physico-chemical parameters of the breeding site in the two environments were determined. *Culex* larvae from houses (LC_{50} = 0,014ppm) were more resistant than *Culex* from rice field (LC50 = 0,0001 ppm) contrary for *Anopheles* larvae from rice field (LC_{50} = 4,35ppm) which appeared more resistant than those from houses (LC50= 2,32 ppm). Mortality was not significantly different between adult *Culex* from the two environments (47%). By cons, *Anopheles* from houses (9,78%) were more resistant than those from rice field (37,11%). Furthermore, according to WHO criteria, *Culex* and *Anopheles* from rice field were both resistant. The same trends were seen with *Culex* and *Anopheles* from houses. Parameters such as conductivity, salinity, redox and pH are significantly different in the two environments contrary for dissolve oxygen and temperature ($p>0.05$). In conclusion, adult mosquitoes are resistant to deltamethrin according WHO interpretation. However, the metabolism of resistance in adult and larvae stages are likely not similar. Molecular analysis wil determine if existing any genetic similarity between *Culex* and *Anopheles* collected in the houses and those collected in the rice fields.

131

TARGET-SITE RESISTANCE MECHANISMS *KDR* AND *ACE-1* PRESENT UNIQUE CHALLENGES TO REGIONAL MALARIA ELIMINATION IN THE AMERICAS

Audrey Lenhart[1], Kelly Liebman[2], Jesus Pinto[3], Lucrecia Vizcaino[1], Carlos Lol[4], Norma Padilla[4], William Brogdon[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention/ASM, Atlanta, GA, United States, [3]Instituto Nacional de Salud, Lima, Peru, [4]Universidad del Valle, Guatemala City, Guatemala

In the region of the Americas, malaria transmission intensity tends to be low and/or focal, and as such, the Americas are seen as a region where malaria elimination may potentially be achieved. However, a variety of anopheline vector species displaying a wide range of breeding, bloodfeeding, and resting behaviors present a challenge for vector control strategies in the Americas. Strategies that successfully control malaria vectors elsewhere are not as well-characterized in the Americas, and little research has been conducted on how insecticide resistance might further compromise efforts for malaria control, and ultimately, elimination. For example, *Anopheles albimanus* are highly resistant to multiple classes of insecticides in coastal Peru, including an area where *Plasmodium falciparum* was recently re-introduced. Our data suggest that voltage-gated sodium channel target site resistance (*kdr*) has been present in *albimanus* throughout Mesoamerica for at least 20 years. To date, the *kdr* polymorphisms we have detected in *An. albimanus* throughout the region are L1014C (associated with permethrin, deltamethrin and beta-cypermethrin resistance in other anophelines) and L1014F (associated with resistance to DDT and a broad range of pyrethroids in other anophelines). We have also identified a novel mutation on the *ace-1* gene and our data suggest that *ace-1* gene duplication and balancing selection are allowing resistant phenotypes to persist at high levels with minimal fitness costs. Even in the absence of routine insecticide application for vector control, resistance selection pressure throughout coastal Mesoamerica and South America is likely maintained through the use of agricultural pesticides, as *An. albimanus* habitats are often associated with lowland crop cultivation. The high frequency of alleles associated with resistance to pyrethroids, DDT, carbamates, and organophosphates in these populations is worrisome and could pose significant vector control challenges as interventions are scaled up as part of regional malaria elimination strategies.

132

WIDESPREAD EMERGENCE OF PYRETHROID RESISTANCE AND PRESENCE OF KNOCK DOWN RESISTANCE (KDR) MUTATIONS IN INDIAN *AEDES AEGYPTI* POPULATIONS

Raja B. Singh[1], Tridebis Adak[1], Neera Kapoor[2], Om P. Singh[1]

[1]National Institute of Malaria Research, New Delhi, India, [2]Indira Gandhi National Open University, New Delhi, India

Aedes aegypti, primary vector of yellow fever in Americas, is the primary vector for dengue and chikungunya in India. Resistance to DDT, temephos, permethrin and deltamethrin has been reported in this species in America, Brazil, China, Thailand, Indonesia, Vietnam and many other tropical and subtropical countries; however there is scanty information about insecticide resistance of this species in Indian populations. We collected immature of *Ae. aegypti* from different geographical regions of India; Haryana, Bangalore, Chennai, West Bengal, Bhopal and Khandwa. Larvae/pupae were allowed to emerge into adults. WHO standard insecticide susceptibility tests for DDT (4%), permethrin (0.75%) and deltamethrin (0.05%) were carried out on 2-3 days old adult females. We found varying susceptibility for different insecticides in different populations. Very high level of resistance against DDT was observed in Kolkata population (12.07% mortality) moderate resistance in Bangalore, Chennai and Haryana (55.13% - 66.16% mortality) and incipient resistance in Bhopal and Khandwa populations (91.11% mortality). Deltamethrin resistance was found to be moderate level in Bangalore, Chennai, (48.48% - 80.83% mortality) and low level in Bhopal, Kolkata and Haryana (82.48% - 83.58% mortality). Permethrin was still effective in most of the population showing moderate level of resistance Kolkata population (74.29 % mortality) and low level in Bangalore, Chennai, Haryana and Bhopal populations (92.31% - 98.37% mortality). Two populations (Haryana and Kolkata) tested for Malathion were found susceptible (100% mortality). Here we also studied a knock down resistance (kdr) mutation reported worldwide F1534C and found it few populations. This mutation was absent in Bangalore, Chennai, Khandwa and Bhopal populations. In Haryana population the frequency for mutant allele is very low 0.095 but the high degree of resistance in Kolkata population can be correlated to the high frequency of mutant allele 0.48. Resistance against pyrethroids is alarming and has negative effect on the success of pyrethroid based personal protection methods. Understanding of resistance mechanism is helpful for effective vector control strategies.

133

INSECTICIDE SUSCEPTIBILITY OF *ANOPHELES GAMBIAE* S.S MOSQUITOES IN IBADAN, SOUTHWEST NIGERIA

Patricia N. Okoye[1], Olusegun G. Ademowo[1], Helen Irving[2], Louise A. Kelly-Hope[3], Charles S. Wondji[2]

[1]Institute for Advanced Medical Research and Training, Ibadan, Nigeria, [2]Vector Group, Liverpool, United Kingdom, [3]Centre for Neglected Tropical Diseases, Liverpool, United Kingdom

The emergence of insecticide resistance in *Anopheles* mosquitoes has great implications for malaria control in Nigeria. This study aimed to determine the dynamics of insecticide susceptibility levels and frequency of knockdown resistance (kdr) mutations (L1014F) in wild *An. gambiae* s.s. (Diptera: Culicidae) from Ojoo and Bodija areas of Ibadan, South-west, Nigeria. Insecticide susceptibility to pyrethroids, organophosphates carbamates and organochlorines was assessed using WHO bioassays. A subset of the mosquitoes exposed to pyrethroids and DDT was used for species and molecular form identification and their kdr genotyping was determined using the Taqman assay. The mosquitoes were resistant to pyrethroids and DDT but completely susceptible to organophosphates and carbamates. All samples were identified as *An. gambiae* s.s. Bodja sample comprised of the S form (91.4%) and the M form (8.1%) while one M/S hybrid was recorded. All the mosquitoes screened in Ojoo belonged to the S form. The 1014F kdr mutation was detected at a frequency of 24.52% and 5.8% in Bodija and Ojoo respectively. No correlation was observed between kdr genotypes and resistance phenotypes. The results indicate that metabolic resistance probably plays an important role in the resistance observed and calls for a need to implement insecticide resistance management strategies.

134

EFFICACY OF OLYSET DUO® (A PYRIPROXYFEN AND PERMETHRIN BI-TREATED NET) AGAINST PYRETHROID RESISTANT *ANOPHELES GAMBIAE* IN BENIN, WEST AFRICA

Corine A. Ngufor[1], Raphael N'Guessan[1], Josias Fagbohoun[2], Abibatou Odjo[2], David Malone[3], Martin Akogbeto[2], Mark Rowland[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Centre de Recherche Entomologique de Cotonou, Cotonou, Benin, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Pyrethroid resistance threatens to undermine the efficacy of longlasting insecticidal nets (LNs) in Africa and without prompt action, the fragile gains in malaria control could be reversed. Alternative compounds which can replace or complement pyrethroids on LNs are urgently needed. Pyriproxyfen (PPF), an insect growth regulator, reduces the fecundity and fertility of adult female mosquitoes. Mixing pyrethroids and PPF on LNs could provide personal protection through the pyrethroid and reduce vector abundance through the sterilising effects of PPF. Experimental hut studies were performed in Benin, West Africa to assess the efficacy of Olyset Duo, a new LN incorporating permethrin (pyrethroid) and pyriproxyfen against pyrethroid resistant *Anopheles gambiae* which are difficult to control with current LNs. Comparison was made with LNs treated with permethrin alone (Olyset Net) and pyriproxyfen alone (PPF LN). Laboratory bioassays were performed with resistant and susceptible strains of An gambiae to substantiate the hut studies. Mortality of wild pyrethroid resistant An gambiae in the experimental huts was significantly higher with Olyset Duo (40-50%) than with Olyset Net (20-30%, $P<0.05$). Olyset Duo also provided significantly higher levels of personal protection than Olyset Net. The reproductive rate of bloodfed pyrethroid resistant mosquitoes surviving in huts with the pyriproxyfen treated LNs was significantly reduced relative to the control (71-100% reduction with Olyset Duo and 91-100% with PPF LN). Cone bioassay and tunnel test results were consistent with the experimental hut studies and showed complete sterilisation and life shortenning effects in mosquitoes surviving exposure to Olyset Duo and PPF LN. The mixture net (Olyset Duo) demonstrated superior performance to the pyrethroid net (Olyset Net) in terms of mortality and personal protection; and also sterilised and reduced the lifespan of surviving mosquitoes. Combining PPF with pyrethroids on bednets shows potential for improved malaria vector control and management of pyrethroid resistance.

135

HOW LONG DO BED NETS LAST? PHYSICAL DURABILITY AND INSECTICIDE ACTIVITY OF LONG-LASTING INSECTICIDAL NETS AFTER THREE YEARS IN USE, CRUZEIRO DO SUL, BRAZIL

Crissiane Costa Reis[1], Marcia Moraes dos Santos[1], Ediane Marli dos Reis[1], Ana Carolina Santelli[2], Camila Damasceno[2], Simone Daniel[3], Oscar Mesones Lapouble[4], Alexandre Macedo de Oliveira[5], Audrey Lenhart[5], Rita Lucrecia Cabarrus[5], **Izis M. Sucupira**[1], Marinete Marins Póvoa[1]

[1]Evandro Chagas Institute, Ananindeua, Brazil, [2]Brazilian Ministry Health, Brasilia, Brazil, [3]Acre Health Secretary, Cruzeiro do Sul, Brazil, [4]OPAS, Brasilia, Brazil, [5]Centers for Disease Control and Prevention, Atlanta, GA, United States

As regional malaria control efforts in the Americas begin to consider the possibility of elimination, the role of vector control tools such as long-lasting insecticidal nets (LLINs) must be well-defined. With the number of malaria cases reported in the Americas yearly, LLINs have become

an integral part of malaria control in the Brazilian Amazon region. The monitoring of LLIN durability is crucial to guide vector control programs and refine distribution and replacement strategies. In 2007, public health authorities in Brazil distributed 7,000 PermaNet 2.0 LLINs in Cruzeiro do Sul, Acre State covering all sleeping spaces in selected areas of the municipality. We evaluated the physical integrity and insecticide content of LLINs distributed in three high-transmission areas of Cruzeiro do Sul municipality after nets have been in use for 3 years. Physical integrity was evaluated by counting and measuring the holes on each of the five panels of the net (4 lateral sides and roof). Cone bioassays were used to assess the bioefficacy of the LLINs and a standard colorimetric assay was used to estimate the amount of deltamethrin available on the surface. Of the 27 evaluated LLINs, the lateral panels showed the greatest amount of physical damage, although most holes (61.3%) were <1.5 cm in diameter. Bioassay results indicated that only two LLINs achieved mosquito mortality >80%. The colorimetric test showed that 59.3% (16/27) of the nets had insecticide on the surface ≥0.36 µg/net, which is considered the minimal efficacy threshold. In summary, most LLINs contained holes. However, most were small, so the LLINs likely still provided some degree of physical protection against host-seeking mosquitoes. More importantly, bioefficacy was severely impaired. The colorimetric data also showed depletion of insecticide levels on the surface of the nets. These data suggested that the lifespan of LLINs in this part of Brazil should be further evaluated at time periods <3 years to help refine the planning of LLIN distribution and replacement strategies.

136

ACCURACY OF GOOGLE EARTH FOR HOUSEHOLD ENUMERATION AND PLANNING OF AN INDOOR RESIDUAL SPRAYING CAMPAIGN FOR MALARIA IN CHIBOMBO DISTRICT, ZAMBIA

Jessie Pinchoff[1], Aniset Kamanga[2], Brian Chirwa[3], Chadwick Sikaala[4], Benjamin Winters[2], Anna M. Winters[2], Daniel J. Bridges[2]

[1]John Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Akros, Lusaka, Zambia, [3]Zambia Integrated Systems Strengthening Program, Lusaka, Zambia, [4]Zambia National Malaria Control Center, Lusaka, Zambia

Satellite imagery is increasingly used for public health research and operations. Indoor residual spraying (IRS) for malaria requires extensive planning including enumeration of structures and procurement of insecticides. Using free satellite imagery from Google Earth may be more efficient and cost effective than ground based processes for planning and monitoring IRS campaigns. In 2014, a 5 x15 km area was selected and all structures were enumerated using Google Earth imagery; location, number of rooms in the house, and wall material based on the roof (tin, thatch or asbestos) was recorded. These were compared to IRS data from 2011 captured by the mSPRAY tool which collects GPS coordinates of households sprayed, the number of rooms in the house and the wall surface type. One hundred structures from the mSPRAY data and 25 visited by field staff were analyzed to compare Google Earth enumeration to ground processes. Google Earth was able to identify household locations within 10-meter accuracy. The number of rooms determined based on Google Earth showed strong agreement to data captured at the household with a mean difference of 0.26 (95% confidence intervals =-0.01, 0.52, p=0.06, 100% agreement to ground visits). Among thatch roof structures, agreement was 100%; tin or asbestos roof structures, however, frequently have unfinished walls meaning they are porous instead of non-porous. This may vary regionally; ground visits will help determine the proportion with finished or unfinished walls. Previously, ground enumerations required 25-30 trained technicians to visit each household in a designated area and conduct an extensive questionnaire for 30 days resulting in an estimated 7,200 structures enumerated (~ 900 person days). In comparison, a single trained GIS technician was able to enumerate the same structures in only 6 days (6 person days) using Google Earth. Google Earth and satellite-based enumerations may be useful and more cost effective in both planning for and conducting an IRS campaign to ensure high IRS coverage.

137

CROSS RESISTANCE TO ALTERNATIVE MOLECULES AFTER LONG-TERM USE OF PERMETHRIN AND TEMEPHOS IN *AEDES AEGYPTI* FROM YUCATAN, MEXICO

Karla L. Saavedra-Rodriguez[1], Julian Garcia-Rejon[2], Maria A. Lorono-Pino[2], Guadalupe Reyes-Solis[2], Adriana E. Flores[3], Gustavo Ponce[3], Saul Lozano-Fuentes[1], Patricia Penilla[1], Barry Beaty[1], Lars Eisen[1], William C. Black, IV[1]

[1]Colorado State University, Fort Collins, CO, United States, [2]Universidad Autonoma de Yucatan, Merida, Mexico, [3]Universidad Autonoma de Nuevo Leon, Monterrey, Mexico

Aedes aegypti chemical control campaigns have applied exclusively two insecticides, permethrin and temefos, during the last 12 years in Mexico. Recent reports of insecticide resistance threaten the effectiveness of such mosquito control strategy. An updated list of insecticides for vector control re-introduced the use of organophosphates, carbamates and other pyrethroids type I and II. Therefore, it is very important to track the susceptibility status to these molecules, as well as cross resistance patterns. We performed bottle bioassays using seven adulticides, including permethrin, bifenthrin, transfluthrin and deltamethrin; and the organophosphates chlorpyrifos and malathion, and one carbamate, bendiocarb. We also ran WHO larval assays using temefos. We calculated the lethal concentrations that killed 50% (LC_{50}) of two Yucatan mosquito collections - Hunucma and Vergel – that differ in permethrin resistance profiles. LC_{50} for each insecticide were used to calculate the resistant ratios (RR) relative to the New Orleans susceptible strain. We found that the Vergel collection is highly resistant to permethrin (RR = 98 fold) and this phenotype also confers resistance to other pyrethroids type I and II (RR= 92-117 fold); however, it remained susceptible to organophosphates and carbamate adulticides (RR = 2 - 7 fold). Hunucma had moderate levels of resistance to pyrethroids (RR= 3 -17 fold) and was also susceptible to organophosphates and carbamates (RR = 3 -5 fold). Vergel and Hunucma were resistant to temefos by 21 and 17 fold relative to New Orleans, respectively. Temefos resistance phenotype in larvae seems to be unrelated with adult resistance against molecules that share the same mode of action, organophosphates and carbamates. These results indicate that a rotation scheme for insecticides could be suitable in these locations.

138

USE OF A SEMI-FIELD SYSTEM TO EVALUATE THE EFFICACY OF TOPICAL REPELLENTS UNDER USER CONDITIONS PROVIDES A DISEASE EXPOSURE FREE TECHNIQUE COMPARABLE WITH FIELD DATA

Peter Sangoro

Ifakara Health Institute, Bagamoyo, United Republic of Tanzania

Before topical repellents can be employed as interventions against arthropod bites, their efficacy must be established. Currently, laboratory or field tests, using human volunteers, are the main methods used for assessing the efficacy of topical repellents. However, laboratory tests are not representative of real life conditions under which repellents are used and field testing potentially exposes human volunteers to potential disease. There is, therefore, a need to develop methods to test efficacy of repellents under real life conditions while minimizing volunteer exposure to disease. A lotion-based, 15% N, N-Diethyl-3-methylbenzamide (DEET) repellent and 15% DEET in ethanol were compared to a placebo lotion in a 200 sq m (10 m × 20 m) semi-field system (SFS) against laboratory-reared *Anopheles arabiensis* mosquitoes, and in full field settings against wild malaria vectors and nuisance-biting mosquitoes. The average percentage protection against biting mosquitoes over four hours under SFS and field setting was determined. A Poisson regression model was

then used to determine relative risk of being bitten when wearing either of these repellents compared to the placebo. Average percentage protection of the lotion-based 15% DEET repellent after four hours of mosquito collection was 84.02% (95% CI 78.58-89.46) in the semi-field experiments and 87.77% (95% CI 83.02-92.05) in the field experiments. Average percentage protection of 15% DEET in ethanol after four hours was 67.08% (CI 51.11-83.05) in the semi-field system and 86.43% (81.19-91.67) in the field. In conclusion, semi-field evaluation results were comparable to full-field evaluations, indicating that such systems could be satisfactorily used in measuring efficacy of topically applied mosquito repellents, thereby avoiding risks of exposure to mosquito-borne pathogens, associated with field testing.

139

ROLE OF *ANOPHELES BALABACENSIS* IN TRANSMISSION OF SIMIAN MALARIA IN KUDAT DIVISION, SABAH, MALAYSIA

Meng Li Wong[1], Indra Vythilingam[1], Cherng Shii Leong[1], Loke Tim Khaw[1], Tock Hing Chua[2], Benny Obrain[2], Heather Ferguson[3], Chris Drakely[4]

[1]University of Malaya, Kuala Lumpur, Malaysia, [2]University of Malaysia Sabah, Kota Kinabalu, Malaysia, [3]University of Glasgow, United Kingdom, United Kingdom, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom

Simian malaria, *Plasmodium knowlesi* is currently a cause of human malaria leading to fatal infections. High proportion of knowlesi malaria cases were reported in Kudat Division. A study was conducted in Kudat division, Sabah, Malaysia from June 2013 to February 2014 to determine the vectors. A total of 1192 anopheline mosquitoes belonging to 13 species were collected from two localities in Pulau Banggi and one in Kudat using human landing catch (HLC). The mosquitoes were dissected, and the mid gut and salivary glands were examined for oocysts and sporozoites. From the study, *Anopheles balabacensis* was the predominant anopheline, consisting 90.74% of the total collection. They bite early in the night which accounts in 61.17% came to bite before 2200 hours. There are 27 *An. balabacensis* positive for malaria parasites, of which 6 was positive for sporozoites, 10 for oocysts and 11 had both. From the ongoing molecular work, mono-infection of *Plasmodium inui* was detected in both midgut and salivary glands while mixed infection of *P. inui+P. knowlesi* and *P.inui+P. cynomolgi* were detected in salivary glands only.

140

ECOLOGICAL NICHE MODEL OF *ANOPHELES DARLINGI* AND *AN. ALBIMANUS* (DIPTERA:CULICIDAE) IN COLOMBIA

Mariano A. Altamiranda, Nelson J. Naranjo, Paula Aguirre, Camilo Orozco, Julian R. Zabala, Margarita M. Correa

Universidad de Antioquia-Colombia, Medellin, Colombia

The potential distribution of two primary malaria vectors in Colombia, *Anopheles albimanus* y *Anopheles darlingi* and the environmental factors that influence habitat occupancy were evaluated. Presence records were obtained from published literature from 1997-2011 and field collections performed between 2012 and 2013. Two ecological niche models (ENM) were developed per species using environmental and topographic layers: elevation, land cover and 15 of 19 bioclimatic layers from WorldClim. The models were evaluated using the area under the curve (AUC). The first model included 15 bioclimatic layers and altitude, while, a land cover layer was incorporated in the second model. AUC for the two *An. albimanus* models was 0.94, with altitude being the most influential environmental variable. These results were consistent with the predicted potential distribution for *An. albimanus*, mainly spanning low land areas of the Atlantic and Pacific Coasts. AUC for *An. darlingi* was 0.97, and altitude was also the variable contributing the most to both models. Land cover was the second variable in contribution to the second *An. darlingi* model (24.4%), which is consistent with previous reports of a strong association of this species with forested covers. The potential distribution range was higher for *An. darlingi* than for *An. albimanus*. In addition, *An. darlingi* showed a highest probability of occurrence in the endemic area Urabá-Bajo Cauca-Alto Sinú. This information contributes to future studies on the eco-epidemiology of these species

141

PHENOTYPIC STABILITY IN EXTRINSIC INCUBATION OF WEST NILE VIRUS STRAINS IN *CULEX TARSALIS*

Mary E. Danforth, William K. Reisen, Philip H. Kass, Christopher M. Barker

University of California Davis, Davis, CA, United States

Temperature is a critical factor that regulates the efficiency with which West Nile virus (WNV) is transmitted by its mosquito vectors. The interaction between rates of WNV extrinsic incubation, vector biting and vector survival are key determinants of the virus's range as well as the potential for its amplification to outbreak levels. Previous studies have suggested that extrinsic incubation rates of WNV are higher among recent WNV strains, while others have questioned this assertion. Here, we compare the founding North American NY99 strain with two other representative isolates from WNV's initial incursion into California's southeastern deserts and a recent strain from a hyperendemic area of California's Central Valley. We expand on a previous degree-day model to compare the probabilistic range of extrinsic incubation periods among these strains, and we had expected that our more recent strains would exhibit accelerated extrinsic incubation compared to NY99, in agreement with the previously described emergence of a selectively advantaged strain in North America. However, at the two temperatures studied (22 and 30°C), we found no difference in the extrinsic incubation period in *Culex tarsalis* between NY99 and the recent California isolates. We present our comparative findings among strains and to earlier literature, along with a model-based assessment of the epidemiological implications for the efficiency of WNV transmission.

142

BITING CYCLES OF YELLOW FEVER AND MALARIA VECTORS ON UDS NAVRONGO CAMPUS IN THE UPPER EAST REGION OF GHANA

Millicent Captain-Esoah

University for Development Studies, Navrongo, Ghana

Yellow fever and malaria are both infectious vector borne diseases with high mortality rates among humans and some animals. While yellow fever has a history of a 10 to 12 years cycle in Ghana with minor ones occurring at three-yearly intervals malaria is found at all times. Behavior such as host seeking, biting activities, resting and disease transmission potentials have considerable epidemiological importance for planning and implementing vector control programmes. However very little information is available on these parameters for both *Aedes* species and *Anopheles* species in the Navrongo area. We studied the comparative biting behavior of *Aedes* and *Anopheles* mosquitoes within the Navrongo campus of UDS. Female adult mosquitoes were collected both indoor and outdoor between the hours of 05.00 and 09.00 GMT, 15.00-17.00 GMT and 18.00-06.00 GMT using human landing collections. All mosquitoes were grouped and identified morphologically using appropriate taxonomic keys. 27 *An. gambiae* complex were identified to species and molecular forms of *An. gambiae* s.s. using PCR-RFLP. Also, the presence of the kdr gene mutation was estimated in the *Anopheles* population. A total of 1072 mosquitoes were collected. Out of this, 61.8 % were *Anopheles*, 22.7 % were *Aedes* and 15.5 % were *Culex*. *An. gambiae* complex was predominant of the *Anopheles* genera constituting 59.6 % whereas *Ae. aegypti* was predominant (85.6 %) of the *Aedes* genera. *Aedes* mosquitoes had a bimodal biting pattern peaking at 05.00 - 07.00 hours GMT in the morning and 17.00 - 19.00 hours GMT in the evening as compared to the *Anopheles* species which had a unimodal biting behaviour peaking at 24.00 - 02.00 hours GMT. All the 27 *An. gambiae* were *An. gambiae*

s. s. M forms The kdr gene mutation frequency in the population was RR 29.6 %, RS 51.9 % and SS 18.5 % (n=27). The information on the vector biology, genetic diversity and feeding behavior as well as resistance of vector mosquitoes are important in planning and implementing effective vector control programmes.

143

DEVELOPMENT AND EVALUATION OF ELECTRIC GRID TRAP FOR SAMPLING OF HOST-SEEKING MALARIA VECTORS

Deodatus F. Maliti

University of Glasgow, Glasgow, United Kingdom

Evidence of malaria vectors changing their behaviors in response to control measures has been documented. These changes include a shift in the biting time and place of biting behavior, where mosquitoes have been observed to bite in the earlier hours of the night before people are under bed nets, or have developed a preference towards outdoor biting to avoid insecticide treated surfaces. These changes could possibly render the current intervention tools such as Insecticide Treated Nets (ITNs) and Indoor Residual Spraying (IRS) inefficient. To be able to study these behavior changes, efficient and safe sampling tools are required to replace the current gold standard human landing catch technique (HLC) which exposes the human subject to mosquito infectious bites. In this work we develop and evaluate an Electric Grid Trap (EGT) to be used in studying the host seeking behaviors of indoor and outdoor biting mosquitoes. This trap was evaluated at the Lupiro village situated in the malaria endemic Kilombero Valley in South-Central Tanzania in comparison to a commercially available bug zapper (CEGT) and the Human Landing Catch technique (HLC) as the reference trap. The study was done in a Latin Square design conducted in experimental huts over a twenty one-night period. The EGT efficiency in collecting indoor and outdoor biting *Anopheles arabiensis* was 21.8% and 59.9% respectively relative to HLC, while for collecting *An. funestus*, EGT was able to perform at 70.3% and 90.5% for indoor and outdoor sampling respectively relative to HLC. The peak biting time estimated by EGT and HLC was the same for indoor sampling of *An. arabiensis* ($z=0.024$, $p=0.98$), but was different for outdoor sampling ($z=-0.35$, $P<0.018$). No difference in the peak biting times for *An. funestus* sampled the two traps indoors ($z=-0.75$, $p=0.45$) or outdoors ($z=-1.72$, $p<0.086$). With improvements, the current prototype of the EGT could be a possible replacement for the HLC and could be used to sample indoor and outdoor biting mosquitoes giving an estimate of their biting behaviors with comparable efficiency to the HLC technique.

144

SUSCEPTIBILITY OF WILD AND LABORATORY-REARED *ANOPHELES DARLINGI* TO INFECTIONS WITH *PLASMODIUM VIVAX* IN THE PERUVIAN AMAZON

Carlos G. Tong[1], Raul Chuquiyauri[2], Marta Moreno[3], Fanny Castro[4], Victor Neyra[2], Jorge L. Marapara[5], Margaret Kosek[6], Joseph M. Vinetz[3]

[1]Universidad Peruana Cayetano Heredia, Iquitos, Peru, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]University of California San Diego, San Diego, CA, United States, [4]Naval Medical Research Center Detachment Unit 6, Lima, Peru, [5]Universidad Nacional de la Amazonia Peruana, Iquitos, Peru, [6]Johns Hopkins School of Public Health, Baltimore, MD, United States

Malaria studies focus on quantify human infectious reservoir, to evaluate transmission reducing interventions or to test the gametocytocidal effects of drugs, are crucial for to the critical evaluation of disease control strategies. Mosquito-parasite relationships are still not well understood and it varies as a function of vector species and parasite strains. *Anopheles darlingi,* the main malaria vector in South America, could present different responses to *Plasmodium* infection from females reared under laboratory conditions or wild An. darlingi specimens. The aim of this study was to compare the susceptibility of laboratory-reared versus wild *An. darlingi* to *Plasmodium vivax* using membrane feeding assays (MFA). The study was carried out in Iquitos (Peru) in 2009-2010 and seven patients with positive thick smears for *P. vivax* malaria were enrolled in the study. Sexual and asexual parasites stages were recorded and subsequent mosquito feeding was performed using the MFA technique. To detect number of oocysts, mosquito midgut dissections were performed 8 days postinfection (pi) and counted by microscopy. To determine the proportion of infectious mosquitoes, salivary glands were dissected 14 days pi and performed an ELISA assay to detect VK210 and VK247 *P. vivax* strains. A total of 1333 An. darlingi females, 701 wild and 632 laboratory-reared, were used for this study. Our results showed that there was no statistically significant difference between oocysts means of wild and laboratory-reared An. darlingi ($p=0.105$). Likewise, no statistically difference was identified between sporozoites detection in the two groups ($p=0.137$, $p=0.170$, respectively). ELISA assays showed that VK210 was the most common strain, representing 59% and 63% in wild and laboratory-reared mosquitoes, respectively. In conclusion, the MFA might be a realistic ex-vivo surrogate technique for measuring first insights into malaria transmission, and provides the basis for human-mosquito interaction studies such as transmission-blocking vaccines studies.

145

SUSCEPTIBILITY TO *PLASMODIUM VIVAX* AND INSECTICIDE RESISTANCE OF *ANOPHELES CAMPESTRIS* AND *AN. SUBPICTUS* ALONG THAI-CAMBODIAN BORDER

Patchara Sriwichai

Mahidol University, Bangkok, Thailand

Changing malaria prevalence from *Plasmodium falciparum* to *P. vivax* along the Thai-Cambodian border has been observed. We have investigated the potential vectors that may contribute to this change in two selected endemic areas, Sa Kaeo and Chantaburi provinces. Two potential vectors, *An. campestris* and *An. subpictus*, were collected by cow bait or human landing catch during the wet and dry seasons of 2012-2013. In Sa Kaeo, 17.8% of 2,355 mosquitoes from 22 species collected were *An. campestris*. In Chantaburi 88% of 433 mosquitoes from 7 species collected were *An. subpictus*. Susceptibility to *P. vivax* for these mosquitoes was studied in laboratory using F1 to F15 progeny and artificial membrane feeding. Both species were found susceptible to *P. vivax*. The parasite developed normally to oocysts in the midguts and sporozoites in the salivary gland. In contrast, development of *P. falciparum* was not observed in either species when compared with An. dirus, the major malaria vector in Asia. Study of insecticide susceptibility indicated that An. campestris (F1 to F3) from Sa Kaeo had potential to resist 0.1% Bendiocarb, 1% Fenitrothion and 4%DDT. An. subpictus (F1-F3) had shown to resist 0.15% Cyfluthrin, 0.05% Lambda-cyhalothrin, 5% Malathion, and 0.05% Deltamethrin. The change in species composition and malaria vector biology on the Thai Cambodian border may contribute to the shift in the *P. falciparum* to *P. vivax* prevalence; therefore, the malaria control program including vector control will have to be planned accordingly.

146

SEASONALITY OF MULTIPLE BLOOD FEEDING BEHAVIOR IN ANOPHELINE MOSQUITOES AND IMPLICATIONS FOR MALARIA TRANSMISSION IN NCHELENGE DISTRICT, ZAMBIA

Smita Das[1], Mbanga Muleba[2], Samantha Eng[3], Maureen Kessler[1], Douglas E. Norris[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Tropical Disease Research Centre, Ndola, Zambia, [3]University of Maryland Baltimore County, Baltimore, MD, United States

As part of the Southern Africa International Centers for Excellence in Malaria Research (ICEMR) project, mosquito collections were performed during August-September 2012 and March-May 2013 in Nchelenge District, Luapula Province, Zambia. Located along the environs of Lake Mweru and Kenani Stream, Nchelenge experiences hyperendemic

transmission and has the highest malaria infection rate in children under the age of 5 years despite implementation of indoor residual spraying (IRS) and long-lasting insecticide-treated net (LLIN) distribution. Center for Disease Control light traps (CDC LTs) and pyrethroid spray catches (PSCs) were performed at three villages along Lake Mweru and two villages along Kenani Stream. The collections revealed that during the dry season, *Anopheles funestus* sensu stricto is the dominant vector near both the lake and stream. In contrast, during the wet season, *An. gambiae* sensu stricto is the dominant vector in the lakeside villages, whereas *An. funestus* s.s. is the primary vector with secondary contribution from *An. gambiae* s.s. in the streamside villages. Both vector species are highly anthropophilic and *An. funestus* has a higher *Plasmodium falciparum* sporozoite rate than *An. gambiae*. In the wet and dry seasons, it was found that the *P. falciparum* infection rate of the vector populations was higher near the streamside villages than those of the lakeside villages. The multiple blood feeding rate for *An. funestus* is higher during the dry season, whereas the multiple blood feeding rate for *An. gambiae* is higher in the following wet season. As a result, the entomological inoculation rates (EIRs) for each vector during the dry and wet seasons are underestimated. The results also suggest that there are spatial differences in vector composition and their multiple blood feeding rates, which contribute to differences in human malaria risk during the dry and wet seasons. Overall, the vector data in Nchelenge present unique opportunities to further our understanding of malaria transmission and implications for malaria control in high-risk areas.

147

INCONSISTENCY IN THE RELATIVE PERFORMANCE OF HUMAN LANDING CATCHES AND LIGHT TRAPS IN SAMPLING ANOPHELINE POPULATIONS ACROSS DIFFERENT AREAS OF AFRICA

Bernadette Huho

Ifakara Health Institute, Dar es salaam, United Republic of Tanzania

The need for surveillance of adult host seeking mosquitoes is of foremost importance in determining levels of disease transmission and for appropriate allocation of interventions. The gold standard for estimating mosquito - human contact rate has thus far been obtained based on Human Landing Catch (HLC), where human volunteers catch mosquitoes that land on their exposed body parts. This approach is necessitates exposure to potentially infectious mosquitoes, such a risk it is unethical calling the need for safer and accurate tools. Centers for disease control light traps (LT) have been used widely in malaria endemic setting as an alternative tool to HLC in estimating human biting rate (HBR). Here, multi sites paired mosquito collections of LT against HLC are evaluated for their consistency in the sampling indoor host seeking mosquitoes. Regression models were applied to determine the site specific as well as the overall LT sampling efficiency and their trend across increasing mosquito density for two major malaria vectors across Africa, *Anopheles gambiae* sensu lato and *Anopheles* funestus sensu lato. Generally, LT were able to collect more mosquitoes than HLC, though the ratio of LT:HLC varied between sites and mosquito density. Across sites LT had an overall sampling efficiency of =1.07 [0.76-1.51] in sampling *An. gambiae* s.l. and =1.78 [0.90-3.44] in sampling *An. funestus* s.l.. There was variation in sampling efficiency of LT across mosquito densities and only in a few locations did LT sample proportionally to HLC. More often LT either underestimated human exposure by under-sampling or over-sampling at high mosquito densities, in particular for An. funestus. Such inconsistency necessitates calibration of LT against HLC for each location and across seasons. Also advises against the use of a single calibration factor across all geographical locations since no evidence of a geographical pattern in the sampling efficiency of LT against HLC was demonstrated.

148

IDENTIFICATION OF THE FIRST OVIPOSITION ATTRACTANT FOR GRAVID MALARIA MOSQUITOES USING NEWLY DEVELOPED METHODS FOR MEASURING OLFACTORY ATTRACTION IN *ANOPHELES GAMBIAE* S.S..

Michael N. Okal[1], Jenny Lindh[2], Manuela Herrera-Varela[1], Paul Ouma[1], Baldwyn Torto[3], Steve Torr[4], Steve W. Lindsay[5], Ulrike Fillinger[1]

[1]International Center for Insect Physiology and Ecology, Mbita, Kenya, [2]Royal Institute of Technology (KTH), Stockholm, Sweden, [3]International Center for Insect Physiology and Ecology, Nairobi, Kenya, [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [5]Durham University, Durham, United Kingdom

Little is known about how *Anopheles gambiae* s.l. find aquatic sites to lay eggs. Some evidence suggests that they use both vision and smell to locate and select breeding sites. Like many nocturnal insects with efficient mechanisms for processing chemical cues, olfaction is suspected to play the major role in this process and has been the subject of many investigations often with contradictory conclusions. Over 2000 gravid mosquitoes were evaluated individually. Every mosquito was given two cups with oviposition media in a cage. We measured the number of eggs laid by individual mosquitoes and determined whether they laid eggs in more than one cup. Thereafter we developed cage experiments that takes into account newly described behaviour. In addition, a semi-field test was developed using modified BG sentinel traps. This test measured olfactory attraction of gravid females to substrates over time and space. Together these two tests enabled the screening and description of an assortment of oviposition substrates. The number of eggs laid by individual mosquitoes varied widely. In addition individual mosquitoes skip-oviposited: laying their eggs in more than one cup on the same night. This is the first report of within-cage skip oviposition in the species and demonstration of how it could lead to an illusion of substrate preference with common egg count bioassays. A new two-tier two-choice cage test that adjusts for large variation and eliminates the risk of artefact preferences as a result of skip oviposition was developed and used to identify the first confirmed attractant (patent pending) for gravid *An. gambiae* s.s. Our study confirms that gravid mosquitoes use olfactory cues to select breeding sites. These findings pave the way for the development of new strategies for controlling malaria vectors; one that will also help target insecticide resistant and outdoor biting populations previously left out by indoor residual spraying and long lasting insecticidal nets.

149

ANOPHELES DARLINGI RESTING BEHAVIOR USING INTERCEPTION SCREENS IN THE PERUVIAN AMAZON

Marlon P. Saavedra[1], Marta Moreno[2], Carlos Tong[3], Joseph M. Vinetz[2], Joseph M. Vinetz[4], Jan E. Conn[5], Jan E. Conn[6]

[1]Asociación Benéfica Prisma, Iquitos, Peru, [2]Division of Infectious Diseases, Department of Medicine, University of California San Diego, San Diego, CA, United States, [3]Laboratorio ICEMR-Amazonia, Laboratorios de Investigacion y Desarrollo, Facultad de Ciencias y Filosofia, Universidad Peruana Cayetano Heredia, Lima, Peru, [4]Instituto de Medicina Tropical "Alexander von Humboldt" and Laboratory de Investigacion y Desarrollo, Departamento de Ciencias Celulares y Moleculares, Universidad Peruana Cayetano Heredia, Lima, Peru, [5]Wadsworth Center, New York State Department of Health, Albany, NY, United States, [6]Department of Biomedical Sciences, School of Public Health, State University of New York, Albany, NY, United States

Anopheles darlingi, the main malaria vector in the Neotropics, is highly anthropophilic. However, extrinsic drivers of host seeking and oviposition are poorly understood. Analysis of anopheline flight patterns within villages could enhance malaria risk assessment and control. Data from preliminary studies using a single barrier screen were problematic because several factors may have affected mosquito behavior. The aim was to

intercept vectors seeking hosts, resting sites, and/or oviposition sites. The design was modified using 4 screens for 2 nights/month (6PM-6AM), January-March 2014, in two riverine localities in the Peruvian Amazon, Lupuna and Cahuide. Screens, 2m high x 15m long, were placed such that the distance from the house to the river/forest was 2-7 meters. Screens were checked hourly and resting anophelines recorded for height (>or <1m above ground) and side of screen (next to forest/house/river). For every collection, screen orientation and wind speed and direction were noted. A subsample of mosquitoes was dissected for parity status and PCRs were performed to determine blood meal source. Our results showed that *An. darlingi* was the most abundant species in both localities, with peak densities between 9PM and 2AM on the village side, suggesting a need for additional investigation on behavior during this time. 75% and 78% of the females in Lupuna and Cahuide, respectively, were captured <1m from the ground, and, by visual inspection, 2% of the mosquitoes had blood fed. These data provide important information about mosquito flying behavior and pose new questions about anophelines seeking shelter, mating and/or oviposition sites.

150

INVESTIGATING THE ROLE OF DIVERSE ANOPHELINES IN TRANSMITTING ARTEMISININ-RESISTANT *PLASMODIUM FALCIPARUM* IN CAMBODIA - A CHALLENGE FOR MALARIA CONTROL AND ELIMINATION

Brandyce St. Laurent[1], Becky A. Miller[1], Nicole Palmer[1], Men Sary[2], Seila Suon[2], Siv Sovannaroth[2], Robert W. Gwadz[1], Jennifer M. Anderson[1], Rick M. Fairhurst[1]

[1]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia

Cambodia is a hotspot for the rapid evolution of artemisinin (ART)-resistant *Plasmodium falciparum* parasites, which endanger the effectiveness of all ART-based combination therapies (ACTs), and harbors an exceptional level of *P. falciparum* and *Anopheles* diversity. Subpopulations of ART-resistant and -sensitive parasites have recently been identified, but the role of diverse anophelines in transmitting them has not yet been investigated. We approached this question by screening field-collected anophelines from three Cambodian provinces for *P. falciparum* infection and infecting multiple Southeast Asian and other regional anophelines with ART-resistant and -sensitive parasite strains from Cambodia. More than 10,000 *Anopheles* mosquitoes from three provinces (Pursat, Western Cambodia, where ART resistance is established; Preah Vihear, Northern Cambodia, where ART resistance is emerging; and Ratanakiri, Eastern Cambodia, where ART resistance is rare) were screened for *Plasmodium* infection. Contrary to the current dogma that only one or two vectors efficiently transmit malaria in this region, we found 14 distinct anophelines infected with *Plasmodium* (at least 4 infected with *P. falciparum* and at least 10 infected with *P. vivax*) using nested PCR. Molecular analysis of rDNA ITS2 loci identified 27 distinct *Anopheles* species and revealed only a 50% concordance between morphological and molecular identification methods. *P. falciparum* infections of An. dirus A isolates from Western Cambodia and An. minimus, *An. gambiae* s.s., and An. stephensi lines were achieved in the laboratory. Rates of infection of these and diverse Cambodian anophelines by ART-resistant and -susceptible parasite isolates will be presented. The ability of multiple vectors to carry ART-resistant parasite subpopulations presents a startling challenge for the control and elimination of falciparum malaria in this region.

151

ANOPHELES FUNESTUS IN MUTASA DISTRICT, ZIMBABWE: THE ROLE OF VECTOR BIONOMICS AND POTENTIAL INSECTICIDE RESISTANCE IN MALARIA TRANSMISSION

Tyler C. Henning[1], Nzira Lukwa[2], Lovemore Gwanzura[3], Douglas E. Norris[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]National Institute of Health Research, Harare, Zimbabwe, [3]University of Zimbabwe, Harare, Zimbabwe

Mutasa District, Zimbabwe is an area marked by seasonal malaria transmission with severe outbreaks occurring during the wet season. A research arm of the International Centers of Excellence in Malaria Research (ICEMR) in Southern Africa began in 2012 to assess malaria burden in the region. This area is characterized as a region of resurgent malaria, having had increasing rates in recent years despite being previously considered as under control. In order to assess the reasons for this transmission dynamic, the ICEMR project seeks to analyze malaria epidemiology, parasite genomics, and entomology, which is of particular importance for this study. Samples were collected during the wet seasons of 2012-2014 using CDC light traps and pyrethroid spray catches, and morphological identification indicates that the predominant malaria vector is *Anopheles funestus* senso lato. Molecular identification of samples from the 2012-2013 collections confirms these results, with samples being *An. funestus* senso stricto or *Anopheles leesoni*, both members of the *An. funestus* species complex. Blood meal analysis indicates that these mosquitoes feed predominantly on human populations. Sporozoite infection rates for 2012-2013 were approximately 5%. Of additional interest is the high level of pyrethroid resistant *An. funestus* as the dominant malaria vector in the region. During collections in December 2013, 80% of mosquitoes were collected in houses that had been recently treated (within 2 months) with pyrethroids used for IRS control methods. Of these samples, 93% were morphologically observed to be blood fed or gravid, suggesting that these mosquitoes had been resting on treated surfaces for many hours. Although the findings are preliminary in nature, they suggest that potentially insecticide-resistant *An. funestus* is the dominant malaria vector in the region and may be contributing to the resurgence of malaria in spite of control efforts. Further collections are scheduled and will help to further elucidate the vector component of this study. With this knowledge it may be possible to determine areas of highest malaria risk and provide information on how to most effectively deploy limited resources to achieve control.

152

INVENTORY AND EVALUATION OF CULICIDAE NUISANCE IN URBAN POST-CONFLICTUAL AREA: CASE OF BOUAKÉ CITY (CÔTE D'IVOIRE)

Bedjou P. N'Dri, Chouaibou Mouhamadou, Benjamin G. Koudou

Centre Suisse de Recherche Scientifique en Côte d'Ivoire, Abidjan, Côte D'Ivoire

Culicidae are vectors of many diseases. They are sources of nuisance by their painful bites,spots and noises. In Côte d'Ivoire malaria remains a major public health problem. Contact between man and the vector is based on the epidemiology of malaria. It is directly modulated by human activities and indirectly through environmental modification. After the politico-military conflict of 2002 in Côte d'Ivoire, many factors may have influenced environment including overcrowding of some cities. Such modification of the environment can impact on the mosquito's composition and nuisance. The objective of this study was to inventory and evaluate the culicidae nuisance in previously overpopulated urban post-conflict area. The study was carried in two quarters of Bouaké city. Ahougnansou was overpopulated quarter before the crisis and less populated after the crisis and Sokoura was overpopulated before and after the crisis. The study was based on human landing catch inside and outside the housing, from 18 pm to 6 am. Mosquitoes were

morphologically identified by genus and species. However, the breeding sites have been characterized and the larvas were collected by "dipping" method and identified the adult stage. A total of 1030 mosquitoes were collected on human landing catch. Four genus of culicidae were identified: *Anopheles, Aedes, Culex* and *Mansonia*. The Culicidae fauna inventoried in these areas consists of 9 species (2 *Anopheles*, 4 *Culex*, 1 *Aedes* and 2 *Mansonia*). In Sokoura the culicidae nuisance was mainly due to *Culex* (98.2%). Mansoni, *Aedes* and *Anopheles* were virtually low. The breeding sites encountered in Sokoura are gutters, drainage channels contained solid wastes. However in Ahougnansou it were the irrigated rice and water drums. The culicidae fauna in Ahougnansou is dominated by *Culex* (48.5%) and *Anopheles* (48 %. The *Aedes* and *Mansonia* were poorly represented. The biting rate in Ahougnansou was 30.6 biting per human per night (b/h/n) and 53.1 b/h/n in Sokoura. Mosquito abundance and biting rate varied by site. The culicidae nuisance remains important. This was due to the environment modification, thus creating favorable conditions of mosquitoes breeding and their adaptation to their new environment. In Sokoura the high density of population involved the *Culex* breeding sites. However in Ahougnansou, the high rate of *Anopheles* is due to irrigated rice exposing the population to the malaria risk transmission.

153

SHIFTING PATTERNS OF *AEDES AEGYPTI* FINE SCALE SPATIAL CLUSTERING IN IQUITOS, PERU

Gonzalo M. Vazquez-Prokopec[1], Genevieve LaCon[1], Amy C. Morrison[2], Helvio Astete[3], Steven Stoddard[4], Valerie A. Paz-Soldan[5], John P. Elder[6], Eric S. Halsey[3], Thomas W. Scott[4], Uriel Kitron[1]

[1]Emory University, Atlanta, GA, United States, [2]University of California Davis, Davis, CA, United States, [3]U.S. Naval Medical Research Unit - 6, Iquitos, Peru, [4]University of California Davis, Davis, CA, United States, [5]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [6]San Diego State University, San Diego, CA, United States

Aedes aegypti abundance is spatially heterogeneous, with some areas and larval habitats producing more mosquitoes than others. There is a knowledge gap, however, regarding temporal persistence of *Ae. aegypti* abundance hotspots. We used a longitudinal entomologic dataset from the Amazonian city of Iquitos, Peru, to (1) quantify spatial clustering patterns of adult *Ae. aegypti* and pupae counts per house, (2) determine overlap between clusters, (3) quantify temporal stability of clusters over nine entomologic surveys spaced 4 months apart. Data from 13,662 household entomological surveys performed in Maynas and Tupac Amaru, two Iquitos neighborhoods differing in *Ae. aegypti* abundance and dengue virus transmission, were analyzed using global and local spatial statistics. Evidence of clustering of pupae presence and abundance was observed in 44% and 11% of surveys, respectively, for Maynas and in 55% and 33% of surveys in Tupac Amaru. The estimated overall mean ± SD clustering distance was 16.6 ± 5.0 m for pupae presence and 10.3 ± 7.8 m for pupae abundance. On average, 3.1% of Maynas and 1% of Tupac Amaru households were members of a cluster of high adult abundance. There was no consistent temporal pattern of adult clusters; i.e., the location of clusters in one survey differed from the location of clusters in future or prior surveys. The probability of finding adults clustering beyond the household was 42% (95% CI,57.8-25.8%). Our analyses indicate that *Ae. aegypti* distribution was highly focal and hotspots of high vector abundance were common on every survey date, but temporally unstable over the period of study. Our findings have implications for understanding *Ae. aegypti* distribution and the design of surveillance activities relying on household-level data. In settings like Iquitos, where there is a relatively low percentage of *Ae. aegypti* in permanent water-holding containers, identifying and targeting key premises will be significantly challenged by shifting hotspots of infestation.

154

EFFECT OF HOLDING CONDITIONS ON THE DETECTION OF CHIKUNGUNYA AND VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN MOSQUITO POOLS

Elizabeth S. Andrews, Michael J. Turell

United States Army Medical Research Institute for Infectious Diseases, Fort Detrick, MD, United States

Emerging and re-emerging arboviruses continue to be a threat to global public health. With the recent introduction of chikungunya virus (CHIKV) into the Caribbean and its potential spread across the Americas, there will be a need to increase surveillance of mosquito populations for viruses. Due to the tropical climate of many of the affected areas, it will be difficult to maintain a cold chain as the samples travel from collection sites to laboratories for testing. We determined how suboptimal holding temperatures affected the ability to detect viruses in pools of mosquitoes. Adult female *Aedes albopictus* and *Ae. taeniorynchus* were inoculated with CHIKV or Venezuelan equine encephalitis virus (VEEV) suspensions, respectively, and placed at 26°C for 7 days. One infected mosquito was then added to a vial of 24 negative mosquitoes and then held at -70°C, -20°C, 4°C, 22°C, or 35°C for selected time intervals. Mosquito pools were triturated in cell culture media and processed for detection of CHIKV and VEEV. Samples were analyzed for both infectious virus by plaque assay and for viral RNA with real-time RT-PCR. At high temperatures the amount of infectious virus decreased rapidly, but virus in samples held at 4°C or lower remained relatively stable. In contrast, viral RNA was detectable from pools held at all temperatures and holding times by real-time RT-PCR, although Ct values increased as temperatures and holding times increased. These findings suggest that if viral RNA detection is the goal of surveillance efforts, then mosquito pools do not need to kept at 4°C. This enhances the feasibility of field-based arbovirus surveillance programs where maintaining a cold chain may not be a possibility.

155

SOCIOECONOMIC AND ECOLOGICAL FACTORS OF *AEDES AEGYPTI* PRODUCTIVITY IN DHAKA, BANGLADESH: IMPLICATIONS FOR DENGUE VECTOR CONTROL

Parnali Dhar Chowdhury[1], C. Emdad Haque[1], Robbin Lindsay[2], Shakhawat Hossain[3], Michael A. Drebot[2]

[1]University of Manitoba, Winnipeg, MB, Canada, [2]National Microbiology Laboratory, Winnipeg, MB, Canada, [3]University of Winnipeg, Winnipeg, MB, Canada

In the absence of commercially available dengue vaccine, the primary option for formulating dengue prevention strategies is vector control programming. Although numerous dengue vector control efforts have been implemented, none of them has proved to be sustainable.In our research,we have argued that the general failure of vector control practices demands consideration of the interrelationships within the socio-economic system and ecological system.The first hypothesis we tested was that most *Aedes aegypti* pupae are being produced in a few types of household containers, and socio-economic characteristics of households are responsible for possession of container types.The second hypothesis tested in our study was that the risk of exposure to dengue virus (DENV) is directly associated with the magnitude of *Aedes* productivity.Two household (n=1200)level entomological surveys were conducted in the City of Dhaka (2011, 2012).During our second survey in 2012, blood samples from human population were also collected from members of the same households.During the 2011 entomological survey,a total of 3,651 immature *Aedes* was counted in 1,501 containers with water in 826 premises.The first hypothesis was supported by the data,revealing that 82.1 % of total *Aedes* pupae were produced from only 9 types of containers. We applied multiple criteria(i.e., container use,two-step cluster analysis based on surrounding ecological variables of containers,and multivariate nominal regression model of container types)to classify the containers and to evaluate the role of household's socio-economic factors

for possessing the specific containers types. Most pupae were 3 of 10 types of container usage categories. The cluster technique revealed that most pupae were produced from in-house,tap water-filled containers with no vegetation nearby,and under shade.Household income(χ2=11.9, df=4, p=0.01)and purpose of storing water(χ2=16.9, df=4, p=0.003)were most important explanatory variables to affect possession of productive containers types.ELISA and PRNT results of collected blood samples showed high seroprevalence (80%)and 95 new DENV infections among survey population.Interestingly, the correlation of number of pupae from a household and seropositivity led us to infer that household income,the purpose of water storage,and indoor potted plants are the most significant factors of *Aedes* productivity and seroincidence.

156

MOSQUITOCIDAL NECTAR DELIVERY: EVALUATION OF CANDIDATE PLANT SPECIES

Christopher Kearney, Zhongyuan Chen

Baylor University, Waco, TX, United States

A transgenic toxic protein delivered via nectar could be used to kill or weaken mosquito males, which feed on nectar, and females, which require nectar for energy to seek a blood meal. In addition, transgenic nectar proteins could be used to deliver any peptide, such as a hormone, a lectin, or antipathogen peptide, directly to the gut of wild mosquito populations. However, most mosquito-attractive plants listed in the literature are not amenable to genetic transformation. We selected plants with known insect/plant symbioses and published plant transformation protocols for evaluation of mosquito feeding attractiveness and the nectar protein profile. In all tests, cages with 20 (10m/10f) *Culex pipiens* or *Aedes aegypti* were tested and delivered similar results. Mosquitoes survived best in cages with Impatiens plants (*Impatiens walleriana*), followed by passion flower vines (*Passiflora edulis*), tropical milkweed (*Asclepias curassavica*), red trumpet flower vines (*Campsis radicans*) and 10% sucrose control, followed by castor bean (*Ricinus communis*). With red dye added to the nectaries, mosquitoes were seen to ingest dye within a few hours, with the speed and percentage of acquisition following the same order of plant species. When Impatiens with dyed nectaries was placed in a large cage with three other species with undyed nectaries plus an undyed sucrose tube, 80% of the mosquitoes were dyed within one day, implying that Impatiens nectar would be sampled by mosquitoes even in a complex garden setting. Finally, the nectar protein profile was examined and Impatiens nectar was found to contain a 20 kDa protein at 3 mg/ml. Since *Impatiens walleriana* has a facile transformation protocol, currently in use in the authors' laboratory, produces seeds easily, and belongs to a mainly tropical genus containing 800-1000 species, it represents an excellent candidate plant system for the creation of a mosquitocidal nectar plant.

157

THE UBIQUITIN PROTEASOME SYSTEM IS REQUIRED FOR VENEZUELAN EQUINE ENCEPHALITIS VIRUS REPLICATION

Moushimi Amaya, Forrest Keck, Lauren Scavone, Kelsey Voss, Charles Bailey, Aarthi Narayanan

George Mason University, Manassas, VA, United States

Proteins destined for degradation are tagged with multiple copies of ubiquitin. Traditionally, the proteasome was restricted to disposing of misfolded proteins or general recycling in the cell. Recently, the ubiquitin proteasome system (UPS) has been depicted in roles such as signal transduction and intracellular trafficking. Many viruses have been implicated in utilizing or modulating the UPS to enhance viral replication and/or to sustain a persistent infection. The mosquito-borne virus Venezuelan Equine Encephalitis Virus (VEEV) belongs to the Togaviridae family and is considered an important biodefense pathogen and select agent. There are currently no approved vaccines or therapies to treat the disease, therefore it is imperative to identify novel targets for therapeutic development. An initial screen of a limited FDA-approved drug library indicated that Bortezomib (Velcade) inhibited replication of the attenuated TC-83 strain of VEEV in infected cells without apparent toxicity to the cell. We hypothesized that a functional UPS is required for efficient VEEV replication. Proteasomal inhibitors deplete free ubiquitin that may be needed to modify viral proteins for efficient viral budding. Bortezomib, a dipeptidyl boronic acid, specifically and reversibly inhibits the 26S proteasome. We have shown that at non toxic concentrations Bortezomib proved to be a potent inhibitor of VEEV replication in the human astrocytoma cell line U87MGs. Bortezomib inhibited both the virulent Trinidad Donkey strain and TC-83 strain of VEEV. Additional studies with virulent strains of Eastern Equine Encephalitis Virus and Western Equine Encephalitis Virus depicted that Bortezomib is a broad spectrum inhibitor of the New World alphaviruses. Time of addition assays showed that Bortezomib may affect early time points in the viral life cycle thus influencing viral replication. Mass spectrometry analyses indicated that VEEV capsid was ubiquitinated, which was validated by western blot. Subsequent studies revealed that capsid is stabilized in the presence of Bortezomib in treated cells while the nuclear and cytoplasmic distribution of capsid did not undergo any marked changes. Ongoing studies are focused on elucidating the mechanism by which Bortezomib inhibits alphavirus replication. This study will aid future investigations in identifying host proteins as potential broad spectrum therapeutic targets for treating VEEV infections.

158

SINDBIS AND BUNYAMWERA VIRUSES CIRCULATE AMONG PATIENTS WITH FEBRILE ILLNESS IN MFANGANO ISLAND OF LAKE VICTORIA WESTERN KENYA

David Ouma[1], Daniel Masiga[1], Burtram Fielding[2], Jandouwe Villinger[1]

[1]icipe-African Insect Science for Food and Health, Nairobi, Kenya, [2]University of Western Cape, Cape Town, South Africa

Bunyamwera and Sindbis viruses are medically important arboviruses in the tropical and subtropical regions of the world, with an estimated 2.5 billion people being at risk. Early detection of any arbovirus infection has great importance for the clinical management of patients, surveillance, and to prevent possible disease outbreak. The aim of this study was to investigate if patients with acute febrile illness without *Plasmodium* detectable by microscopy or RDTs were arbovirus positive. Patients whose ages ranged from 13 to 70 years with a mean age of 29 were used for the study. There was preponderance of females (65%) over males (35%) among the patients studied. Whole blood was sampled (n=105) from consenting febrile patients at Sena Health Centre, Mfangano Island of Lake Victoria, Kenya. Information on body temperature, type of occupation (indoor/outdoor), clinical manifestation, nature of exposure (direct or indirect) to animals and type of treatment received were collected using a briefed close ending questionnaire. Arbovirus specific RNA sequences extracted directly from blood were amplified by reverse transcriptase, multiplex PCR and amplicons resolved by High Resolution Melt (HRM) for virus detection and differentiation. Four patients (3.8%) tested positive for bunyamwera virus while two representing (1.9%) had Sindbis virus infection. Among all the patients studied, 102 (97.1%) received anti-malarial treatment while 3(2.9%) were treated with antibiotics. These findings demonstrate the presence of arboviruses among febrile illness cases in Western Kenya and the possibility of these viruses being the aetiological agents of in the cases exist. In addition, inadvertent use of antimalarial and antibiotics treatments for malaria negative cases should be discouraged. This study underscores the need to differentially diagnose febrile illnesses for arboviruses to guide and direct appropriate treatment and management.

159

DEVELOPMENT OF EILAT VIRUS, A HOST-RESTRICTED ALPHAVIRUS, AS A VACCINE VECTOR

Jesse Erasmus

University of Texas Medical Branch, Galveston, TX, United States

Eilat virus (EILV) is an alphavirus isolated from a pool of mosquitoes collected in Israel. It replicates efficiently in insect cells but is unable to replicate in vertebrate cells. EILV is host-restricted at two points in its replication cycle: 1) attachment/entry, and 2) viral RNA replication. The host-restricted nature of EILV provides an opportunity to develop it as a potential "pseudoinactivated" vaccine vector. Our goal was to develop EILV chimeras as a new platform for alphavirus vaccines. For proof-of-concept, we chimerized EILV with eastern equine encephalitis virus (EEEV), a highly virulent alphavirus that can cause fatal disease outbreaks in both humans and equids with high case-fatality rates. Our central hypothesis was that a chimeric alphavirus containing the non-structural protein genes of EILV and the structural protein genes of EEEV will retain the vertebrate host restriction of EILV and provide safe, effective protection against lethal challenge with EEEV. To test this hypothesis, we generated a chimeric EILV/EEE infectious cDNA using standard cloning techniques, and rescued the virus in insect cells. We then performed immunogenicity and safety experiments in mice. After a single vaccination, EILV/EEE protected mice against lethal challenge with EEEV and produced a higher titer of neutralizing antibodies when compared to a commercial EEE vaccine for horses. Additionally, EILV/EEEV showed no neurovirulence in suckling mice after intracranial inoculation. These results suggest that the chimeric EILV-based alphavirus vaccine platform represents a safe and efficient approach to protect against EEEV and other highly pathogenic alphaviruses in mice.

160

DEVELOPMENT OF A CHIKUNGUNYA VIRUS RNA REFERENCE REAGENT FOR STANDARDIZATION OF NUCLEIC ACID TESTS

Germán Añez, Daniel A. Heisey, Maria Rios, CHIKV RNA Reference Reagent Working Group

Food and Drug Administration, Bethesda, MD, United States

Chikungunya virus (CHIKV) is an Alphavirus transmitted by the mosquitoes *Aedes aegypti* and *Aedes* albopictus, the same vectors that transmit Dengue viruses (DENV), a group of four Flaviviruses that often co-circulate with CHIKV in the same geographical area. CHIKV has caused explosive epidemics in Africa, Asia and Indian and Pacific Ocean islands. It recently appeared in the Caribbean islands, posing risk to the rest of the Americas. Some CHIKV human infections are asymptomatic. Most cause a febrile illness similar to that of DENV, characterized by high fever, polyarthralgia, headache, back pain, myalgia, nausea, vomiting, and rash. There are no vaccines or specific treatments for DENV or CHIKV. However, DENV infections require prompt differential diagnosis and not infrequently hospitalization to prevent fatalities. Laboratory diagnosis for these viruses is made by serology, viral isolation or by nucleic acid test (NAT), the most sensitive method. There are no FDA-approved CHIKV diagnostic or blood screening assays. The lack of a reference reagent for CHIKV is a barrier for proper evaluation of NAT assays. This work aims to produce a well-characterized CHIKV RNA Reference Reagent (CHIKV-RR) for use as a standard for evaluation of performance of existing assays, and to facilitate the development of NAT assays that fulfill the requirements for blood screening. The CHIKV-RR candidate was produced by (a) expansion of a CHIKV clinical strain in Vero cells; (b) heat-inactivation (HI) of the viral stock; (c) preliminary in-house titration of the HI CHIKV stock; (d) validation of results in external collaborative studies; (e) formulation of the Center for Biologics Evaluation and Research of the U.S. Food and Drug Administration CHIKV-RR. Preliminary results showed that the CHIKV stock has a concentration of ~10^6 PCR-detectable units/ml. The final formulation of the CHIKV-RR was shipped to collaborators for a second round of testing. External results are expected within 3 months, when it will be subjected to statistical analysis for assignment of a final number of units.

161

EFFECT OF THE LYSOSOMOTROPIC COMPOUNDS ON CHIKUNGUNYA VIRUS *IN VITRO*

John T. Nuckols[1], Yan-Jang S. Huang[2], Alexander J. McAuley[3], Kate M. Horne[4], Stephen Higgs[2], Dana L. Vanlandingham[2]

[1]Medical Countermeasure Systems, Joint Vaccine Acquisition Program, Fort Detrick, MD, United States, [2]Biosecurity Research Institute, Kansas State University, Manhattan, KS, United States, [3]Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, United States, [4]Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, United States

Alphaviruses are mosquito-borne viruses that primarily alternate between the arthropod vectors and vertebrate hosts. These viruses utilize the class-II fusion protein-mediated viral membrane fusion in the acidic intracellular compartments during the process of viral entry. The treatment of lysosomotropic compounds has been extensively used to characterize the viral entry of alphaviruses in mammalian cell lines and have been found effective for the inhibition of viral entry. Such characterization has not been performed for the analysis of chikungunya virus (CHIKV) entry in mosquito cell lines. In this study, three lysosomotropic compounds, ammonium chloride, chloroquine and monensin were tested to evaluate the inhibitory effect of viral entry *in vitro*. The entry of CHIKV is assessed by the fluorescent signals from the expression of the green fluorescent protein (GFP) genetically engineered into the viral genome. The inhibition of viral entry was assed at concentrations which maintained the viability of the cells. The effective $dose_{50}$ of each compound was calculated based on the percentage of reduction in the fluorescent signals and the concentrations of the compounds. The results and the possible mechanisms responsible for the entry of CHIKV and alphaviruses into mosquito cells will be discussed.

162

INSIGHTS INTO THE SPREAD OF CHIKUNGUNYA IN BANGLADESH FROM A SEROPREVALENCE STUDY

Abu Mohd Naser[1], Henrik Salje[2], Mahmudur Rahman[3], Mohammed Ziaur Rahman[1], James D. Heffelfinger[4], Stephen P. Luby[5], Emily Gurley[1]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]Johns Hopkins School of Public Health, Baltimore, MD, United States, [3]Institute of Epidemiology Disease Control and Research, Dhaka, Bangladesh, [4]Global Disease Detection Branch. Division of Global Health Protection, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]Stanford University, Stanford, CA, United States

Chikungunya virus is an emerging infectious disease in Southeast Asia. The first chikungunya outbreak in Bangladesh was reported in 2008 in Chapai Nawabganj, a district in northwest Bangladesh bordering India. Two more outbreaks occurred in 2011 in the same district. It is unclear whether these outbreaks are markers of large-scale transmission throughout the district or isolated events. We conducted a serosurvey in Chapai Nawabganj to better understand chikungunya dispersal and to determine factors associated with exposure. Between March — May 2013, we randomly selected 40 communities and selected all household members to participate in the study until we enrolled 30-50 individuals per community. We collected a blood sample and socio-demographic characteristics and typical travel behavior from all participants. We used IgG ELISAs (NovaLisaTM, Dietzenbach, Germany) to identify past chikungunya infection. We used a multivariate logistic regression model with a random intercept for each community to identify risk factors. Overall 6.7%

(87/1290) of individuals had evidence of chikungunya infection. Half of the communities, located in the north of the district, had no seropositive cases at all. Communities in the south, nearby previous outbreak sites, had up to 56% seropositivity. Individuals < 20 years were 2.5 (95% confidence interval [CI], 1.5-4.2) times more likely to be seropositive than older individuals. Having another seropositive individual in the same household increased the risk of previous infection 1.4 (95% CI, 1.2 - 2.0) times and each additional seropositive individual within the community (but outside the home) increased the risk of past exposure 1.3 (95% CI, 1.2 - 1.3) times. Gender and travel history were not associated with past exposure. Very high proportions of people infected in some communities and no infections in communities in a small geographic area suggests that transmission was very intense in outbreak communities, but the virus failed to transmit beyond outbreak communities. Research into spatial difference in vector ecology may help us understand heterogeneities in outbreak risk across this area.

163

DISSECTING E2 PROTEIN DOMAINS INVOLVED IN ALPHAVIRUS CELL TROPISM

James D. Weger-Lucarelli, Matthew T. Aliota, Jorge E. Osorio

University of Wisconsin Madison, Madison, WI, United States

Alphaviruses represent a diverse set of arthropod borne viruses, many of which are important veterinary or human pathogens. One of the most medically relevant alphaviruses, Chikungunya virus (CHIKV), is the cause of an ongoing outbreak of arthritic disease in the Caribbean islands and South America and its spread to North America appears likely. Other alphaviruses such as Venezuelan equine encephalitis virus (VEEV) and Semliki Forest virus (SFV) cause encephalitic disease. The role of viral genetics determining these different disease phenotypes is unclear. E2, the alphavirus receptor binding protein, has been implicated as a critical determinant of tissue tropism and host range. For example, previous reports have demonstrated that domains A and B of E2 contain residues important for host range expansions; however, little research has been performed to implicate which critical domain (e.g., A, B, and C) of this protein confer variations in cell tropism. Therefore, we created chimeric viruses between a normally arthritic virus (CHIKV) and a normally encephalitic virus (VEEV or SFV) to probe the effect of each domain on cell tropism both *in vitro* and *in vivo*. Chimeric CHIKV/VEEV were not viable, likely due to high sequence dissimilarity between the viruses. In contrast, CHIKV/SFV chimeras were rescued successfully and replicated well in several cell lines, albeit to lower levels than parental CHIKV. All chimeric viruses were attenuated as compared to parental CHIKV. When delivered intracranially to type-1 interferon deficient mice, the virus containing domain B and to a lesser extent domain A from SFV caused neuron degeneration and demyelination, similar to parental SFV. The chimera containing domain A from SFV appeared to cause more perivascular cuffing, similar to SFV. Transfer of these two domains from SFV to CHIKV altered tropism to reflect that of SFV. This indicated that the different domains of E2 may be playing an important role in determining cell tropism for alphaviruses. Further study of these domains may provide useful insight into alphavirus pathogenesis.

164

ELUCIDATING THE MECHANISM BY WHICH THE INTERFERON-INDUCED EXORIBONUCLEASE, ISG20, RESTRICTS CHIKUNGUNYA VIRUS REPLICATION

Christopher M. Weiss, Chengqun Sun, William B. Klimstra, Kate D. Ryman

University of Pittsburgh, Pittsburgh, PA, United States

Chikungunya fever is an important emerging arboviral disease characterized by painful and often persistent, flaring polyarthritis and arthralgia affecting more than six million people worldwide. The causative agent, chikungunya virus (CHIKV), is a positive sense, RNA virus from the genus *Alphavirus*, family Togaviridae, whose geographic distribution has recently increased to include Europe, the Caribbean and South America. Currently, there are no approved vaccines or antivirals available for the treatment of CHIKV or related arthritogenic alphaviruses. We have previously demonstrated a crucial role for type I interferon (IFN) in the protection against musculoskeletal CHIKV disease in mice. Using a systems biology approach for identifying novel interferon stimulated genes (ISGs) with potent anti-alphaviral activity, our lab has identified a subset of genes with antiviral activity against CHIKV and its relatives. Among the genes identified with the greatest anti-alphaviral activity was the 20 kDa, nuclear exoribonuclease, ISG20. Using an inducible over-expression system and dominant-negative inhibitor, we have characterized this antiviral activity against CHIKV infection and identified early genomic translation as an early target of ISG20 function. Interestingly, over-expression of ISG20 also controlled the upregulation of a subset of other ISGs, independently of type I IFN production. Ongoing experiments are examining the mechanism, direct or indirect, by which ISG20 abrogates CHIKV genomic translation and the extent of host regulatory networks influenced by ISG20 upregulation. Our results indicate a crucial role for ISG20 in IFN-mediated protection against CHIKV infection and may reveal targets for future therapeutic intervention.

165

MODELING THE POTENTIAL FOR A CHIKUNGUNYA OUTBREAK IN THE MIAMI METRO AREA

Michael A. Robert[1], Rebecca C. Christofferson[2], Christopher N. Mores[2], Helen J. Wearing[1]

[1]University of New Mexico, Albuquerque, NM, United States, [2]Louisiana State University, Baton Rouge, LA, United States

Once found only in parts of Africa, southeastern Asia, and the Indian subcontinent, chikungunya has spread globally in the past decade with major outbreaks occurring in parts of Europe, La Réunion Island, and most recently in the Caribbean. The expansion of the disease has been facilitated in part by global travel, increased vector competence due to mutations in the virus, and increased ranges of habitats of the primary vector species, *Aedes aegypti* and *Ae. albopictus*. Expansion of the disease into the Western Hemisphere coupled with recent outbreaks and autochthonous transmission of dengue fever (which is vectored by the same species) in the U.S. raises concern that a chikungunya outbreak in the U.S. is imminent. Southern Florida, including the densely population Miami metropolitan area, is particularly susceptible to an outbreak due to the tropical climate, cohabitation of *Ae. aegypti* and *Ae. albopictus*, and heavy travel between the region and tropical locations worldwide, particularly Central and South America and the Caribbean. To assess the potential for an outbreak in the Miami metro area, we developed a mathematical model parameterized to study chikungunya dynamics in this region. We utilize the model to study the impact that timing, location, and size of an introduction have on the ability of an introduction to lead to an outbreak. Further, we study the influence of variation in viral strains and differential competence between vectors on disease dynamics. We calculate the probability that introductions will lead to outbreaks in the Miami metro and discuss the potential for the disease to spread from Southern Florida to other parts of the U.S. We also highlight the flexibility of the model by proposing its utility in studying the potential for outbreaks of other diseases, such as dengue fever, in the same region.

166

HOST-PATHOGEN INTERACTION DYNAMICS OF HUMAN ASTROCYTES INFECTED WITH VENEZUELAN EQUINE ENCEPHALITIS VIRUS

Alan Baer[1], Danielle Swales[2], Nicole Waybright[2], Lindsay Lundberg[1], Jonathan Dinman[3], Jonathan Jacobs[2], **Kylene Kehn-Hall**[1]

[1]George Mason University, Manassas, VA, United States, [2]MRIGlobal, Rockville, MD, United States, [3]University of Maryland, College Park, MD, United States

Venezuelan Equine Encephalitis Virus (VEEV) is an emerging arthropod-borne virus responsible for causing acute encephalitis, and often death, in animal and human hosts. VEEV is a priority pathogen that has previously been weaponized. Due to its continued environmental persistence in the Americas, it represents a significant threat to U.S. public health and economic security. The increased circulation and spread in the Americas of VEEV, and other encephalitic arboviruses such as Eastern Equine Encephalitis Virus (EEEV) and West Nile virus (WNV), underscores the need for research aimed at characterizing the pathogenesis of viral encephalomyelitis as a foundation for the development of novel medical countermeasures. In this study, we have sought to characterize the host-pathogen dynamics of VEEV in the human neuronal cell line U87MG by carrying out RNA sequencing of poly(A)+ mRNAs. We aim to identify critical alterations in the host transcriptome that take place within the first 24 hours following VEEV infection. Triplicate samples were collected at 4, 8, and 16 hours post-infection and RNA-Seq data acquired using an Ion Torrent PGM. Significant differentially expressed genes were part of the following super pathways: immune response IFN alpha/beta signaling, immune response IL-2 activation and signaling, regulation of nuclear SMAD2/3 signaling, and development glucocorticoid receptor signaling. Specifically we observed an increase in interferon regulated genes IFIT1, IFIT2, IFIT3, and OASL following VEEV infection. We also observed an increase in EGR-1 and differential expression of a number of genes that are involved in the EGR-1 pathway including ADAMT21, ATF3, KLF6, MYC, JUN, and PTGS2. Data from these studies will be leveraged towards identifying specific host mRNA transcripts or pathways suitable for therapeutic intervention, as well as provide mechanistic details regarding how alphaviruses manipulate the host transcriptome to facilitate replication.

167

ASSESSING DENGUE VIRUS-INDUCED CHANGES IN GENE EXPRESSION PROFILES VIA RIBOSOME PROFILING

Mariana Leguia[1], Diana Juarez[1], Michael Torres[1], Eric Halsey[1], Daniel G. Bausch[1], Anton Vila-Sanjurjo[2]

[1]U.S. Naval Medical Research Unit - 6 (Peru), Washington, DC, United States, [2]Universidade de Coruña, A Coruña, Spain

A majority of the research on dengue (DENV) virus-induced changes in gene expression has focused on the role of the adaptive immune response, which is undeniably important. However, epidemiological data suggest that the host's genetic background may also contribute important susceptibility factors that could exert their effect in a manner independent from the adaptive immune response. Cellular responses with the potential to make a difference between life and death outcomes are ultimately mediated by the actions of proteins encoded in the genome. Thus, an understanding of differential global gene expression at the proteome level is essential to understand how DENV infection can result in dramatically different disease outcomes. Ribosome profiling is a new technique that enables direct measurements of protein expression at the whole cell level. In so doing, it generates all the information needed for a comprehensive understanding of how global gene expression may influence particular disease phenotypes. We have recently completed the first ribosome profiling-based study of DENV-2 infected human cells. Our results indicate that ribosome profiling is a powerful tool to study changes in cellular dynamics upon DENV infection. Specifically, we are able to pinpoint differentially regulated genes and corroborate previously identified putative predictors of disease progression. As a whole, these data sets elucidate differentially regulated genes in the context of the host's genetic background. Furthermore, this approach has the potential to enable discovery of genes not previously associated with particular disease states, and in so doing, lead to the development of improved vaccines, diagnostics and therapeutics.

168

POTENTIAL PROTECTIVE ACTIVITY OF DENGUE NANOVACCINE DELIVERY BY BCG/CHITOSAN NANOPARTICLE

Taweewun Hunsawong[1], Saradee Warit[2], Panya Suninthaboon[3], Butsaya Thaisomboonsuk[1], In-Kyu Yoon[1], Stefan Fernandez[1], Sukathida Ubol[4]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand, [3]Department of Chemistry, Faculty of Science, Mahidol University, Bangkok, Thailand, [4]Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand

In this study, we report the development and testing of a novel dengue nanovaccine (DNV) composed of UV-inactivated DENV-2 (UVI-DENV-2), strain 16681 and BCG/chitosan nanoperticles (BCG/CS-NPs). We prepared BCG/CS-NPs by emulsion-polymerization method, yielding 299.1 ± 1.67 nm particles in size. The adjuvant properties of BCG/CS-NPs were tested using primary human immature DCs. A significant increase in the activation markers of DCs (CD80, CD86 and HLA-DR) and stimulation of IL-12 production were observed. By these properties, we used BCG/CS-NPs as an adjuvant and vaccine delivery system for the UVI-DENV-2, a vaccine immunogen. The inactivation and antigenicity of UVI-DENV-2 were confirmed by plaque assay and ELISA using specific monoclonal antibodies (4G2, 3H5 and 2H2). To prepare DNV, we loaded UVI-DENV-2 into BCG/CS-NPs which yielded DNV of 372.0 ± 11.21 nm particle in size. We also showed that up to 98.6% of UVI-DENV-2 found on DNV. We tested the immunogenicity of DNV in Swiss albino mice. Mice were vaccinated with three DNV doses, 15 days apart. The results showed that DNV stimulated anti-dengue IgM/IgG antibodies along with dengue-neutralizing antibodies in a dose-dependent manner, peaking 15 days post-dose three when using 10 µg of DNV per dose. We also demonstrated the cell-mediated immunity properties of DNV by flow cytometry. We showed an increase in the frequency of interferon (IFN)γ-producing CD4+T cells and CD8+ T cells after stimulation with 10 µg of DNV. We developed a mouse dengue challenge model to test the protective qualities of the DNV. Mice were vaccinated with three doses (10 µg each) of DNV and challenged with 10^6 PFU of a mouse-adapted DENV-2 (NGC strain) 14-day post dose three. Serum and spleen were collected at various dates post-challenge to measure viremia by RT-PCR. The decreasing of viremia in vaccinated mice indicated a potential use of the DNV in protecting against dengue virus infection.

169

COMMUNITY KNOWLEDGE AND ATTITUDES AND HEALTH WORKERS' PRACTICES REGARDING NON-MALARIA FEBRILE ILLNESSES IN EASTERN TANZANIA

Beatrice Chipwaza

Nelson Mandela African Institute of Science and Technology, Arusha, United Republic of Tanzania

Although malaria has been the leading cause of fever for many years, with improved control regimes malaria transmission, morbidity and mortality have decreased. Recent studies have increasingly demonstrated the importance of non-malaria fevers, what has significantly improved our understanding of etiologies of febrile illnesses. A number of non-malaria febrile illnesses including Rift Valley Fever, dengue fever, Chikungunya

virus infection, leptospirosis, tick-borne relapsing fever, Brucellosis and Q-fever have been reported in Tanzania. This study aimed at assessing the awareness of communities and practices of health workers on non-malaria febrile illnesses. Twelve focus group discussions with members of communities and 14 in-depth interviews with health workers were conducted in Kilosa district, Tanzania. Transcripts were coded into different groups using MaxQDA software and analyzed through thematic content analysis. The study revealed that the awareness of the study participants on non-malaria febrile illnesses was low and many community members believed that most instances of fever are due to malaria. In addition, the majority had unrealistic beliefs about the possible causes of fever. In most cases, non-malaria febrile illnesses were considered following a negative Malaria Rapid Diagnostic Test (mRDT) result or persistent fevers after completion of anti-malaria dosage. Therefore, in the absence of mRDTs, there is over diagnosis of malaria and under diagnosis of non-malaria illnesses. Shortages of diagnostic facilities for febrile illnesses including mRDTs were repeatedly reported as a major barrier to proper diagnosis and treatment of febrile patients. Our results emphasize the need for creating community awareness on other causes of fever apart from malaria. Based on our study, appropriate treatment of febrile patients will require inputs geared towards strengthening of diagnostic facilities, drugs availability and staffing of qualified health workers.

170

NEW INSIGHTS INTO THE MOLECULAR EVOLUTION OF DENGUE VIRUS TYPE 4 IN PUERTO RICO OVER TWO DECADES OF EMERGENCE

Estelle M. Martin

JABSOM, Honolulu, HI, United States

Dengue has emerged globally as a human health problem since the 1950s and is now the major arboviral disease infecting hundreds of million people annually. While some cases are asymptomatic, others develop a febrile illness (dengue fever) or even progress to severe dengue which can be fatal. Dengue is caused by any of four related viruses (DENV-1 to -4) that are maintained in endemic transmission in large urban centers of the tropics by *Aedes* mosquitoes. Since the late 1960s, Puerto Rico (PR), a major population center in the Caribbean, has experienced increasingly severe epidemics following the introduction of multiple dengue serotypes. A particularly severe outbreak in 1998 was dominated by a novel DENV-4 strain that evolved in PR, replacing earlier forms and spreading throughout the region. Here we show that this DENV-4 strain is genetically distinct based on unique changes in the NS2A and NS5 genes. Its replacement of earlier forms in Puerto Rico progressed rapidly from 10% of samples in 1994 to 95% in 1997, suggesting that strong natural selection played a role in its fixation. This study confirms that dengue viruses evolve through rapid lineage turnover driven in part by natural selection, important contributors to its ongoing emergence in human populations. *In vitro* experimental infection did not support higher replication rate as the cause of severity in the 1998 outbreak, however similar experiments are now being analyzed from *Aedes aegypti* mosquitoes.

171

POOR CORRELATION BETWEEN BLEEDING AND THROMBOCYTOPENIA IN DENGUE

Ananda D. Wijewickrama, Jayathra Liyanagamage, V. P. Jayaweera

Infectious Diseases Hospital, Angoda, Sri Lanka

Thrombocytopenia is a common finding in Dengue. Advice for prophylactic platelet transfusions in some guidelines has led to many un-necessary platelet transfusions. Threshold of platelet transfusions is often set at $20x10^9$/L, an arbitrary figure set without clinical evidence. Hence, it is important to study the relationship of bleeding in Dengue to low platelet count. We studied all the patients with dengue infection who were admitted under and referred to the principal investigator at Asiri Hospitals PLC, Colombo, during a period of 24 months from 1st of June 2011. Dengue infection was confirmed by positive antigen and/or antibody test. DHF was diagnosed in patients who had ultrasonically confirmed fluid leakage. Study included 254 patients with 142(55.9%) males and 112(44.1%) females. Mean age was 28.28 yrs. While 118 of them had DHF, 136 had DF. Bleeding manifestations were seen in 47(18.5%) patients; GI bleeding (n=6, 2.4%), gum bleeding (n=4, 1.6%), PV bleeding (n=31, 12.2%) and occult bleeding (n=6, 2.36%). Bleeding in DHF patients was significantly higher than that of DF patients. (DHF- 36, DF - 11, p=0.000). Bleeding was significantly associated with low platelet count when all the Dengue patients were considered with a mean platelet count of $34.02x10^9$/L (range 80-$5x10^9$/L) in bleeders and $53.66x10^9$/L in those who had no bleeding (p=0.000). When only the patient with platelet counts <80x109/L were analysed, there was a significant difference in the two groups of patients with platelet counts >20 and <$20x10^9$/L (p=0.022). However, when the patients with minor bleeds (gum bleeding) were excluded, there was no significant difference between these two groups (p=0.136). Mortality rate in this study was zero. Of the total, 33(12.99%) had platelet counts <$20x10^9$/L, but only 10 of them had any bleeding. None had prophylactic platelet transfusions. Therapeutic blood transfusions were required in 18(7.1%) and 01 had both platelet and blood transfusions as they were hemodynamically unstable. Mean lowest platelet count of this group was $30.44x10^9$/L and 56% had lowest platelet counts above $20x10^9$/L. This study confirms that the transfusion threshold of $20x10^9$/L in Dengue is arbitrary and relationship with clinically significant bleeding in Dengue and low platelet counts is not straight forward. Therefore, recommendations on prophylactic platelet transfusions need to be reconsidered.

172

DENGUE HEMORRHAGIC FEVER VIRUS DETECTION IN *AEDES* MOSQUITOES, IBADAN NIGERIA

Seyi B. Olaniran, Bernard Onoja, Adedayo Faneye, Clement Meseko, Johnson A. Adeniji

University of Ibadan, Ibadan, Nigeria

Dengue Hemorrhagic Fever is cause by arthropod-borne virus that is transmitted to humans through mosquitoes bite. The subtropical climate of southern Nigeria is often hot and humid which promotes mosquitoes breeding. The consequences are reports of feverish illnesses most often attributed to malaria or label as fever of unknown origin when treatment is intractable. This study was carried out in Ibadan, southwest Nigeria to Detect Dengue Hemorrhagic Fever Virus in mosquito vectors and as a possible cause of fever of unknown origin in order to shed more light on the ecology of infectious disease in the locality. Five hundred and ten anthropophillic mosquitoes were captured, identified and processed for detection of arboviruses by molecular techniques. Nucleic acid was extracted from mosquitoes pool following homogenization in cell culture medium. Extracted RNA was thereafter transcribed to cDNA with random Hexamers. Thereafter, primers that primarily target alpha and flaviviruses were used for gene amplification. Subsequently, gene specific primers for yellow fever, West Nile, Chikungunya, rift valley and dengue viruses were used to amplify conserved regions of the different gene segments. Amplified products were thereafter separated in agar gel electrophoresis and viewed in UV light with a transilluminator. Out of 17 mosquitoe pools, 7 *Aedes* species pool tested positive for Dengue Haemorrhagic Fever Virus. DENV-1 was found in 6 pools, DENV-2 in 0 pools, DENV-3 in 0 pools and DENV-4 was tested positive in 5 pools. We detected Dengue Haemorrhagic Fever virus in *Aedes* mosquitoes in the study area, this provide recent data on Dengue Haemorrhagic Fever Virus ecology in Nigeria. It is also an indication that arboviruses may contribute to the burden of fever of unknown origin in the sub-region and should be considered for public health interventions.

173

DEVELOP ALGORITHMS TO DIFFERENTIATE PRIMARY AND SECONDARY DENGUE INFECTIONS USING A SINGLE SPECIMEN IN ACUTE PHASE

Hanh Tien T. Nguyen[1], Lam K. Phung[1], Kieu T. Nguyen[1], Stephen Whitehead[2], Cameron Simmons[1], Marcel Wolbers[1], Bridget Wills[1]

[1]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, [2]Laboratory of Infectious Diseases, National Institutes of Allergy and Infectious Diseases, Bethesda, MD, United States

Dengue is the most important arboviral disease affecting humans. It can be caused by any one of four dengue viral serotypes, DENV1-4. Among symptomatic cases clinical presentation varies widely, ranging from mild febrile illness to severe and fatal disease. Although the pathogenesis is poorly understood, there is good epidemiological evidence indicating that heterotypic secondary infections are associated with development of severe disease. Therefore differentiating clearly between primary and secondary infections is crucial for studies on dengue pathogenesis. However the current methods are not ideal - often only a single specimen is available, and none of the available methods take into account the evolving nature of the immune response over time during the acute illness. The aim of this study was to develop a model to distinguish primary from secondary dengue infections using a single specimen obtained early in the illness course. The results of plaque reduction neutralization tests (PRNT) at 6 months after fever onset, when the acute immunological response had settled, were taken as the gold standard for classifying the recent infection as either primary or secondary. Daily plasma samples obtained during the acute illness were assayed for a) IgG using commercial PanBio Indirect ELISA kits, b) anti-whole dengue virus IgG and IgM in-house Capture ELISAs, and c) anti-dengue E protein IgG in-house indirect ELISA. Using logistic regression models were developed according to the day of illness (DOI), with separate models prepared for DOI 3; DOI 6; both DOI 3 and DOI 6; and for all days from DOI 3 to DOI 6. The models were internally and temporally validated. Preliminary results show that simple univariate logistic models based on the commercial PanBio Indirect ELISA or the anti-whole dengue virus IgG in-house Capture ELISA using DOI 3 samples performed well (AUCs (95% CI) of 0.88 (0.82-0.95) and 0.82 (0.74-0.9) respectively). Performance of the early acute phase models was as good as both convalescent, and dual phase, models.

174

PRESENCE OF DENGUE FEVER IN SEMI-URBAN AREAS OF TWO HEALTH DISTRICTS IN BURKINA FASO

Antarou Ly

Research Institute for Health Sciences, Ouagadougou, Burkina Faso

The global incidence of dengue has grown dramatically in recent decades. In Africa, particularly in Burkina Faso, it has been neglected or ignored, the last description of acute cases were back in 1982. All fever cases are systematically considered presumptive malaria cases. During a regular populations survey, unexplained persistent fevers facing anti malaria treatment were observed. We therefore conducted an epidemiological investigation for non-malaria cases. The study was conducted in a panel of febrile children 0-10 years old, with temperature of 37.8°C or higher, living in semi- urban areas of Kaya and Zorgho. Rapid tests from SD Bioline Dengue Duo (AgNS1-IgG/IgM) were added to a panel of malaria rapid test. Samples on filter paper were taken from every patient with a rapid dengue test positive and for every tenth negative patient to perform qTR-PCR analyses. A total of 264 children were included, 153 from Kaya and 111 from Zorgho. Among them, 6.7% (18/264) had positive results in the two districts. In Kaya, 9.8% (15/ 153) positive cases were reported and 2.7% (3/ 111) in Zorgho. From all positive results, 11.1% were NS1 antigen (2/18) and 88.9% IgG/IgM (16/18) antibodies. The qRT-PCR results show the presence of DENV2, further analyses are ongoing to characterize the virus and to define the possible presence of other serotypes. Thirty years after from its last report, dengue presence has been document in febrile and symptomatic children, from semi- urban areas of Burkina Faso. Due to the contextual/local limitations, it was important to explore different ways to obtain the information and to analyze the samples. Therefore, filter paper use was a feasible tool to perform molecular analyses needed to confirm the presence of the virus. These findings reveal the need to address research and actions towards non-malaria diseases in the region. In this case thinking about dengue as one of the causes for febrile diseases. (More data about the viral sequencing and epidemiological description of the cases will be available for presentation at the conference)

175

MOLECULAR DETECTION OF DENGUE VIRUS SEROTYPES CO-CIRCULATING WITHIN MOSQUITOES IN PERI-DOMESTIC ECOSYSTEMS IN NIGERIA

Anyebe Bernard Onoja, Adekunle Johnson Adeniji, Olufemi David Olaleye

University of Ibadan, Ibadan, Nigeria

Dengue Virus (DENV) causes devastating health impacts with an estimated 500,000 infections annually. The first DENV-1 isolate in Africa was reported from Nigeria. Reports of importation of DENV from West Africa into Europe have been increasing, yet the burden of the disease in Africa has not been estimated. Surveillance activities are weak or non-existent in many African countries. There is currently no vaccine against DENV, although several research activities are underway for an effective tetravalent vaccine. In this study we provide recent epidemiological data of co-circulating DENV serotypes in mosquitoes involved in peridomestic transmission. This is by far the most elaborate study in Nigeria with comparison between habitats where non-human primates are kept and aboreal habitats without nonhuman habitats where humans visit. The modified CDC light trap was used to catch both *Aedes albopictus* and *Ae. aegypti* from Agodi garden Ibadan; University of Ibadan zoo, botanical garden, botany forest Oyo State. They were sorted and identified morphologically into 26pools of 50mosquitoes each. It was screened after RNA extraction using primers designed to target and amplify the nonstructural proteins region of the genome using a thermalcycler and the amplicon was observed using a trasilluminator. Temperature of diurnal activity was between 26-36oC. The number of positive pools are as follows; DENV-1 (11), DENV-2 (14), DENV-3 (16), DENV-4 (10). DENV was detected in all 26pools and the minimal rate of infection was DENV-1 (22.2%), DENV-2 (44.4%), DENV-3 (77.8%) and DENV-4 (44.4%). *Aedes albopictus* was found to be more abundant all year round compared to *Aedes aegypti*, both competent in transmitting DENV. Co-circulation of four DENV serotypes within peridomestic cycles has been established in Nigeria. There is the need for immunological investigation of host factor interaction within the area, this may enhance vaccine production. Also, sustained surveillance activities in hospitals are encouraged to confirm clinical cases and improve patient care.

176

THE ROLE OF DENGUE VIRUS MATURATION IN VECTOR INFECTION

Christopher Roundy, Andrea Troupin, Tonya Colpitts

Tulane University, New Orleans, LA, United States

Dengue, a mosquito-borne flavivirus, affects up to 100 million people a year with over 40% of the global population currently at risk. Infections range from mild febrile illness to severe dengue hemorrhagic fever and dengue shock syndrome, both of which can be lethal. Present primarily in tropical and subtropical regions, dengue's expansion into the U.S. and other areas has become a major public health concern. Due to antibody-dependent enhancement of a secondary infection, a dengue vaccine should contain all serotypes or risk putting vaccinated individuals at higher risk for severe disease. While progress has been made to develop a vaccine with the four established serotypes, the discovery of a fifth

possible serotype presents a significant setback. With no vaccine or specific treatment for dengue, understanding the viral interaction with the mosquito vector is particularly important. Knowledge regarding dengue pathogenesis in the mosquito could lead to novel treatments and preventative measures. Dengue is a positive-stranded RNA virus which contains three functional proteins: capsid protein C, envelope protein E, and membrane protein M. Immature virus within the cell is coated with immature M protein (prM). This protein is cleaved by furin during exocytosis, which results in mature particles coated with E protein. This process is imperfect and can lead to the release of viral particles in various stages of maturation. The infectivity of these particles has been well-documented in mammalian cells, with the addition of prM-antibody rendering immature particles infectious. However, since mosquitoes lack antibody receptors, the mosquito-specific infectivity of immature virus particles remains unclear. We and others have found that completely immature dengue particles are not infectious in mosquito cells *in vitro* yet the *in vivo* infectivity remains to be seen. It is possible that enzymes or other factors may render the immature stages of the virus infective to the mosquito. Recent and ongoing experiments have given us valuable insight into the function of these immature virus particles when it comes to vector infectivity. Results point to exciting new roles for virions in various stages of maturity when taken up by the mosquito vector.

177

HIGH PREVALENCE OF FLAVIVIRUS EXPOSURE AMONG PREGNANT WOMEN IN MYSORE, INDIA

Nandita Ganesh[1], Kavitha Chandrappa[2], Bhavana Niranjankumar[2], Julie Brichard[1], Ginny Gildengorin[1], Purnima Madhivanan[3], Desiree LaBeaud[1]

[1]Children's Hospital and Research Center, Oakland, Oakland, CA, United States, [2]Public Health Research Institute, India, Mysore, India, [3]Florida International University, Miami, FL, United States

Dengue is a flavivirus infection whose prevalence has been steadily growing in recent years, especially in the developing world. It has also been shown to affect pregnant women by increasing the rate of cesarean section deliveries and risk of pre-eclampsia. This pilot study describes the baseline incidence of dengue in a sample of pregnant women in Mysore, India. While 349 samples (89%) were seropositive for dengue, there was no significant association with demographic factors that were studied. The high numbers of flavivirus infection however, indicate that further research is crucial to better understanding and implementation of preventive measures.

178

MOLECULAR CHARACTERIZATION OF DENGUE VIRUSES IN THE PHILIPPINES 2008-2013

Ma. Jowina H. Galarion, Brian E. Schwem, Joy Ann Petronio, Raul V. Destura

National Institutes of Health, Manila, Philippines

Dengue is the most important arboviral disease in the Philippines with over 166,000 cases from January to November 2013. All four serotypes of dengue are found in the Philippines. However, to our knowledge, there have been no studies to characterize dengue strains at the molecular level in the Philippines. Molecular characterization is important to monitor changes in circulating strains. The aim of this study was to characterize the dengue strains circulating in the Philippines from 2008-2013. To date, 34 serotype 1, 18 serotype 2, and 24 serotype 3 samples have been sequenced and analyzed. Substitution rates were analyzed using BEAST software giving mean rates of 1.99x10-3 substitutions/site/year for serotype 1, 1.3136x10-3 substitutions/site/year for serotype 2, and 8.3243x10-4 for serotype 3. Single Breakpoint Recombination (SBP) and Genetic Algorithm for Recombination Detection (GARD) analysis showed potential breakpoints at 157 in capsid-premembrane gene of serotype 1 in seven samples and 194 in CprM gene and 587 in the Env gene of Serotype 3 in 4 samples. Initial analysis of the recombinant sequences of serotype 3 indicates recombination between genotypes I and IV. The recombination in both dengue serotypes 1 and 3 was first detected in samples from 2010 and serotype 1 recombinants were also detected in samples from 2011. The occurrence of these recombinants may be linked to virulence and improved transmission. These are probable factors in the evolution and dynamics of the dengue virus in the Philippines. No recombination was found in serotype 2 samples. More samples will be sequenced and analyzed with a target completion date of June 30, 2014.

179

VIROLOGICAL AND SEROLOGICAL INVESTIGATION OF THE FIRST DENGUE FEVER OUTBREAK IN ETHIOPIA

Mesfin M. Tsegaye

Ethiopian Public Health Institute, Addis Ababa, Ethiopia

Dengue fever is transmitted by the bite of a mosquito infected with one of the four dengue virus serotypes. In recent years, transmission has increased predominantly and has become a major international public health concern. In Ethiopia Dengue Fever has never been reported or laboratory confirmed previously. Unusual increment of febrile cases of unknown etiology was reported from Dire Dawa town, in eastern Ethiopia, in September 2013. Serological and virological investigations were carried to confirm or refute if arbo-viruses is the causative agent of the outbreak. Fifty serum samples were obtained from acute febrile illness patients who visited health facilities and also active case search for patients in convalescent phase of illness. The laboratory investigation of the serum samples included testing for the presence of IgM against arbo-viruses; namely flavi viruses (Yellow fever, Dengue, West Nile and Zika viruses) and Rift Valley Fever, Crimean-Congo Hemorrhagic Fever and Chikungunya viruses by ELISA as well as PCR testing for the presence of viral nucleic acids and nested PCR for sero-typing of dengue viruses. The results of the laboratory investigation showed that 15 of the 50 tested samples were positive for IgM against Dengue infection by ELISA (30%) and 11 were also positive by PCR (22.0%), one case was positive by both techniques giving a total dengue infection positivity rate of 50%. Relatively lower rate of Flavi-virus cross reactive IgM positivity was also observed for yellow fever (22%), Westnile (20%), and Zika (10%) viruses as compared to dengue. None of the samples were positive for IgM against rift valley fever, Crimean-Congo Hemorrhagic Fever and Chikungunya. Except for dengue, none of samples were positive by PCR for the tested viruses. Sero-typing of the virus revealed that the infection was caused by dengue virus sero type 2. It is evident from the laboratory investigation that the acute febrile illness outbreak was caused by Dengue fever virus sero type 2 and this is the first laboratory finding confirming the first dengue outbreak in Ethiopia.

180

INTRACELLULAR COMPARTMENTALIZATION OF DENGUE VIRUS DURING ANTIBODY-DEPENDENT INFECTION

Eugenia Ong, Summer Zhang, Hwee Cheng Tan, Kuan Rong Chan, Eng Eong Ooi

Duke-NUS Graduate Medical School, Singapore, Singapore

Dengue virus (DENV) continues to put billions at risk of life-threatening disease annually. Infection is enhanced when DENV is opsonized with sub- or non-neutralizing antibodies that augment entry into monocytes and dendritic cells through Fc-gamma receptors (FcγRs), a process termed antibody-dependent enhancement of DENV infection (ADE). It has also been suggested that besides augmenting entry, ADE occurs through other intrinsic factors activated by FcγR-mediated signaling. We recently reported a role for leukocyte immunoglobulin-like receptor B1 (LILRB1) in downregulating activating FcγR-mediated induction of the interferon stimulated genes (ISG). Co-ligation of LILRB1 by antibody-opsonized DENV recruits the phosphatase SHP-1 to dephosphorylate Syk and hence reduce ISG induction. In this work, we test the hypothesis that reduced Syk

signaling would also lead to differences in the compartmentalization of DENV-containing phagosomes, which may influence the outcome of ADE. We observed that higher levels of phosphorylated Syk permitted faster phagocytic trafficking of DENV immune complexes through Rab-5, Rab-7 and LAMP-1 compartments. When Syk activity was inhibited either by LILRB1 co-ligation or by piceatannol, a Syk-selective inhibitor, phagosomes displayed reduced levels of trafficking markers. Further interrogation of proteomic changes in phagosomes isolated under differential levels of Syk phosphorylation provides insights into how phagosomes are trafficked for ADE. Collectively, our data suggests that antibody-dependent infection enables DENV to be trafficked into compartments that may be more congenial for replication.

181

A DENGUE HUMAN INFECTION MODEL TO EVALUATE THE PROTECTIVE EFFICACY OF THE LIVE ATTENUATED TETRAVALENT DENGUE CANDIDATE VACCINE TV003

Anna P. Durbin[1], Dan Elwood[1], Cecilia Tibery[1], Beulah Sabundayo[1], Cathy Larsson[2], Adrienne Jarvis[2], Matthew Jo[1], Janece Lovchik[1], Marya Carmolli[2], Ben McElvany[2], Cassandra Ventrone[2], Stephen S. Whitehead[3]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]University of Vermont, Burlington, VT, United States, [3]National Institutes of Health, Bethesda, MD, United States

Dengue virus (DENV) has become the most important arbovirus worldwide with estimates of as many as 400 dengue infections occurring annually. These infections result in approximately 100 million symptomatic cases of which more than 2 million are classified as severe disease and approximately 21,000 result in death. Neutralizing antibody, the accepted immunologic endpoint chosen to move candidate DENV vaccines forward, was not predictive of protection against DENV illness in the field in a recently completed Phase 2b efficacy trial of the lead DENV candidate vaccine CYD. These results have made it more difficult for vaccine manufacturers to determine which candidates should be further evaluated in large efficacy trials in endemic areas where vaccine failure could predispose subjects to more severe disease should they subsequently become infected with DENV. A dengue human infection model (DHIM) would be useful in down-selecting candidate vaccines prior to testing in endemic areas as well as identifying putative correlates of protection. We have developed a DHIM to evaluate the protective efficacy of the live attenuated tetravalent dengue vaccine TV003 developed by the U.S. National Institutes of Health. In Phase I clinical trials, TV003 induced neutralizing antibody to DENV-1, DENV-2, DENV-3, and DENV-4 in 92%, 76%, 97%, and 100% of vaccinees, respectively. It induced seroconversion to all four DENV serotypes in 74% of vaccinated subjects and to three or more serotypes in 98%. Because the seroconversion rate to DENV-2 was lowest, we sought to evaluate protection elicited by the vaccine against a heterotypic strain of DENV-2. We vaccinated 24 flavivirus-naïve subjects with a single dose of TV003; an additional 24 subjects received a placebo. Six months later, all 48 subjects received a challenge dose of 1,000 PFU of rDEN2Δ30, an under-attenuated DENV-2 vaccine candidate. DEN2Δ30 was previously demonstrated to induce viremia in 100% of inoculated subjects with a mean peak virus titer of 2.5 log10 PFU/mL. In addition, 80% of subjects developed a maculopapular rash and 40% developed neutropenia. The study is powered to detect 60% efficacy of TV003 to prevent viremia caused by rDEN2Δ30 (the primary outcome). Secondary outcomes include protection against rash and neutropenia. Preliminary clinical, virological, and efficacy outcomes of the trial will be presented.

182

CHARACTERIZATION OF CELLULAR RESPONSES TO A TETRAVALENT LIVE-ATTENUATED DENGUE VACCINE IN NON-HUMAN PRIMATES AND FLAVIVIRUS-NAÏVE HUMAN VOLUNTEERS

Charalambos Partidos[1], Haiyan Chu[1], Yuping Ambuel[1], Eva Rakasz[2], Sarah George[3], Dan T. Stinchcomb[1], Jorge Osorio[1]

[1]Takeda Vaccines, Inc., Madison, WI, United States, [2]Primate Research Center, University of Wisconsin Madison, Madison, WI, United States, [3]St. Louis University, St. Louis, MO, United States

We have developed a live attenuated tetravalent dengue vaccine candidate based on an attenuated dengue 2 virus (DENV-2) and three chimeric viruses containing the pre-membrane and envelope genes of DENV-1, -3 and -4 expressed in the context of the attenuated DENV-2 genome. The cellular responses elicited by this vaccine were analyzed in: 1) non-human primates after a single subcutaneous administration, and 2) flavivirus-naïve human volunteers vaccinated twice (day 0, 90) via the subcutaneous or intradermal routes. Using peptide arrays and intracellular cytokine staining, we demonstrated that the vaccine elicits $CD4^+$ and $CD8^+$ T cell responses targeting the non-structural NS1, NS3 and NS5 proteins of DENV-2, and E proteins of each DENV serotype. Both T cell subsets produced IL-2, IFN-γ, and TNF-α, and expressed the CD107a marker. $CD8^+$ T cell responses in humans were highest on day 90 after the first immunization and were still detectable on day 160 post-secondary immunization. In both species, $CD8^+$ T cells were multifunctional, producing ≥2 cytokines simultaneously, and cross-reactive to the NS proteins of the other serotypes. Overall, these findings highlight the immunogenic profile of our candidate dengue vaccine and support the further evaluation of clinical samples from ongoing phase II clinical trials.

183

ORGAN-HOMING TARGETS OF ANTIBODY SECRETING CELLS DURING ACUTE DENGUE VIRAL INFECTION

Nattawat Onlamoon, Ampaiwan Chuansumrit, Kulkanya Chokephaibulkit, Kanchana Tangnararatchakit, Chonnamet Techasaensiri, Nopporn Apiwattanakul, Ladawan Khowawisetsut, Tipaporn Sae-Ung, Kasama Sukapirom, Kovit Pattanapanyasat

Mahidol University, Bangkok, Thailand

B cell responses play important roles during dengue viral infection due to the ability to produce both protective and disease enhanced antibody. Although many studies demonstrated a major expansion of antibody secreting cells (ASC), the precise mechanisms for this massive increase in the frequency of ASC and their mobilization remain unclear. The analysis of cell surface homing molecules on ASC may provide some clues for the organ or tissue distribution of these cells. In this study, the blood samples from dengue infected patients were analyzed by polychromatic flow cytometry for B cell subset distribution based on the expression of CD21, CD27 and CD38 and the expression of cell surface homing molecule on ASC including CCR7 and CD62L (to the regional lymph nodes), CXCR3 and ICOS (to the lung), CCR10 (to the skin), beta7, CCR9 and CD103 (to the gut tissue), CCR2 and beta1 (to the central nervous system) and CXCR4, CD122, CD132 and CD137 (to the bone marrow). The results showed that the frequencies of ASC (CD21-/CD27hi/CD38hi) varied from 16.1% to 87.1% of total B cells depend on the time when samples were collected. At the time of defervescense, ASC had a highest frequency (47.9%) when compared to naïve (32.5%), resting memory (9.3%), activated memory (4.4%) and tissue memory (6.1%) B cells. Frequencies of ASC remained higher than other B cell subsets even 2 days after defervescense date. Although high frequencies of ASC were observed, the results showed no significant difference when compared between patients with dengue fever and dengue hemorrhagic fever. When the expressions of homing markers were determined, the results showed high frequencies of ASC that expressed CD62L (79.4%), CXCR3 (71.9%), CCR10 (99.8%), beta7 (75.7%), CXCR4 (84.13%) and CD132 (99.9%). Almost half of

ASC expressed CCR2 (48.3%) whereas only a minor population expressed CCR7 (29.4%) or CCR9 (22.8%). Low frequencies of ASC expressed beta1 (3.9%) or CD122 (5.7%) whereas ICOS, CD103 and CD137 could not be observed (less than 1%). The results suggested that large proportions of ASC may distributed to various organs and tissues including lymph nodes, lung, skin, gut and bone marrow whereas minor proportions mobilized to the central nervous system. The results obtained in this study provide novel insights on the target distribution of ASC to specific organ and tissue where it plays protective or disease enhanced roles during acute dengue infection.

184

HOUSEHOLD LEVEL ECONOMIC BURDEN OF DENGUE VIRUS INFECTION IN PUERTO MALDONADO, PERU

Gabriela Salmon-Mulanovich[1], David L. Blazes[2], Andres G. Lescano[1], Joel M. Montgomery[3], Daniel G. Bausch[1], William K. Pan[4]

[1]U.S. Naval Medical Research Unit - 6, Callao, Peru, [2]Navy Medicine Professional Development Center, Bethesda, MD, United States, [3]Division of Global Disease Detection, Centers for Disease Control and Prevention, Nairobi, Kenya, [4]Duke Global Health Institute and Nicholas School of Environment, Duke University, Durham, NC, United States

Dengue virus (DENV) is an arbovirus with global distribution. DENV was reintroduced to Peru in the 1990s and has been reported in Puerto Maldonado (population ~80,000) in the Peruvian southern Amazon Basin since 2000. This region also has the highest human migration rate in the country, much of it from areas where DENV is not endemic. Several studies have reported on the economic burden of dengue disease from societal, health care system or governmental perspectives but few have focused on the financial burden at the household level. We therefore sought to assess the household income diverted to costs incurred when a household member contracts DENV and to compare costs between recent migrants (RM) and longer-term residents (LTR), defined respectively as residency in Puerto Maldonado for less than or greater than 5 years. We administered a standardized questionnaire to persons diagnosed with dengue disease at Hospital Santa Rosa in Puerto Maldonado from December 2012 to March 2013. We compared direct and indirect medical costs and compared between RM and LTR. Demographic data, socioeconomic characteristics and assets were also compared between RM and LTR. Of the 80 persons who completed the survey, 28 (35%) were RM. Each dengue disease episode cost the household an average of US$ 105 (SD=107), representing 24% of their monthly income. Indirect costs were the greatest expense (US$ 56, SD=87), especially lost wages. LTR households had a higher average monthly income than RM ones (p=0.041) and were significantly more affluent based on wealth index (p=0.002). Costs did not differ significantly between RM and LTR households. The study highlights the very significant financial burden incurred by households when a family member suffers dengue disease, especially for RM since their overall monthly income is lower.

185

COMPARING FLUORESCENT FOCUS ASSAYS (FFAS) WITH PLAQUE ASSAYS (PAS) IN DENGUE CONFIRMED CASES

Juan Sulca[1], Kanya C. Long[2], Thomas W. Scott[3], Carolina Guevara[1], Marita Silva[1], Elizabeth Castillo[1], Amy C. Morrison[1]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Andrews University, Berrien Springs, MI, United States, [3]University of California Davis, CA, United States

Plaque formation assays and mosquito inoculations are two widely used techniques to quantify dengue viruses. Each of these techniques has its limitations; for example, mosquito inoculations are sensitive but require regular maintenance of a mosquito colony, and the sensitivity of plaque assays (PA) depends on the type of cell line used and the ability of a particular virus to induce a desired cytopathic effect (CPE). The sensitivity of C6/36 *Aedes albopictus* cells to dengue virus (DENV) infection has been well documented; however, DENV causes little apparent CPE in this cell line. Fluorescent focus assays (FFA) are a variation of PA where focused areas of infection are visualized by detecting viral antigen with monoclonal antibody. To quantitate DENV infections in cell culture, we performed serial dilutions of all four DENV serotype seeds, infected Vero 76 and C6/36 cells using the FFA in 8-well chamber slides, and performed PA using BHK-21 clone 15 cells in 24-well plates. We also titrated 8 and 15 samples positive for DENV-2 and DENV-4 by viral isolation, respectively. Seed viruses showed higher titers of DENV-1, DENV-3 and DENV-4 using FFA in 2-3 days in both cells lines compared with 5-7 day in PA. Compared with PA, FFA in C6/36 cells resulted in higher titers of DENV in 14 samples (61%), similar titers in 2 samples (9%), and lower titers in 2 samples (9%). Only 3 samples (13%) were positive by FFA in Vero cells. Although more expensive than PA, FFA does not require the development of CPE in the infected cell monolayer. In conclusion, C6/36 cells are ideal for DENV titration in humans, increasing sensitivity and producing more accurate quantifiable DENV results.

186

DESIGNING GOAL-ORIENTED DISEASE SURVEILLANCE NETWORKS: A DENGUE VIRUS CASE STUDY

Samuel V. Scarpino[1], Lauren A. Meyers[2], Michael Johansson[3]

[1]Santa Fe Institute, Santa Fe, NM, United States, [2]The University of Texas at Austin, Austin, TX, United States, [3]Centers for Disease Control and Prevention, San Juan, PR, United States

With public health facing increasing budget constraints, disease surveillance is at a critical juncture. To design effective surveillance under resource constraints, we propose a four-step process that produces a surveillance system through systematic evaluation and integration of candidate data streams. This methodology quantifies the performance of individual data streams in terms of the specified surveillance objectives, and prioritizes them for incorporation into surveillance systems. We demonstrate the utility of this method by designing a multi-objective Dengue surveillance network for Puerto Rico. Networks designed using this approach were able to effectively monitor regional and local Dengue outbreaks, track serotype dynamics, and provide early warning for large outbreak years. This system was predicted to perform at least as well as the existing system and only utilized 20% of the existing providers.

187

LONGITUDINAL ANALYSIS OF SERUM AVIDITY FOLLOWING SECONDARY DENGUE VIRUS TYPE 2 INFECTION IN PATIENTS WITH DIFFERENT DISEASE SEVERITY

Louis Lau, **Angela M. Green**, Eva Harris

Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States

The four dengue virus serotypes (DENV1-4) are responsible for the most prevalent mosquito-borne viral illness in humans. DENV causes a spectrum of disease ranging from self-limiting dengue fever (DF) to severe, life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Secondary (2°) DENV infection is associated with more severe disease, which is attributed to cross-reactive antibodies and T cells. However, antibodies and T cells can also mediate protection. The effectiveness of the antibody response is dependent on both the affinity and avidity of the antibody/antigen interaction, where avidity depends on the antibody isotype and the number and affinity of individual binding sites. We sought to determine how antibody avidity developed over time following 2° infection with DENV2 and whether avidity differed according to disease severity. We analyzed sera from 41 2° DENV2-infected subjects (DF, n=15; DHF, n=15; DSS, n=11) from an ongoing hospital-based study of pediatric dengue in Managua. IgG avidity against DENV2 whole virion antigen (DENV2 strain N172) was measured in samples collected during the acute and convalescent phases as well as 3, 6, 12, and 18 months post-illness using a urea avidity enzyme-linked immunosorbent assay

(ELISA). With a stringent cutoff of background absorbance <0.2 OD, a positive control absorbance of >5X background OD, and positive control within one standard deviation from a quality control plate, the avidity was calculated as a ratio between percent IgG bound in 9M urea-treated wells compared with PBS-treated wells. Longitudinal analysis of serum IgG avidity against DENV2, regardless of disease severity, parallelled our previously published kinetics of avidity in 2° DENV3 infections. Our data show a substantial increase in avidity from acute to convalescent phase followed by a decrease in avidity from the convalescent phase to 3-months post-infection, then a plateau. During the acute phase, individuals who would develop more severe disease demonstrated higher serum avidity against DENV2. In contrast, at 18 months post-infection, sera from individuals with more severe disease displayed lower avidity when compared to sera from cases with less severe disease. Taken together, these data show that the kinetics of serum avidity following 2° DENV2 and DENV3 infection are similar. In addition, the data suggest that serum avidity levels may correlate with disease severity.

188

QUAD-COLOR B CELL FLUOROSPOT: A NOVEL APPROACH FOR ANALYZING THE SEROTYPE SPECIFICITY AND CROSS-REACTIVITY OF ANTIBODY-SECRETING CELLS IN DENGUE VIRUS-INFECTED INDIVIDUALS

Alexandros Hadjilaou, Angela Green, Josefina Coloma, Eva Harris

Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States

The 4 dengue virus serotypes (DENV1-4), members of the flavivirus genus, cause the most prevalent mosquito-borne viral illness in humans, with >3 billion people at risk for infection and up to 96 million cases annually worldwide. DENV infection can result in self-limiting dengue fever or more severe life-threatening disease. Severe disease is thought to be associated with the presence of serotype cross-reactive antibodies (Abs) that enhance viral uptake into target cells during a heterologous secondary infection. In contrast, type-specific Abs after a primary infection are important for homotypic protection. However, little is known about the protective role of cross-reactive Abs. To date, studies have analyzed DENV-specific B cells using ELISPOT assays and flow cytometry or by generating monoclonal Abs (MAbs) from memory B cells (MBCs) or plasma cells. We have developed a novel approach to determine the frequency of serotype-specific and serotype cross-reactive MBC responses on a per-cell basis. Using a modified version of an ELISPOT and applying recent advances in fluorescent detection (FluoroSpot), we can evaluate MBC specificity to all 4 DENV serotypes simultaneously in individual cells. The MBCs from cryopreserved peripheral blood mononuclear cells (PBMCs) of DENV-exposed individuals were stimulated *in vitro* for 3-5 days to become Ab-secreting cells and then incubated in ELISPOT plates coated with a human IgG capture Ab for 1-2 days. Subsequently, OptiPrep-purified virions from the four serotypes (Nicaraguan DENV1-4) were added to bind to DENV serotype-specific and cross-reactive Abs secreted by the Ab-secreting B cells. Visualization was achieved using four mouse anti-E Domain III serotype-specific MAbs that were labeled with different fluorophores. Serotype-specific MBCs and MBCs cross-reactive to 2, 3, or 4 DENV serotypes were detected in different individuals, with type-specific and cross-reactive MAb hybridomas used as controls. We are currently applying this method to analyze longitudinal samples (acute, convalescent, and 3, 6, 12, and 18 months post-illness) from primary and secondary DENV infections in a hospital-based dengue study in Nicaragua. This new technique will further the understanding of how MBC specificity evolves over time, how the serotype-specific and cross-reactive responses associate with disease outcome, and how serial infections with heterotypic serotypes drive MBC specificity.

189

CO-INFECTION WITH INFLUENZA AND DENGUE VIRUS LEADS TO SEVERE DISEASE VIA MODULATION OF THE HOST RESPONSE

Michael A. Schmid[1], Karla N. Gonzalez[2], José Peña[3], Eva Harris[1]

[1]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States, [2]Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua, [3]Lawrence Livermore National Laboratory, Livermore, CA, United States

Both influenza and dengue are major health problems worldwide. In 2009, Nicaragua experienced a large dengue epidemic, even though the dominant dengue virus serotype (DENV3) and virus clade remained the same between 2008 and 2011. In addition, the 2009 dengue epidemic was atypical, with early onset of compensated shock. The only identifiable epidemiological difference in 2009 was the influenza A-H1N1 pandemic, which was shifted from the usual seasonal influenza peak and instead overlapped with the dengue epidemic for 8-10 weeks. We hypothesized that sequential or co-infection of influenza H1N1 and DENV modulated the immune response, leading to the atypical presentation and more severe dengue disease. We then established a mouse model of dual infection with Nicaraguan pandemic H1N1 influenza clinical isolate A/NI/5227/2009 (intranasal) and the virulent DENV2 strain D220 (intravenous). DENV suppresses the interferon response, replicates, and causes disease in humans but not in wild-type mice. Thus, we used C57BL/6 mice lacking the interferon-α/β receptor (*Ifnar*$^{-/-}$) that were susceptible to DENV2 and influenza virus infection. Influenza virus infection followed after two days by DENV2 caused 90% lethality in *Ifnar*$^{-/-}$ at virus doses that caused mild disease during infection with either virus alone. DENV2 infection followed two days later by influenza H1N1 infection caused 50% lethality in *Ifnar*$^{-/-}$ mice. The viral load of DENV2 and influenza virus H1N1 was similar in the lungs and other tissues of *Ifnar*$^{-/-}$ mice infected with one or both viruses; thus, viral load does not appear to explain the decreased survival of *Ifnar*$^{-/-}$ mice sequentially infected with the two viruses. We are currently investigating the inflammatory response via Nanostring nCounter gene expression analysis of mRNA in mice infected with one or both viruses to establish immunological pathways that determine disease outcome. Preliminary results suggest differential expression of inflammatory genes in the lungs of mice infected with both viruses compared to mice infected with only one virus. Our study may inform treatment and vaccine strategies in endemic areas where dengue and influenza viruses co-circulate.

190

A NATURALLY OCCURRING SINGLE POINT MUTATION IN THE ENVELOPE PROTEIN CHANGES THE NEUTRALIZATION PROFILE OF DENV2 ASIAN 1 VIRUSES

Chunling Wang[1], Leah Katzelnick[2], Magelda Montoya[1], Eva Harris[1]

[1]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States, [2]Centre for Pathogen Evolution, University of Cambridge, Cambridge, United Kingdom

The four serotypes of dengue virus (DENV1-4) cause the most medically important arthropod-borne viral disease worldwide. Each serotype is comprised of several genotypes. A DENV2 genotype replacement event in Vietnam, where the previously dominant Asian-American (AA) genotype was entirely replaced by the Asian 1 (A1) genotype, was observed during 2003-6. We investigated the possible role of pre-existing immunity in driving genotype replacement by generating reporter virus particles (RVPs) representing the two genotypes and analyzing their neutralization profiles by patient sera collected in Vietnam prior to (1997-8) and after (2006-7) the replacement event. The Vietnamese 2007 samples neutralized the A1 better than the AA genotype, as did sera from post-secondary DENV2 infections in a Nicaraguan cohort. However, among A1 isolates

that shared the 5 amino acid changes in charge or side chain between the E genes of A1 and AA -- N83K, D203N, T226K, G228E, and H346Y -- one A1 isolate had a different neutralization profile when analyzed using antigenic cartography with non-human primate antisera. Six A1 isolates were poorly neutralized by both homologous and heterologous antisera, but the outlier A1 was strongly cross-neutralized by DENV1 and DENV3 antisera and had a distinct substitution, K160Q. This mutation was observed in strains isolated at the end of the Vietnam DENV2 A1 epidemic, in a clade that persisted for multiple years. To test if this amino acid substitution determined differences in neutralizing antibody titers with human antisera, the A1 RVP with 160Q was mutated to K. Interestingly, the dominant neutralizing phenotype was lost in the Q160K A1 RVP when tested with Nicaraguan cohort antisera from post-secondary DENV2 infections (1.7-fold reduction, $p<0.001$), post-primary DENV3 infections (2.4-fold reduction, $p<0.001$), and post-primary DENV1 infections (1.5-fold reduction, $p<0.05$). We are analyzing changes in neutralization titer to 160K and 160Q variants by a panel of monoclonal antibodies sensitive to virion structure to determine if mutations at position 160, located in a valley on the surface of E Domain I, alter the global structure of the virus. In sum, our findings from independent analyses indicate that a clade of viruses circulating for multiple years in Vietnam had a single point mutation in the E protein (K160Q) that significantly altered polyclonal neutralization, in particular by heterologous antisera.

191

DIAGNOSTIC UTILITY OF ATYPICAL LYMPHOCYTES TO IDENTIFY DENGUE AND SEVERE DENGUE

Gamaliel Gutierrez[1], **Lionel Gresh**[1], Douglas Elizondo[1], Maria Angeles Pérez[2], Angel Balmaseda[3], Eva Harris[4]

[1]Sustainable Sciences Institute, Managua, Nicaragua, [2]Hospital Infantil Manual de Jesús Rivera, Ministerio de Salud, Managua, Nicaragua, [3]Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua, [4]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States

Dengue is the most prevalent mosquito-borne viral disease of humans worldwide. Dengue virus (DENV) infection can cause a range of outcomes, from asymptomatic infections and undifferentiated fever to dengue fever and severe illness. Prompt and accurate diagnosis of dengue, as well as the identification of severe cases, remains a challenge, in particular in resource-limited settings. Atypical lymphocytes, aka Downey cells or reactive lymphocytes, have been described in several infectious, autoimmune, and idiopathic disorders, including severe dengue. Atypical lymphocytes can be identified morphologically and quantified in blood smears simply using a light microscope. Here, we analyzed the diagnostic value of atypical lymphocytes, quantified as percentage of total lymphocytes, for the identification of 1) confirmed dengue cases and 2) severe dengue cases among suspected dengue cases. A total of 1,522 suspected dengue cases, aged 6 months to 14 years and enrolled in a prospective, hospital-based study in Managua, Nicaragua, from August 2005 to November 2013 were included (964 laboratory-confirmed dengue-positive and 558 dengue-negative cases). Daily measurements of atypical lymphocytes were performed (total: 4,416; range: 1-6 per case). On days 1 and 2 of illness, no difference was observed in the proportion of atypical lymphocytes between dengue-positive and dengue-negative cases. However, from day 3 on, the proportion of atypical lymphocytes was significantly higher in dengue-positive cases ($p<0.01$). On day 5, the best cut-off value for atypical lymphocytes was estimated to be ≥2%, with a sensitivity of 64.6% (95%CI: 61.0-68.8) and a specificity of 62.3% (57.1-67.3) for dengue positivity. We then compared severe dengue cases, defined using the 2009 WHO classification, vs. non-severe cases. A significant difference was not observed between these groups on days 1-3; however, on days 4-7, the proportion of atypical lymphocytes was higher in severe dengue cases ($p<0.01$). At day 5, the best cut-off that differentiated severe from non-severe cases was ≥3% of atypical lymphocytes, with a sensitivity of 66.7% (60.8-72.1) and a specificity of 52.0% (47.3-56.7). Our results suggest that atypical lymphocytes can be used to aid in the diagnosis of dengue and severe dengue. Additional signs, symptoms, and blood parameters could be used in combination with atypical lymphocytes to improve their diagnostic utility.

192

LOWER CHOLESTEROL LEVELS ARE ASSOCIATED WITH SEVERE DENGUE OUTCOME

Hope Biswas[1], Aubree Gordon[1], Douglas Elizondo[2], Andrea Nuñez[3], Crisanta Rocha[4], Maria Angeles Pérez[4], Angel Balmaseda[3], Eva Harris[5]

[1]Division of Epidemiology, University of California Berkeley, Berkeley, CA, United States, [2]Sustainable Sciences Institute, Managua, Nicaragua, [3]Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua, [4]Hospital Infantil Manual de Jesús Rivera, Ministerio de Salud, Managua, Nicaragua, [5]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States

Dengue virus is a flavivirus of worldwide importance, with approximately 3.97 billion people across 128 countries at risk of infection and up to 96 million dengue cases annually. Previous studies have shown that lipids and lipoproteins may play a role in modifying virus infectivity and the host's immune response to infection. However, the relationship between total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and severe dengue outcome is unclear. We analyzed data from 789 laboratory-confirmed pediatric dengue cases and 447 other febrile illnesses (OFI) in a prospective hospital-based study in Managua, Nicaragua, between August 2005 and January 2013, and used three different classifications of dengue severity: World Health Organization (WHO) 1997, WHO 2009 and standardized intervention categories. To examine the association between total serum cholesterol at first presentation and risk of severe dengue outcome, relative risks and 95% confidence intervals (CIs) were calculated using modified Poisson models with robust standard errors, adjusted for day of illness and other confounders. We found that total cholesterol, HDL-C and LDL-C decreased over the course of illness and differed across disease outcome groups. Greater decreases were observed in LDL-C compared to HDL-C. Multivariate models showed that lower levels of total serum cholesterol were associated with severe dengue outcome compared to OFI and mild dengue outcome. At first presentation, for each 10 mg/dL decrease in total serum cholesterol, the risk of severe dengue outcome increased by 14% (95% CI: 6-22%) using the WHO 1997 classification, 6% (95% CI: 2-11%) using the WHO 2009 classification, and 3% (95% CI: -0.02-9%) using standardized intervention categories. Models using the cut-point of total serum cholesterol <85mg/dL had cross-validated areas under the curve (AUC) ranging from 0.7-0.8. These results indicate the relatively specific ability of cholesterol to discriminate patients with severe dengue outcome from patients with OFI and mild dengue outcome when they first present to the hospital.

194

MOLECULAR SURVEILLANCE OF DENGUE VIRUS (SEROTYPES 1 TO 4) AND ITS SEVERAL GENOTYPES IN A MEDIUM SIZE CITY OF SOUTHEASTERN BRAZIL, DURING EIGHT EPIDEMICS YEARS

Danila Vedovello[1], Tatiana E. Colombo[1], Tauyne M. Pinheiro[1], Leonidas M. Pires[1], Joice M. Biselli[2], Betania Drumond[3], Paula Rahal[2], Maurício L. Nogueira[1]

[1]FAMERP, São José do Rio Preto, Brazil, [2]UNESP, São José do Rio Preto, Brazil, [3]UFJF, São José do Rio Preto, Brazil

Dengue is a viral infection transmitted by the bite of an infected *Aedes* mosquitos. This illness is endemic throughout the tropics and subtropic (100 countries worldwide) with a geographic distribution similar to that of malaria, but more risk in urban and residential areas. The four serotypes

of dengue virus (DENV 1-4) (family Flaviviridae, genus Flavivirus) are antigenically and genetically distinct and their genotypes vary in virulence. Wherefore, the detection and analysis of spatial and temporal transition are essential. In this work we looked the Dengue viruses circulation in Sao Jose do Rio Preto (a 434K habitants city) from 2006 to 2014. São José do Rio Preto show subquente tropical and humid weather, ideal for the proliferation of vector and has annual epidemics and hyperendemicity situations. We used serum samples of suspected DENV patients provided by the Public Health to profile DENV circulation by PCR. We analyzed 2.162 cases from January 2006 to January 2014. We amplified 1372 (63,45%) samples of DENV: 389 (28,35%) were positive for DENV-1, 177 (12,90%) for DENV-2, 494 (36,00%) for DENV-3, 312 (22,74%) DENV-4 and 4 (0,29%) DENV-1/4 coinfection. Studying the movement of DENV from 2006 to 2014 we get to see the introduction, extinction and re introduction of serotypes in the city. Up to now, we got 20 sequences of DENV-1 (inferred E protein) and 5 sequences of DENV-1 (full genome); 26 sequences of DENV-2 (E protein) and 4 sequences of full genome; and 31 sequences of DENV-4 (E protein) and 4 sequences of full genome. The phylogenetic reconstruction of serotypes 1, 2 and 4 show that the samples identified in this study grouped with genotypes that circulating in Brazil (genotypes V, Asian American and American, for types 1, 2 and 4 respectively). We have identified two lineages of DENV 1 circulating in the region ad the others serotypes showed no lineages divergences. Some E protein aa substitutions were detected between the two strains of DENV-1. This data shows that the phylodinamics of dengue circulation can be much more complex than expected even in a medium size city and reinforces the importance of DENV constant surveillance.

195

COMPARISON OF DENGUE TRANSMISSION INTENSITY ESTIMATES OBTAINED FROM DIFFERENT DATA TYPES

Natsuko Imai[1], Simon Cauchemez[2], Ilaria Dorigatti[1], Neil M. Ferguson[1]

[1]Imperial College London, London, United Kingdom, [2]Insitut Pasteur, Paris, France

With more than 2.5 billion individuals at risk and an estimated 390 million infections per year globally, the vector-borne viral infection dengue continues to be a major public health problem. Prior knowledge of local dengue transmission is essential to design effective vector control and vaccination strategies. Yet estimates of global distribution and transmission intensity remain highly ambiguous. Most of the models estimating dengue transmission risk have utilised notification data which depend heavily on the quality of the surveillance system. Furthermore since the majority of dengue infections are asymptomatic, assessing the true burden of dengue is difficult. We estimated the force of infection (λ) by fitting a catalytic model to age-stratified seroprevalence IgG data from 7 countries using a Metropolis-Hastings Markov Chain Monte Carlo algorithm. We also estimated λ and the probability of detection of sequential infections by fitting a catalytic model to case-notification data from Puerto Rico. We find that the estimates of the force of infection vary substantially depending on the type of data fitted, with seroprevalence data resulting in higher estimates of λ on average, and that reporting rates and sequential infections play a major role in how dengue burden is currently assessed. Although IgG data are unable to differentiate between the dengue serotypes, they can identify past asymptomatic infections, are easy and cheap to obtain and can provide an insight into local dengue dynamics. On the other hand, case notification time series data, although subject to reporting bias, is more readily available. Combining the two data sources allows a more robust estimation of dengue transmission intensity and helps to identify areas where clinical dengue surveillance may require improvement.

196

ESTIMATING DENGUE INTER-SEROTYPE INTERACTIONS FROM AGE-STRATIFIED SEROTYPE SPECIFIC DATA

Natsuko Imai[1], Simon Cauchemez[2], Ilaria Dorigatti[1], Neil M. Ferguson[1]

[1]Imperial College London, London, United Kingdom, [2]Insitut Pasteur, Paris, France

With an estimate of 390 million infections per year globally, dengue continues to be a major public health problem. While the majority of infections are asymptomatic, the presence of multiple serotypes circulating simultaneously in a population increases the risks of severe infection due to antibody-dependent enhancement (ADE). Although costly compared to IgG ELISAs, plaque reduction neutralisation tests (PRNTs) can determine the infecting serotype of past infections, are cheaper than PCR and are considered the gold standard for routine serotyping. The serotype with the highest titre is regarded as the most recent infecting serotype. However due to the cross-reactivity of anti-dengue antibodies it is difficult to definitively determine primary and secondary infecting serotypes from PRNTs alone. Thus it is essential not only to identify populations previously exposed to dengue and thus at risk of severe dengue upon heterologous infection, but to estimate the degree of immune-mediated inter-serotype interactions. We developed a catalytic model to estimate serotype-specific forces of infection (λ) and the level of interaction between serotypes (susceptibility enhancement-inhibition following a primary infection) from cross-sectional PRNT data. The model was fitted to multiple PRNT datasets from 1985 - 2010 using a Metropolis-Hastings MCMC algorithm. We find that if one serotype has a significantly higher λ than the others, the basic reproduction numbers are driven by the dominant serotype. We can infer the probable order of sequential heterologous infections by comparison of the interaction parameters assuming interaction was dependent solely on the primary or secondary infection strain. All estimates were comparable to previously published values of R0 estimated from incidence data. Our models provide an insight into dengue transmission intensity in different countries at different time points and highlight the complex transmission dynamics of dengue.

197

METHOD DEVELOPMENT AND CHARACTERIZATION OF *IN VITRO* RELATIVE POTENCY (IVRP) ASSAY FOR A TETRAVALENT RECOMBINANT SUBUNIT ENVELOPE-BASED DENGUE VACCINE CANDIDATE

Liman Wang[1], Cindy Pauley[1], Kuo Yin[1], Andrew Bett[1], Adam C. Finnefrock[1], John Loughney[1], Tingyi Zhou[1], Keith Gottlieb[2], Sarah Daijogo[2], Man Tran[2], My-Lien Pham[2], Beth-Ann Griswold Coller[3], Zhi-qiang Zhang[1]

[1]Merck Co., Inc., West Point, PA, United States, [2]Focus Diagnostics, Inc., Cypress, CA, United States, [3]Merck Co., Inc., North Wales, PA, United States

Dengue is a widespread and potentially fatal disease, with 50 - 100 million infections per year, and over 2.5 billion persons at risk globally. Dengue has four serotypes and a potential vaccine should elicit strong and balanced neutralizing responses to each type to reduce the risk of dengue hemorrhagic fever. Dengue envelope glycoprotein has been the focus of subunit-vaccine development as it is the primary target of neutralizing antibodies. We have developed a tetravalent (DEN1, 2, 3, 4) recombinant subunit envelope (E) protein based Dengue vaccine candidate. An *in vitro* relative potency (IVRP) assay has been developed as an alternative to the *in vivo* immunogenicity assay to support product release and characterization. In this method, Dengue antigens are detected by neutralizing monoclonal antibodies (mAbs) specific for E protein of each serotype. The mAbs for IVRP were selected by neutralization activity, binding affinity and kinetics, and stability-indicating attributes. These mAbs were further characterized by other methods including Biacore and Western blot. The IVRP assay uses a sandwich ELISA format, and measures relative potency of the Dengue

antigen sample as compared to a reference standard. The IVRP method is highly specific for each serotype, has a precision of 10-20% RSD, and can be completed in 2-3 days. The relationship between antigenicity measured by the IVRP and immunogenicity measured by the mouse potency assay was evaluated by testing several heat stressed Dengue antigen samples in both methods. The results showed that stressed samples had decreased antigenicity by IVRP and decreased immunogenicity by mouse potency assay relative to untreated control samples. This study also suggests that the IVRP assay is stability-indicating. With good specificity, precision, stability-indicating attributes, and quick turnaround, the IVRP assay is suitable for use as potency release and stability assay for subunit based Dengue vaccine candidates. Furthermore, this method may serve as a good alternative to the variable and time-consuming mouse potency assay for vaccine product release for licensure in the future.

198

MOSQUITO SALIVA FROM THE DENGUE VECTOR *AEDES AEGYPTI* MODULATES ENDOTHELIAL CELL PERMEABILITY *IN VITRO*: A NEW MECHANISM FOR VIRAL DISSEMINATION?

Henry Puerta Guardo[1], Dustin Glasner[1], Matthew Aliota[2], Laura D. Kramer[2], Eva Harris[1]

[1]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States, [2]Wadsworth Center, New York State Department of Health, Slingerlands, NY, United States

Dengue is the most important mosquito-borne viral disease, affecting >40% of the world's population, especially in tropical and sub-tropical areas where the main mosquito vector, *Aedes aegypti*, circulates. Infection with any of the four dengue virus serotypes (DENV1-4) causes a wide spectrum of illness from dengue fever to severe, potentially fatal disease mainly associated with plasma leakage, hemorrhage and organ failure. The infection initiates when a DENV-infected female *Aedes* mosquito probing for blood injects saliva, together with virus, into the skin of its human host. Mosquito saliva is known to enhance replication and pathogenesis of many arthropod-borne pathogens. In DENV infection, *Ae. aegypti* saliva has been shown to both hinder infection of dendritic cells *in vitro* and increase DENV titers in infected mice. However, the early events after DENV inoculation into the skin and the role of mosquito saliva in regulating virus dissemination is not well understood. Here we examine the ability of salivary gland extract (SGE) from female *Ae. aegypti* to modulate endothelial cell permeability *in vitro*. Briefly, cultures of human pulmonary microvascular endothelial cells (HPMEC) grown on a transwell permeable membrane system as a model of endothelial barrier function *in vitro* were exposed to SGE (1 SGE unit=5 µl) through either the apical (AP) or basolateral (BL) membrane, and endothelial permeability was examined by continuously measuring the trans-endothelial electrical resistance (TEER). A sharp reduction in TEER after treatment with 2 SGE units was detected with both AP and BL treatment (60%, $p<0.001$ and 30%, $p<0.05$ reduction, respectively). The AP effect was greater after the second dose of SGE compared to BL (TEER ratio: 0.35-0.66), while the BL effect persisted for a longer time (up to 48 hours). These results suggest distinct mechanisms for modulating endothelial permeability by SGE. A complex mixture of anti-hemostatic, anti-inflammatory, and immunomodulatory compounds has been identified in saliva of blood-sucking arthropods. Experiments to determine the effect of SGE on the intercellular junctions by confocal microscopy and to identify the mechanism(s) conditioning the increased endothelial permeability *in vitro* are ongoing. These findings may suggest a new biological mechanism associated with modulation of endothelial permeability that may facilitate systemic viral dissemination.

199

THE ROLE OF UBIQUITIN-PROTEASOME PATHWAY IN DENGUE VIRUS EGRESS: LEARNING FROM THE *AEDES AEGYPTI* MIDGUT

Milly M. Choy, Summer L. Zhang, Hwee Cheng Tan, October M. Sessions, Duane J. Gubler, Eng Eong Ooi

Duke-NUS Graduate Medical School, Singapore, Singapore

The mosquito-borne dengue virus (DENV) is a cause of significant global health burden. In the mosquito vector, the virus first establishes infection in the midgut before systemic spread to the organs to cause persistent infection. In the midgut, however, infectious DENV titers reaches a peak approximately 8 days after an infectious blood meal but decreases thereafter despite continual viral RNA replication. The mechanism that decouples viral RNA replication and infectious particle production in the midgut may constitute a novel innate immune response employed by *Aedes* mosquitoes against DENV. Using RNA inference studies, we show *in vivo* that down-regulation of ubiquitin proteasome pathway (UPP)-related genes, including proteasomal subunits, ß2 and ß5 decouples RNA replication from infectious titer production. Mechanistically, inhibition of proteasomal function prevented virus egress but not DENV assembly by exacerbating endoplasmic reticulum (ER) stress within the unfolded protein response. The translational attenuation due to increased ER stress led to reduced protein levels of Exoc7, an exocyst complex component required for exocytosis. Our study thus identified a mechanism that contributes to the mosquito midgut barrier. This mechanism also appears to be amenable for clinical translation as inhibition of UPP in primary monocytes with the licensed proteasome inhibitor, bortezomib, inhibited DENV titers even at low nanomolar drug concentration. In conclusion, we suggest here that functional UPP is necessary for virus egress through exocytosis and the inhibition of which could be an effective therapeutic strategy.

200

ALARM SIGNALS FOR DENGUE OUTBREAKS: A MULTI-CENTER STUDY IN ASIA AND LATIN AMERICA

Leigh Bowman[1], David Benitez[2], Giovanini Coelho[3], Lokman Hakim[4], Piero Olliaro[5], Axel Kroeger[5], Max Petzold[6], Silvia Runge-Ranzinger[5], Gustavo Tejeda[7], Philip J. McCall[1], Luong Chan Quang[8]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Universidad Autonoma de Yucatan, Merida, Mexico, [3]Ministry of Health, Rio de Janeiro, Brazil, [4]Ministry of Health, Kuala Lumpur, Malaysia, [5]World Health Organization-TDR, Geneva, Switzerland, [6]University of Gothenburg, Goteborg, Sweden, [7]Ministry of Health, Mexico City, Mexico, [8]Pasteur Institute, Ho Chi Minh City, Vietnam

Dengue outbreaks threaten both endemic countries and those with low or no stable transmission. Outbreaks are detected late, and response is often inadequate; early detection of outbreaks is challenging, since no established set of indicators exist for defining an outbreak. Candidate alert signals have been defined, but a systematic literature review found no satisfactory analyses or validations of these indicators and our expert meetings in 2012 and 2013 found that no country uses dengue outbreak alerts routinely for an early response. Within the context of a WHO/TDR-led Research Work Package, part of the European Union funded IDAMS research consortium (International Research Consortium on Dengue Risk Assessment, Management and Surveillance; www.idams.eu), a model dengue outbreak contingency plan based on the available evidence collected through country case studies and literature reviews, is being developed. The aim is to test the validity of alarm signals for dengue outbreaks through a retrospective study in 5 countries (Brazil, Mexico, Dominican Republic, Vietnam and Malaysia), including a large number of districts and 5-10 years of observation. The following candidate alerts are being explored for their ability to predict dengue outbreaks with a defined probability: increased number of dengue cases compared to previous years; entomological indices; levels of sero-positivity in blood samples;

changes in predominant serotype, age shift; climate data (temperature, rainfall and relative humidity). For each covariate, the Shewart Method is used to quantify correlations between outbreak 'alarms' with known dengue outbreaks. Information about the ongoing analytical process and the results to date will be presented and discussed.

201

USE OF LOCAL OCEANOGRAPHIC INFORMATION TO PREDICT DENGUE IN ECUADOR

Bonny Bayot[1], Anna M. Stewart-Ibarra[2], Martha Barahona[3], Johnny Chavarría[4], Mercy Borbor[3]

[1]Escuela Superior Politécnica del Litoral, Centro Nacional de Acuicultura e Investigaciones Marinas, Guayaquil, Ecuador, [2]Center for Global Health and Translational Science, SUNY Upstate Medical University, Syracuse, NY, United States, [3]Escuela Superior Politécnica del Litoral, Facultad de Ingeniería Marítima, Ciencias Biológicas, Oceánicas y Recursos Naturales, Guayaquil, Ecuador, [4]Universidad Estatal Península de Santa Elena, La Libertad, Ecuador

Dengue fever, a mosquito-borne viral disease, has increased in distribution, prevalence and severity throughout the tropics and subtropics. In this study, we explore the use of local oceanographic variables to predict dengue transmission in the southern coastal of Ecuador. We hypothesize that local oceanographic conditions, such as invasion of warmer water masses from the Panamá Bay and strengthening of the cold Humboldt Current, interact with the El Niño-Southern Oscillation (ENSO) to influence local climate and dengue transmission. We developed a statistical mixed effects model to evaluate the influence of local oceanographic variables (weekly sea surface temperature (SST) and sea subsurface temperature in front of Puerto Bolívar and other coastal localities), ENSO, and local climate on the dengue standardized morbidity ratio from the city of Machala, El Oro Province (weekly SMR, 2003-2013). We found that SST at Puerto Bolivar, El Oro Province, was positively associated with dengue (3-month lag). We also found that the Coastal El Niño index, which was developed in this study using the SST of El Niño 1+2 region, was positively associated with dengue (6-month lag). These findings indicate that local oceanographic variables provide some predictive lead for forecasting dengue outbreaks, and consequently, the potential to be integrated into operative tools of epidemic management, such as climate-driven early warning systems.

202

CLINICAL AND HEMATOLOGICAL PARAMETERS THAT DIFFERENTIATE DENGUE VERSUS OTHER FEBRILE ILLNESSES IN VENEZUELA

Adriana Tami[1], Zoraida I. Velasco-Salas[1], María F. Vincenti[1], Erley F. Lizarazo[2], Gloria M. Sierra[2], Hans Burgerhof[3], Paula Triana[2], Diamelis Guzman[2], Yosnaida Delgado[2], Mery Tortolero[2], Iris Liendo[2], Daria Camacho[2], Maritza Cabello de Quintana[4], Eduardo Guerra[2], María J. Aguero[2], Jan C. Wilschut[1]

[1]Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands, [2]Instituto de Investigaciones Biomédicas de la Universidad de Carabobo (BIOMED-UC), Maracay, Bolivarian Republic of Venezuela, [3]Faculty of Medical Sciences, Epidemiology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands, [4]Laboratorio Regional de Diagnóstico e Investigación del Dengue y otras Enfermedades Virales, Maracay, Bolivarian Republic of Venezuela

Dengue is the most important vector-borne viral disease worldwide. Dengue can be asymptomatic or present a wide range of manifestations from mild dengue fever to more severe disease. To date, there are no vaccines or antiviral treatments for dengue. At the early stage of the disease, general signs and symptoms of dengue can be confused with other febrile illness (OFI) and a late dengue diagnosis can be fatal. Dengue in Maracay, Venezuela, is hyperendemic with co-circulation of the 4 serotypes. In this setting, a longitudinal observational study was set up in 2010 to identify clinical and laboratory parameters that could differentiate patients with dengue versus OFI. Patients presenting with fever and dengue clinical criteria were recruited from 3 designated health centers. Dengue infection was confirmed by IgM ELISA and/or RT-PCR. Patients were followed daily with clinical examination and sequential blood sampling at determined intervals up to 30 days. Severe cases were treated in a tertiary hospital and followed daily until discharge. Hematological parameters and serum levels of selected biochemical markers were determined in acute phase blood samples. Between August 2010 and August 2013, 254 individuals met the inclusion criteria of which 44% were positive for dengue, 31% of positive patients developed alarm signs, while only 6% developed severe dengue. All four serotypes were detected in patients, with DENV-3 predominating. Using logistic regression and mixed effect models clinical, hematological and biochemical parameters where analyzed comparing the first 3 days with days 4-7 after fever onset. Rash, hemorrhagic manifestations including a positive tourniquet test, a concomitant decrease in platelets (~10,000 platelets/day) and white blood cell count and the absence of sore throat were associated with dengue infection in the first 3 days of the disease. A higher proportion of patients with hemorrhagic manifestations were present in days 4-7 after fever onset. A final model of determinants will be presented.

203

GLOBAL ECONOMIC COST OF DENGUE CASES TREATED IN THE MEDICAL SYSTEM

Donald S. Shepard, Yara A. Halasa, Eduardo A. Undurraga

Brandeis University, Waltham, MA, United States

As the most important vector-borne viral disease, dengue is a serious and growing global public health problem, with 2.5 billion (b) people at risk. To estimate the global cost of symptomatic dengue cases treated in the professional health-care system, we reviewed the literature for numbers and cost of dengue cases by country. Using Bhatt et al. (2013) and Brady et al. (2012), we identified 134 countries with active dengue transmission. Shepard et al (2010 and 2013), supplemented by recent field data from India, Mexico, and Philippines, provided the number of dengue cases in the Americas and Southeast Asia. We adjusted the WHO reported dengue cases for South Asia using an expansion factor estimated for India (282). For the rest of the world, we multiplied the number of apparent dengue infections from Bhatt et al. (2013) by 16.25%--the ratio of cases in medical settings to total cases projected in Southeast Asia by Bhatt et al. (2.92 million (m)/17.96 m). To estimate the economic cost of dengue illness (both direct and indirect), we reviewed the literature for 5 studies reporting overall economic cost of dengue complemented by recent field studies from 3 countries. Collectively, these provided the per capita cost of dengue illness for 58 countries. We estimated the log of this per capita cost as a function of log of GDP per capita and log of dengue incidence per 100,000 population ($p<.0001$, $R2=0.66$) and calculated estimated values for the remaining 76 countries. Bootstrapping the selection of 58 countries used with replacement generated a range of estimates. Multiplying the cost per capita times the population gave the aggregate cost of dengue by country, region, and globally. Preliminary results show a total of 30 m locally transmitted symptomatic dengue cases annually treated in the medical system in 134 countries with transmission. The estimated annual global aggregate cost of dengue illness is $8.65 b (range: $3.98-$8.80 b) or $1.58 (range: $0.73-$1.61) per capita. Of this, $3.77 b (44%) was incurred in 8 countries in South Asia; $1.95 b (23%) in 28 countries in East Asia and the Pacific, $2.47 b (29%) in 44 countries in Latin America and the Caribbean; $0.31 b (4%) in 42 countries in Sub-Saharan Africa, $0.14 b (2%) in 6 countries in the Middle East and North Africa, $5.6 m (0.06%) in 4 countries in Europe and Central Asia; and $3.4 m (0.04%) in North America. If control strategies could reduce dengue appreciably, billions of dollars would be saved globally.

204

VERTICAL TRANSMISSION OF YELLOW FEVER TO INFANTS VIA BREASTFEEDING

Daniel E. Mauck[1], David R. DeSalvo[1], David P. Adams[2]

[1]AASU, Savannah, GA, United States, [2]AASU/Georgia Regents Medical University, Savannah, GA, United States

Before the development of a vaccine in 1937, Yellow Fever was one of the most feared and lethal diseases in human history. The live, attenuated Yellow Fever vaccine provides lifelong immunity and is safe for those with a developed immune system. Breastfeeding mothers, however, should not receive the vaccine as it may cause Yellow Fever in infants. Six cases of Yellow Fever transmitted via breastfeeding have been reported. These cases occurred when Yellow Fever vaccine was administered to breastfeeding mothers with infants from 10 to 15 days old. Serum and cerebrospinal fluid were positive for the Yellow Fever virus, and nucleotide sequences confirmed identical viral profiles between vaccine and cases. Such cases suggest acute central nervous system infection with Yellow Fever through breastfeeding. Further research is needed to evaluate more fully the risk of vertical transmission of such viruses via this route. Although vertical transmission of Dengue Fever and West Nile has been documented previously, little information exists regarding transmission of Yellow Fever via breast milk.

205

CHARACTERIZATION OF A MUTATION IN THE YELLOW FEVER VIRUS E LINKER REGION IN *AEDES AEGYPTI* MOSQUITOES

Yan-Jang S. Huang[1], John T. Nuckols[2], Kate M. Horne[3], Alan D. Barett[4], Stephen Higgs[1], Dana L. Vanlandingham[1]

[1]Biosecurity Research Institute, Kansas State University, Manhattan, KS, United States, [2]Medical Countermeasure Systems, Joint Vaccine Acquisition Program, Fort Detrick, MD, United States, [3]Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, United States, [4]Department of Pathology, University of Texas Medical Branch, Galveston, TX, United States

The linker region of the flavivirus envelope (E) protein connects the C-terminus of the domain I and the N-terminus of the domain III and was first hypothesized to accommodate the mechanical forces resulting from structural rearrangement that occur during viral membrane fusion. Available evidence further demonstrated the importance of the linker region in viral particle assembly and secretion of progeny virions. These conclusions were derived from *in vitro* models since all the mutations in the linker region of DENV-2 E protein are lethal and prevent production of infectious virions, thereby precluding *in vivo* studies. We evaluated the phenotypic effect of a methionine to isoleucine mutation generated at position 299 in the yellow fever virus (YFV) E gene that occurred during serial passage of the wild-type Asibi strain to derive the attenuated 17D vaccine virus. Using the YFV reverse genetics system and *Aedes aegypti*, we characterized the infection, replication and dissemination phenotypes of the mutant and parental viruses in mosquitoes. Mosquitoes were orally infected with virus produced from various YFV infectious clones: Asibi, 17D, Asibi E M299I, and a 17D/Asibi M-E chimera with and without the M299I mutation and sampled at 7, 10, and 14 days post infection to evaluate replication kinetics and assess infection and dissemination phenotypes. The phenotypes of the 17D strain, the Asibi strain and the 17D/Asibi M-E chimera were similar to the phenotypes observed in our previous reports. The results of the characterization of the M299I mutation will be discussed.

206

MERCADEO VIRUS: A NOVEL INSECT-SPECIFIC-FLAVIVIRUS

Jean P. Carrera[1], Hilda Guzman[2], Yamilka Diaz[1], Rolando Torres[3], Steven Widen[2], Thomas G. Wood[2], Scott C. Weaver[2], Lorezo Caceres[3], Nikos Vasilakis[2], Robert B. Tesh[2]

[1]Department of Virology and Biotechnology Research, Gorgas Memorial Institute of Health Studies, Panama, Panama, [2]Institute for Human Infection and Immunity, Department of Pathology and Center for Tropical Disease, University of Texas Medical Branch, Galveston, TX, United States, [3]Department of Medical Entomology, Gorgas Memorial Institute of Health Studies, Panama, Panama

The genus *Flavivirus* (*Flaviridae, Flavivirus*) is a heterogeneous group of viruses; some of them are important arthropod-borne-virus for both human and veterinary medicine. Recently an explosion of insect-specific-flavivirus has been documented**.** Although, many flaviviruses have been isolated from humans, field-collected-mosquitoes and birds in Panama, insect-specific flavivirus has never been detected in this Central American country. During 2011, mosquitoes were collected in Darien province close to the border of Colombia, where an outbreak of alphaviral encephalitis in both human and equines was reported in 2010. A number of virus isolates were obtained in C6/36 mosquito cell culture from specific pools of *Culex Melanoconion spp.* and *Cx. Culex spp*. Structural and antigenic characterization placed these viruses in the flavivirus group and their complete genome sequence was obtained using Illummina next generation sequencing. Phylogenetic analysis based on the ORF of two isolates shows that our isolates grouped within the insect-specific clade of flaviviruses, closely related to cell fusing agent and Nakiwogo virus that were isolate in Uganda. Based on the location of the mosquito collection site, we named this virus strains as "Mercadeo virus". Here, we report the discovery and characterization of an insect-specific flavivirus in Panama. Our results highlight the importance of combined viral isolation, structural, antigenic and genetic characterization for virus discovery and taxonomic designation.

207

HEAT SHOCK COGNATE PROTEIN 70 ISOFORM D IS REQUIRED FOR CLATHRIN-DEPENDENT ENDOCYTOSIS OF JAPANESE ENCEPHALITIS VIRUS IN C6/36 CELLS

Tsong-Han Yang, Tien-Huang Chen, Chao-Fu Yang, **Wei-June Chen**

Chang Gung University, Tao-Yuan, Taiwan

Japanese encephalitis virus (JEV) generally enters host cells via receptor-mediated clathrin-dependent endocytosis that requires the involvement of the 70 kDa heat shock protein (Hsp70). Hsc70 is one member of the Hsp70 family and is mainly constitutive; thus, it may be expressed in the absence of stress. In C6/36 cells, Hsc70 is up-regulated in response to JEV infection. Since Hsc70 shows no relationship with viruses attaching to the cell surface, it probably does not serve as the receptor although it has been reported to exist on the cell face. In contrast, Hsc70 is evidently involved in virus penetration and the resultant acidification of intracellular vesicles, suggesting that it is highly involved in clathrin-mediated endocytosis. The effects of Hsc70 occur particularly at a late stage of viral entry into host cells. Furthermore, we found that Hsc70 is composed of at least three isoforms, with isoform D being the one that helps JEV penetrate C6/36 cells via clathrin-mediated endocytosis. This study provides relevant evidence that sheds light on the regulatory mechanisms of JEV infection in host cells.

208

ANALYSIS OF SEROLOGICAL SURVEYS FOR YELLOW FEVER IN AFRICA

Tini Garske[1], Maria Van Kerkhove[1], Sergio Yactayo[2], William Perea[2], Christl Donnelly[1], Neil Ferguson[1]

[1]MRC Centre for Outbreak Analysis and Modelling, London, United Kingdom, [2]World Health Organization, Geneva, Switzerland

Yellow fever can be a devastating disease that occurs both in large epidemics and in sporadic endemic cases across large parts of Africa and Latin America, with over 90% of the global burden being attributed to Africa. However, assessing the extent of yellow fever virus circulation in the endemic countries in Africa is very difficult due to under recognition of both asymptomatic infections and mild cases with non-specific febrile illness that occur in the majority of persons, and under reporting of severe cases who develop jaundice or haemorrhage . We reviewed the published and unpublished available literature for reliable serological surveys for yellow fever in Africa and identified six suitable studies published since 1980 from five African countries including Nigeria, Cameroon, the Central African Republic, the Democratic Republic of the Congo and the Congo. We analysed the age-dependent sero-prevalence of YF neutralizing antibodies to estimate the annual risk of infection to a sero-naive person that would give rise to the observed seroprevalence profiles and obtained estimates between 0.25% and 2.2% in the five countries; these estimates are similar to those previously established from Nigeria during the 1970s. Using this annual risk of infection, we estimate that there are a total of 180,000 (95% CI 54,000 to 440,000) severe cases annually in these five countries, around 52% of which occur in Nigeria and 43% in the DRC. These case estimates indicate a disease burden several orders of magnitude higher than official case notifications to WHO. This illustrates the challenges of achieving highly sensitive syndromic disease surveillance systems and highlights the utility of systematically collected serological data to assess the true extent of virus circulation. Well-designed serological studies are an indispensable component in evaluating the true extent of the yellow fever disease burden. Indeed, the WHO and partners are currently conducting serological studies in YF endemic countries to assess transmission intensity and inform control strategies.

209

INFERRING THE YELLOW FEVER FORCE OF INFECTION FROM THE OBSERVED AGE DISTRIBUTION OF CONFIRMED CASES

Tini Garske[1], Maria Van Kerkhove[1], Sergio Yactayo[2], William Perea[2], Christl Donnelly[1], Neil Ferguson[1]

[1]MRC Centre for Outbreak Analysis and Modelling, London, United Kingdom, [2]World Health Organization, Geneva, Switzerland

Yellow fever is an arboviral disease, transmitted between humans and non-human primates and mosquitoes of various *Aedes* spp. in Africa. Disease severity shows a broad spectrum with high rates of fatality amongst the more severe cases, but symptoms tend to be non-specific, particularly in milder cases leading to huge underreporting and highly uncertain disease burden. As yellow fever is a sterilising infection, the age distribution of observed cases can give clues to the yellow fever transmission intensity. Using data on confirmed human cases of yellow fever reported to the Yellow Fever Surveillance Database covering 20 countries in western and central Africa in conjunction with population demographics and age-dependent vaccination coverage we estimate the force of infection that could give rise to the observed age distribution of cases, using a number of different assumptions regarding the age-dependence of the exposure. Point estimates of the force of infection are low, but comparison with estimates derived from other data suggest upper confidence bounds obtained might put useful bounds on the extent of transmission. Results are sensitive to the age distribution of the population and vaccination coverage and strongly depend on the age-dependence of exposure assumed, with models assuming age-independent force of infection fitting better in western Africa, but a higher exposure in adults than children fitting better in central Africa. In western Africa, the annual risk of infection to a susceptible person was estimated to be below 2.3%, while the annual infection risk in central Africa was estimated to be below 0.5% for children and 1.2% for adults. The fact that in different areas different assumptions about the age-dependence of the infection risk gave the best fit to the data could be interpreted as indiscriminate exposure in villages in western Africa, where the transmission intensity is thought to be highest, but more limited occupational exposure through activities such as wood clearing in central Africa with a lower overall transmission intensity.

210

ROLE OF ENDOGENOUS FLAVIVIRAL GENETIC ELEMENTS IN A VECTOR MOSQUITO

Michael J. Conway, Maya O. Tree, Kristopher J. Keift, Nico Conti

Central Michigan University College of Medicine, Mount Pleasant, MI, United States

Remnants of ancestral flaviviral infections can be detected throughout the genomes of *Aedes albopictus* and *Ae. aegypti*, both major disease vectors throughout the world. Although flaviviral genetic elements in *Ae. albopictus* are riddled with stop codons, NCBI BLAST analysis of complete flaviviral polyproteins against the *Ae. aegypti* genome identified two genes (AAEL007866 and AAEL017001) that corresponded to flavivirus NS1 and NS5 proteins. We call these proteins *Aedes aegypti* NS1 (AeNS1) and NS5 (AeNS5). AeNS1 and AeNS5 are most related to the insect only flavivirus Kamiti River virus (KRV). Genetic alignment against wild-type NS1 and NS5 proteins revealed that AeNS1 and AeNS5 are not complete proteins. AeNS1 is missing a conserved tip of unknown function, and AeNS5 retains only the RNA dependent RNA polymerase finger domain, which is thought to interact with RNA templates. The MTase domain, catalytic site, and the priming loop are missing. We hypothesized that AeNS1 and AeNS5 are dominant negative proteins that were co-opted by *Ae. aegypti* to interfere with flavivirus replication. Accordingly, overexpression of AeNS5 inhibited KRV replication. Interestingly, AeNS5 overexpression had no effect on DENV2 replication. We hypothesize that this is due to the large evolutionary gap between insect only and pathogenic flaviviruses. These studies highlight the role of viruses in the evolution of eukaryotic organisms, and implicate AeNS5 as an evolution-guided template for the design of dominant negative proteins that target major viral pathogens.

211

IDENTIFICATION OF THE FIRST CASE OF IMPORTED ZIKA FEVER TO THE UK: A NOVEL SAMPLE TYPE FOR DIAGNOSTIC PURPOSES AND SUPPORT FOR A POTENTIAL NON-VECTOR-BORNE ROUTE OF TRANSMISSION

Pasco T. Hearn[1], Barry Atkinson[2], Roger Hewson[2], Tim Brooks[1]

[1]Rare and Imported Pathogen Laboratory, Porton Down, United Kingdom, [2]Public Health England, Porton Down, United Kingdom

Zika virus (ZIKV) is a flavivirus related to Dengue, is transmitted by the *Aedes* mosquito and normally causes a self-limiting illness characterised by fever, rash, headache, conjunctival suffusion, myalgia and joint pains. It was discovered in 1947 at Zika Forest, near to Entebbe, Uganda. More recently it has been found outside Africa in outbreaks such as those of French Polynesia and the Cook Islands. We report the first case of imported Zika Fever to the UK, highlight novel samples for testing and a theoretical mode of non-vector-borne transmission. A couple travelled to the Cook Islands during what was thought to be a Dengue outbreak in February 2014. Within 6 days of exposure, both 'Patient 1' and his wife, 'Patient 2', had developed fatigue, followed 48 hours later by fever, headache, aching joints and a widespread maculopapular rash. Both reported symptoms resolving by day 5 of rash onset. In 2011, Foy et al described likely sexual transmission of ZIKV. A scientist from Colorado, travelling back from Senegal whilst incubating the virus, reportedly passed it to his wife, in whom clinical and serological evidence supported the diagnosis. On day 1 of rash onset, Patient 1 had serum sent to the Rare

and Imported Pathogens Laboratory at Porton Down and a panel of serological tests based on stated travel was performed. Dengue testing revealed positive IgM, but negative IgG and PCR. This pattern has been reported previously due to cross-reactivity between the flaviviruses. The sample was then tested by PCR for ZIKV and found to be positive. Further samples were taken on day 28 after rash onset. Blood and urine from both patients were tested in parallel, along with a semen sample from Patient 1. The semen was the only sample found to be positive for ZIKV by PCR. The reasons for persistence in the semen are not yet clear, but this case is significant not only as the first case of Zika Fever imported to the UK, but also as support for the possibility of sexual transmission, though not in this case, and highlights an additional sample type for the confirmation of infection in the future.

212

IXODES SCAPULARIS SALIVA ENHANCES *POWASSAN* VIRUS TRANSMISSION TO THE HOST, INFLUENCING ITS DISSEMINATION AND THE COURSE OF DISEASE

Meghan E. Hermance, Rodrigo I. Marques dos santos, Saravanan Thangamani

The University of Texas Medical Branch, Galveston, TX, United States

Powassan virus (POWV) is transmitted to humans by infected ticks, and successful transmission of POWV can occur within one hour of *Ixodes scapularis* attachment. Successful tick feeding is facilitated by a collection of pharmacologically active factors/proteins in tick saliva which are secreted into the feeding pool that the tick creates on the vertebrate host. The skin serves as the interface of host-virus-tick interactions; therefore, we sought to understand how tick saliva immunomodulates the tick-host interface and whether it facilitates POWV transmission and dissemination. To achieve this objective, groups of naïve BALB/c mice were infected intradermally in the hind footpad with either POWV alone or with POWV plus *Ixodes scapularis* salivary gland extract (SGE). A third group consisted of mice injected with media only. Mice from each group were sacrificed at 1, 3, 5, 7, and 8 days post infection (dpi). Organs were harvested from each sacrificed mouse and viral titers were determined by detecting POWV using quantitative real-time PCR. Starting at 3 dpi, mice infected with POWV+SGE had viremias that were at least 0.5Log greater than the viremias detected in the mice receiving POWV only. At 3 and 5 dpi the viremias of the POWV+SGE group were statistically significantly higher than the POWV only group. A similar pattern of higher viral loads in the POWV+SGE group was detected in the popliteal lymph nodes; however, there was no significant difference between groups on any of the days. POWV was not detected in the brains of mice until 5 dpi. From 5 dpi until experiment completion, the POWV+SGE infected mice had higher titers than the POWV only group, with 5 dpi being statistically significant. The onset of clinical symptoms occurred one day earlier (4 dpi) in the POWV+SGE group. Overall, this data indicates that *I. scapularis* SGE enhances POWV transmission to the host and influences the course of disease. To further examine the effect that *I. scapularis* saliva in the presence of POWV has on the host's immune response, we fed POWV-infected and uninfected *I. scapularis* nymphs on naïve mice for 3 or 6 hours. 5-um sections were taken near the mouthparts of each feeding tick and infiltrating immune cells and POWV-infected cells at the tick bite site were identified. The location, timing, identity, and quantity of immune cells determined via histology enabled us to define the quality and kinetics of the host response to tick-borne POWV infection.

213

OUTBREAK OF YELLOW FEVER VIRUS DISEASE IN UGANDA IN 2010-2011: WERE OTHER INFECTIONS INVOLVED IN THE OUTBREAK?

Julius J. Lutwama[1], John T. Kayiwa[1], Annet M. Nankya[1], Anne Miyingo-Kezimbira[2]

[1]Uganda Virus Research Institute, Entebbe, Uganda, [2]Makerere University, Kampala, Uganda

An outbreak of yellow fever in northern Uganda was confirmed on 23 December 2010 after samples tested at the US Centers for Disease Control and Prevention (CDC) were positive for Yellow Fever Virus (YFV). Uganda is endemic for YFV however nearly 40 years had passed since the previous recorded transmission of YF in Uganda. The first documented outbreak in the country was in 1939-41. Single cases of yellow fever disease were documented in 1952, 1959, 1964 and 1971. Cases of illness of unknown cause had been reported since October 2010 from twelve districts in northern Uganda. These cases mainly concerned males aged 20 to 34 years mostly presenting with severe frontal headache, fever, lethargy, abdominal pain, diarrhoea and vomiting, as well as with hemorrhagic signs. Blood and tissues samples from patients were sent to the U.S. CDC Special Pathogens laboratories in Atlanta, Georgia. Yellow fever virus was identified as the culprit. Then the CDC laboratory in Ft. Collins, Colorado, confirmed the finding. Overall, 181 cases met the YF suspected case definition; there were 45 deaths (case fatality rate 24.9%). Molecular sequencing revealed 92% homology to the YF virus strain Couma (Ethiopia), East African genotype. Suspected YF cases had fever (100%) and unexplained bleeding (97.8%), but jaundice was rare (11.6%). Over 200 blood samples were tested by RT-PCR and by IgM ELISA, including many samples from people who met the suspected case definition. However, only a total of 13 cases were laboratory confirmed from five districts. It was during a dry spell and the limited number of mosquitoes collected were not positive for YFV. Although this is acknowledged as the largest YF outbreak ever reported in Uganda. It remains unclear if all of the cases of disease were caused by YFV given that only a small number of suspected cases were laboratory confirmed to be YFV infections; and they were randomly scattered in five districts. There could have been other flaviviruses which probably were not tested for.

214

MOSQUITO SALIVA-MEDIATED ENHANCEMENT OF YELLOW FEVER VIRUS INFECTION

Alan M. Watson[1], Michael J. Conway[2], William B. Klimstra[1], Kate D. Ryman[1]

[1]University of Pittsburgh, Pittsburgh, PA, United States, [2]Central Michigan University, Mt. Pleasant, MI, United States

Mosquito-borne viruses like those in the genus Flavivirus must interact with both the mosquito and mammalian host in order to complete its life cycle and, to this end, have evolved complex strategies to replicate and disseminate within these different environments. Dengue and West Nile viruses take advantage of the complex mix of proteins in mosquito saliva in order to enhance infectivity in the mammalian host following the bite of an infected mosquito. Our recent work indicates that a non-catalytic serine protease is responsible for mosquito saliva-mediated enhancement of infectivity of these viruses and can be abrogated by the addition of a serine protease inhibitor or specific knockdown of the protein. We now report that in a murine model of the closely related yellow fever virus (YFV), early infection is enhanced in the presence of mosquito salivary gland extract (SGE) by as much as 5,000-fold both at the subcutaneous site of infection and in visceral tissues. However, despite this early enhancement, mice infected in the presence or absence of SGE, develop similar peak virus load and manifestation of clinical disease. Surprisingly, animals infected in the presence of SGE were more likely to survive infection, suggesting the development of a more effective or less damaging adaptive

immune response. We are currently examining the specific role that SGE-enhancement of infection plays in the development of cell-mediated and humoral immunity toward YFV.

215

YELLOW FEVER RE-EMERGENCE AFTER FIFTY YEARS, ETHIOPIA, 2013

Debritu M. Abegaz, abraham Lilay, Abyot Bekele, Daddi Jimma

Ethiopia Field Epidemiology Training Program, Addis Ababa, Ethiopia

In Ethiopia from 1960-1962 there was an outbreak of 100,000 cases of yellow fever (YF), which caused 30,000 deaths. Since 1962, however, no cases of yellow fever had been reported in Ethiopia. In May 2013 we received reports of a suspected outbreak of yellow fever from South Omo Zone situated within the Rift Valley in southern Ethiopia. We investigated to confirm the outbreak, identify risk factors and institute control measures. We used a clinical case definition to identify suspected YF cases and conducted a medical record review at all hospitals and health facilities in the Zone. Laboratory confirmed cases were confirmed by IgM serology at the Institut Pasteur in Dakar. We also conducted an age-matched case control study that included 30 suspected cases and 60 controls, who were neighbours of suspected cases without symptoms of disease. We identified 6 laboratory confirmed and 124 suspected YF cases from 28 November 2012 to 6 June 2013 from four rural districts of South Omo Zone. There were 53 deaths (case fatality rate of 41%). The overall attack rate was 30/100,000, and the highest attack rate (45/100,000) was from South Ari District. The median age of the 130 cases was 27 years, and 73 (56%) were males. Significant risk factors associated with illness included attending a mass gathering (OR=4.0; 95% CI=1.3-6.2) and presence of a person with YF in the village (OR=3.7; 95% CI=1.5-9.4). Working in a forest was a non-significant risk factor (OR=0.92; 95%CI=0.4-2.4). We confirmed the first outbreak of yellow fever in Ethiopia in 50 years. The outbreak was contained with a mass vaccination campaign of more than 500,000 people. We recommended heightened surveillance for hemorrhagic illness throughout Ethiopia.

216

TAU ABNORMALITIES, INFLAMMATION AND AXONAL DAMAGE IN MURINE CEREBRAL MALARIA

Henry Shikani, Mahalia Desruisseaux

Albert Einstein College of Medicine, Bronx, NY, United States

Cerebral malaria (CM) is a potentially fatal complication of disease associated with *Plasmodium falciparum* infection. Despite complete clearance of the parasite with anti-malarial treatment, approximately 20% of CM survivors develop long-term neurological deficits; however, the mechanisms that mediate this are not well understood. Neuronal injury has been linked to neurocognitive impairment in several neurodegenerative diseases and may contribute to the deficits seen in CM. In this regard, damage to neuronal axons has been observed in both human and murine experimental CM (ECM). Furthermore, improper regulation of tau, an axonal protein important for microtubule stability and cytoskeletal organization, has been demonstrated in mouse and human disease. We hypothesized that the neuronal injury observed in ECM results, in part, from abnormalities in tau. Improper regulation of tau results in an increase in its phosphorylated levels. We quantified protein levels of two forms of phosphorylated tau known to be pathological in Alzheimer's disease (Ser396/404; Ser202) in several brain regions of mice with ECM and compared our findings with uninfected mice and mice infected with a less neurotropic malarial strain. In the same regions, we also quantified the level of SMI 32, a marker of axonal damage. Phosphorylated tau and SMI 32 were elevated throughout the brains of mice with neurological disease. Treatment of ECM mice with the immunotherapeutic PHF-1 antibody, which clears phosphorylated tau in mouse models of Alzheimer's disease, prevented axonal damage in certain brain regions, suggesting that this protein is contributing to the neuronal injury in ECM. Abnormal tau regulation has previously been linked to dysregulated inflammation, a common feature of CM. We hypothesized that the aberrant tau phosphorylation in ECM is associated with the hyper-inflammation which typically occurs. mRNA levels of several inflammatory cytokines in the brains of our infected mice were found to be consistently elevated during neurological disease. The increases in these cytokines may contribute to the atypical tau regulation in ECM. Our goal is to further establish abnormal tau as a hallmark of CM. This protein may prove to be a viable target to ameliorate both the neuronal damage and subsequent neurocognitive impairment which occur during disease.

217

CRYOPRESERVATION OF *PLASMODIUM VIVAX* SPOROZOITES

Rapatbhorn Patrapuvich, **Kaewta Lerdpanyangam**, Rachaneeporn Jenwithisuk, Siriwan Rung-in, Narathatai Yimamnuaychok, Jetsumon Sattabongkot Prachumsri

Mahidol Vivax Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Plasmodium vivax represents a great challenge to malaria control because of the ability to relapse of dormant form known as hypnozoite in the liver. Research efforts to understand hypnozoite biology are limited due to the availability of *P. vivax* sporozoites. Mahidol Vivax Research Unit (MVRU) is one of a few centers in the world that has facilities to produce the sporozoites by feeding mosquitoes on blood from *P. vivax*-infected patients. The ability to cryopreserve sporozoites is therefore essential to support liver-stage malaria research community. Hydroxyethyl starch 6% (HESTAR), a currently used cryoprotectant in cryopreservation of *P. vivax* sporozoites at MVRU, retains sporozoite viability up to 30% after thawing. In this study, the protective effect of different combinations of cryoprotectants on sporozoite viability and infectivity was investigated in order to improve the cryopreservation protocol. Sporozoites were harvested from infected mosquitoes and cryopreserved at slow freezing rate of -1°C/min to final temperature of -80°C before plunging in liquid nitrogen. Frozen sporozoites were thawed in a 37°C water bath for a few seconds and viable sporozoites were counted under hemocytometer. Addition of 10% DMSO and 250 mM sucrose into HESTAR solution increased number of viable sporozoites after thawing. The combination of PBS, 0.5% BSA, 250 mM sucrose, and 10% DMSO demonstrated highest sporozoite recovery. However, reduction of sporozoite infectivity was observed in all cryoprotectants tested.

218

FOLIC ACID METABOLISM IS DISRUPTED IN A NON HUMAN PRIMATE MALARIA MODEL

Jorge L. Salinas, Karan Uppal, Monica Cabrera-Mora, ViLinh Tran, Mary R. Galinski, Dean P. Jones, Alberto Moreno

Emory University, Atlanta, GA, United States

Plasmodium falciparum is a leading cause of severe malaria. Due to the challenge of studying *P. falciparum* pathophysiology in human populations, non-human primate (NHP) models have been developed. As part of an effort by the Malaria Host-Pathogen Interaction Center (MaHPIC), we hypothesized that high-resolution metabolomics (HRM) would further our understanding of the biochemical changes ensuing in the early phase of *P. coatneyi* infection in *Macaca mulatta*. As part of a larger intervention study, we collected serum samples from 4 healthy animals at 2 time points: 15 days before experimental infection (baseline) and 5 days after challenge (early infection). All animals developed malaria after intravenous injection of $4x10^4$ *P. coatneyi* infected erythrocytes/kg obtained from an infected donor monkey. At the early infection blood collection, parasitemias were low and animals did not require antimalarial treatment. Samples underwent randomization followed by HRM. We used xMSanalyzer for data extraction and obtained >20,000 metabolites (unique *m/z* with retention time). Paired analyses for differences due to infection were performed using the Linear Models for Microarray package (LIMMA, R

programming) with adjustment for multiple testing by the Benjamini-Hochberg false discovery rate (FDR) method. 33 metabolites altered during early *P. coatneyi* infection at FDR<0.2 were tested for pathway enrichment using Mummichog. Results showed 2 metabolites in the folic acid pathway (p<0.08): methylene-tetrahydrofolate (THF) and methyl-THF being less abundant in the early infection period. Pathway enrichment analysis with the addition of the top 100 correlated metabolites (33+100 with absolute Spearman correlations between 0.83-0.91 at FDR<0.2) confirmed the enrichment of the folic acid pathway (p<0.005) and pathways related to the metabolism of N-glycans, glycosphingolipids, amino sugars, squalene and pyrimidine (p-values <0.005). This first report of the metabolomic analysis of NHP malaria exposed alterations in the folic acid metabolism and other biochemical pathways. These will serve as a reference for future NHP and human malaria metabolomics studies.

219

THE EXPRESSION OF *PLASMODIUM FALCIPARUM* INVASION LIGANDS AND THEIR RECEPTORS IN CLINICAL ISOLATES FROM TIMIKA, PAPUA, INDONESIA

Leily Trianty[1], Boni Sebayang[1], Yusrifar Tirta[1], Andreas Kusuma[1], Retno Utami[1], Daniel Lampah[2], Ferryanto Chalfein[2], Pak Prayoga[2], Jutta Marfurt[3], Alida Harahap[1], Nicholas Anstey[3], Ric Price[3], Rintis Noviyanti[1]

[1]Eijkman Institute for Molecular Biology, Jakarta Pusat, Indonesia, [2]Malaria Research Laboratory, Timika, Papua, Indonesia, [3]Menzies School of Health Research, Darwin, Australia

Plasmodium falciparum invasion is a complex process involving several parasite ligands and their receptors expressed on the red blood cell surface. We reported various receptors used by the parasite ligands during their invasion based on their sensitivity to neuraminidase (N), trypsin (T) or chymotrypsin (C). Sixty nine isolates were subjected to various enzyme treatments including 50 mU/ml neuraminidase, 1 mg/ml trypsin, or 1 mg/ml chymotrypsin. Eight invasion profiles were found in this study showing receptor sensitivity or resistance to those enzymes. Most field isolates in Timika invaded red blood cells through type A receptor that was resistant to all enzyme treatments (NrTrCr; 28,99%) and type B that was sensitive to neuraminidase and trypsin, but resistant to chymotrypsin (NsTsCr; 21,74%). The expression of two invasion ligands; *P. falciparum* Duffy binding ligand (PfDBL) and *P. falciparum* reticulocyte homolog (PfRh) were quantified from the schizonts stage of each isolate. We employed quantitative real-time reverse-transcription polymerase chain reaction (QRT-RT-PCR) to detect the expression of PfDBL family including EBA-140, EBA-175 and EBL-181 and PfRh genes such as Rh-1, Rh-2a, Rh-2b. We demonstrated that EBA-140, Rh-1 and EBA-175 were the major invasion ligands expressed in *P. falciparum* of Timikan isolates. The current study strengthens the support to include these invasion proteins into the malaria vaccine platform. The presence of red cell polymorphisms including the Southeast Asian Ovalocytosis (SAO), Gerbich negativity, and variant hemoglobin (HbE) were not found to affect parasite invasion.

220

EFFECTS OF LOW MOLECULAR WEIGHT HEPARIN AND ANTIMALARIAL DRUGS ON ROSETTE FORMATION AND CYTOADHERENCE OF *PLASMODIUM FALCIPARUM*

Somporn Saiwaew

Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

The main pathophysiology of severe malaria is due to the mature stage of *Plasmodium falciparum*–infected red cells sequestered in the capillary vessels of the brain and other vital organs known as sequestration. This sequestration is due to cytoadherence, the ligands-receptors interactions and rosette formation which are defined as the adhesion of two or more uninfected red cells to the infected cells. Many studies have shown that the heparin sulfate, which is negatively charged through sulfate groups, was used in the adjunctive treatment of malaria. However, there is no report on the effects of low molecular weight heparin (Sevuparin) on the cytoadherence of *P. falciparum* isolated from patients. We therefore investigated the effects of low molecular weight heparin on rosette formation and cytoadherence of *Plasmodium falciparum i*solates in Thailand. *P. falciparum*-infected blood samples at trophozoite stage was incubated with Sevuparin at 37°C for 30 minutes and an hour before performing the rosetting and cytoadherence assay, respectively. The results shown that Sevuparin (62.5-1000 µg/mL) inhibit rosette formation of *P.falciparum* (N=43) *in vitro*, (P<0.01) in dose dependent manner. The concentration of Sevuparin at 400 µg/mL significantly inhibited cytoadherence (N=24) (P=0.02). This study provides the novel knowledge for understanding the pathophysiology of malaria and improving treatment of malaria patients. The low molecular weight heparin with less anticoagulation activities might be used as adjunctive treatment in severe malaria patients.

221

THE IMPACT OF DYSREGULATED ANGIOGENESIS ON BIRTH OUTCOMES IN EXPERIMENTAL PLACENTAL MALARIA

Vanessa Tran[1], Ana Cabrera[1], Kevin C. Kain[2]

[1]Sandra Rotman Laboratories, Sandra Rotman Centre for Global Health, University Health Network - Toronto General Hospital, Toronto, ON, Canada, [2]Sandra Rotman Laboratories, Sandra Rotman Centre for Global Health, University Health Network - Toronto General Hospital; Tropical Disease Unit, Division of Infectious Diseases, Department of Medicine, University of Toronto, Toronto, ON, Canada

Each year ~125 million pregnant women are at risk for malaria infection. Placental malaria (PM) due to *Plasmodium falciparum* infection has a profound impact on maternal and child health, including preterm birth and low birth weight (LBW), and results in an estimated 100 000 infant deaths per year. Previous work by our lab showed that PM-mediated LBW outcomes in both human and murine studies are associated with dysregulated angiogenesis including modifying the angiopoietin (Ang)-Tie2, endoglin, and VEGF pathways. Coordinated expression of these angiogenic factors is required for normal placental development during pregnancy. Furthermore, recent studies have implicated complement activation (e.g. C5a) in mediating these changes. Although we uncovered a role for angiogenesis and complement in the pathobiology of PM, the temporal regulation of these factors has not been precisely mapped. Thus, the objective of this study is to compare the kinetics and levels of factors that regulate vasculogenesis and angiogenesis including VEGF, soluble VEGF Receptor-1 (sFlt-1), endoglin, Placental Growth Factor, Ang-1, and Ang-2, required for the normal placental vascular development and gestation. Using the established BALB/c mouse model of PM, we will measure maternal, fetal, and placental levels of the various angiogenic factors throughout gestation by qRT-PCR and ELISA. Further, to establish a causal role for Ang-1 in mediating PM outcomes, we will use heterozygous and conditional Ang-1 knockout mice to determine the impact of Ang-1 deficiency on angiogenesis and fetal growth in an experimental PM model. Finally we will investigate the therapeutic utility of angiogenic factors such as recombinant Ang-1 and determine if adverse birth outcomes in PM can be reversed by interventions to restore normal angiogenesis. Overall, these studies will improve our understanding of the specific roles of the angiogenic factors in normal and malaria-complicated pregnancies and may help identify novel interventions to prevent poor birth outcomes associated with PM.

222

DC8-EXPRESSING *PLASMODIUM FALCIPARUM* - INFECTED ERYTHROCYTES BIND EPCR UNDER PHYSIOLOGICAL SHEAR STRESS BUT DO NOT INHIBIT ACTIVATED PROTEIN C GENERATION BY EPCR

Mark R. Gillrie[1], Marion Avril[2], Andrew Jay Brazier[2], Joe Smith[2], May Ho[1]

[1]University of Calgary, Calgary, AB, Canada, [2]Seattle Biomedical Research Institute, Seattle, WA, United States

Plasmodium falciparum-infected erythrocytes (IRBCs) expressing the domain cassettes (DC) 8 and 13 of the cytoadherent ligand PfEMP1 bind under static conditions to the endothelial protein C receptor (EPCR). It has been proposed that by interfering with EPCR functions, IRBC adhesion could promote localized coagulation and/or vascular permeabilitycontributing to the pathogenesis of cerebral malaria. Whether EPCR binding occurs under physiological shear stress or has any functional consequences has not been addressed. In this study, we examined the adhesion of the parasite clone IT4var19 expressing DC8 to primary human lung (HLMEC) and dermal (HDMEC) microvascular endothelial cells in a flow chamber assay. In addition, we used CIDRα1.1-coated beads, representing the EPCR-binding domain of DC8, to examine the functional consequence of EPCR binding on HLMEC barrier dysfunction in a transwell assay and activated protein C (APC) generation with an amidolytic APC generation assay. Our results showed that the low expression of EPCR on HLMEC mediated IT4var19 IRBC binding at 1 dyne/cm2. IRBC adhesion was inhibited by the mAb RCR-252 and EPCR mRNA knockdown by 35 and 50% respectively. In comparison, adhesion of IT4var19 to HDMEC, which was 5-6-fold higher than to HLMEC, was completely independent of EPCR. CIDRα1.1-coated beads had no direct effect on endothelial permeability, but inhibited thrombin-induced endothelial barrier dysfunction by 55% in an EPCR-dependent manner. No effect was found for the beads or IT4var19 IRBC on endothelial EPCR-dependent APC generation. Overall, the findings suggest that while EPCR contributes to IT4var19 adhesion to HLMEC under shear stress, its role may vary according to the microvasculature. CIDRα1.1 inhibition of thrombin activity could occur through an EPCR-dependent switch of thrombin-PAR-1 signaling from proinflammatory to cytoprotective signaling as suggested for APC mutants that engage EPCR but do not have proteolytic activity. Additional studies with clinical parasite isolates will further define the clinical relevance of PfEMP1-EPCR interactions.

223

FUNCTIONAL MICROENGINEERED MODEL OF THE HUMAN SPLENON-ON-A-CHIP

Aleix Elizalde-Torrent[1], Luis Rigat-Brugarolas[2], Maria Bernabeu[1], Mariana De Niz[1], Lorena Martin-Jaular[1], Carmen Fernandez-Becerra[1], Antoni Homs-Corbera[2], Josep Samitier[2], Hernando A. del Portillo[1]

[1]CRESIB (Barcelona Center for International Health Research), Barcelona, Spain, [2]Institute for Bioengineering of Catalonia, Barcelona, Spain

We have developed a newfangled microfluidic device that mimics the hydrodynamic behavior and filtering functions of the splenon, the minimal functional unit of the red pulp of the spleen able to maintain its filtering functions. Unlike any other previous microfluidic devices, the human splenon-on-a-chip incorporates for the first time two compartments with different flow velocities (according to the physiological flow division) and two physical barriers representing the reticular mesh and the interendothelial slits (IES) where cells first slow down increasing the hematocrit and then traverse the IES in a unidirectional matter. To validate the use of this platform, several experiments were carried out with different types of red blood cells (RBCs). As a proof of concept, we described that old RBCs showed less deformability than freshly drawn RBCs when traversing the microconstrictions. Posterior analysis allowed studying the passage and deformability of infected RBCs through the IES using peripheral blood of BALB/c mice experimentally infected with the *Plasmodium yoelii* 17X-GFP strain. Results showed that infected reticulocytes are significantly more deformable than non-infected reticulocytes, in agreement with the higher deformability of reticulocytes parasitized by *P. vivax*, a human malarial parasite with reticulocyte tropism. These results suggest that the device is able to reproduce physiological conditions and to distinguish different types of RBCs by means of deformation/mechanical properties. Presently, we are determining if there is hemolysis during the pass of blood through the device, if there is pitting in the absence of macrophages and the rheological properties of malarial infected RBCs.

224

ORGAN-SPECIFIC TISSUE FACTOR ACTIVITY IS SIGNIFICANTLY ALTERED IN EXPERIMENTAL MALARIA

Tara C. Bracken, Demba Sarr, Julie M. Moore

University of Georgia, Athens, GA, United States

Sequestration of *Plasmodium falciparum*-infected erythrocytes in the brain or the maternal blood space of the placenta results in two severe clinical manifestations of this disease, cerebral (CM) and placental malaria (PM), respectively. Hyperinflammation consisting of increased expression of Tumor Necrosis Factor-α (TNF) and activation of macrophages in affected organs are associated with CM and PM. It is well established in other models of disease that inflammatory damage leads to systemic activation of coagulation and severe thrombosis. Recent evidence showing the existence of a procoagulant state in patients suffering from CM or PM indicates the inflammation-coagulation cycle may play a significant role in malaria pathogenesis and may provide useful diagnostic and therapeutic targets. However, the extent to which coagulation is responsible for the pathogenesis of these diseases is incompletely understood. Thus, nonpregnant C57BL/6J mice were infected with 10^6 *P. berghei* ANKA, a virulent murine malaria species capable of inducing CM, or 10^6 *P. chabaudi* AS, a non-CM strain, then serially sacrificed between days 3 and 6 post-infection and assayed for TF procoagulant activity in brain, liver, and lung tissues. TF activity was significantly higher in brain (>1400-fold; P<0.01) of mice that succumbed to CM 5 and 6 days post-infection relative to uncomplicated malaria cases. In mice sacrificed on days 3 and 4 post-infection, all three organs exhibited a trend toward reduced TF activity, most notably in the brain (50-fold decrease). Preliminary data show reductions in TF activity at day 4 of *P. chabaudi* AS infection in the brain (>360-fold, P = 0.057), lung (>1200-fold, P = 0.016), and liver (>4400-fold, P = 0.057) compared to uninfected controls. Increased TF activity in the brains of mice that succumb to CM, but not those with uncomplicated *P. berghei* ANKA infection, suggests a significant role for coagulation in the pathogenesis of experimental CM. Ongoing experiments seek to further elucidate critical cellular sources of TF with the use of TF-floxed/cre mice. In addition, the importance of TNF in malaria-associated TF activity is being assessed with TNF and TNF receptor (TNFR) null mutant mice. Finally, to determine the effect of TF-driven coagulation on inducing placental pathology during PM, placentae from TF-, TNF-, and TNFR-modified *P. chabaudi* AS-infected mice are also being assessed.

225

MALARIA-RELATED ANEMIA IN COLOMBIA

Mary Lopez-Perez[1], Yoldy Benavidez[1], Álvaro Álvarez[1], Juan B. Gutierrez[2], Alberto Moreno[3], Sócrates Herrera[1], Myriam Arévalo-Herrera[1]

[1]Centro de Investigación Científica Caucaseco, Cali, Colombia, [2]Institute of Bioinformatics, University of Georgia, Athens, GA, United States, [3]Emory Vaccine Center, Emory University, Atlanta, GA, United States

Anemia and other hematological changes associated with malaria infections vary with the level of malaria endemicity, demographic variables and malaria immunity, among other factors. Information on cofactors associated with malaria-related anemia in Latin America is scarce. This

study aimed at characterizing erythrocytes indices in patients living in endemic areas of Colombia with unstable malaria transmission. A total of 929 malaria patients were enrolled between 2011 and 2013 in three areas of Colombia: Tierralta, Tumaco and Quibdó. Demographic and epidemiological information related to malaria was obtained at the time of diagnosis, and automated complete blood cell counts were performed on samples collected from all participants. Multiple linear regression analyses were then carried out to determine the relationships between erythrocytes indices and independent variables. *Plasmodium falciparum* was found to be the most prevalent species in Tumaco (84%) and Quibdó (70%), whereas *P. vivax* was predominant in Tierralta (92%). Variable degrees of non-severe anemia (Hb 7.1- 10.9 g/dL) were present in 21% of the patients, with similar distribution in the three study sites. Severe anemia (Hb <7 g/dL) was almost absent (0.3%). Afro-descendants from Quibdó presented lower hemoglobin levels than other ethnic groups regardless of the parasite species. Only in Tumaco, patients with *P. vivax* displayed lower hemoglobin levels than those found in *P. falciparum*-infected patients. In Tierralta, hemoglobin levels, hematocrit and RBC counts were negatively associated with days of illness. Moreover, in Tierralta and Quibdó, a direct relationship was found between the number of previous malaria episodes and hemoglobin levels. Both *Plasmodium* species appear to have similar potential to induce malarial anemia, but distinct cofactors at each endemic setting in Colombia seem to modify this clinical manifestation. Early diagnosis and prompt treatment are likely preventing more frequent and serious anemia in Colombia, and the target age in these low transmission settings has shifted towards adolescents-young adults. Moreover, previous malaria history appears to induce protection against anemia development.

226

HEME INDUCED ACTIVATION OF TOLL-LIKE RECEPTOR 4 IS ASSOCIATED WITH REDUCED HEMATOPOIETIC STEM CELL VIABILITY AND APOPTOSIS IN SEVERE MALARIA

Carmen M. Dickinson-Copeland, Nana O. Wilson, Mingli Liu, Ming-Bo Huang, Craig V. Bond, Michael D. Powell, Gale Newman, Shailesh Singh, Jonathan K. Stiles

Morehouse School of Medicine, Atlanta, GA, United States

Plasmodium falciparum infections are responsible for malaria associated mortalities ranging between 700,000 and 900,000 cases annually. Approximately 30% of the deaths occur after treatments indicating an urgent need for adjunctive therapies. Endothelial progenitor cells ($CD34^+$/$VEGRF^{2+}$) play an important role in the repair and regeneration of vascular endothelium as well as in neovascularization during cerebrovascular diseases, yet they are depleted by an unknown mechanism in severe malaria. Hematopoietic stem cells (HSC) are $CD34^+$ precursors to EPC and are decreased in the peripheral blood of individuals with Cerebral Malaria, a severe form of malaria associated with significantly high mortalities. We have shown that overproduction of free heme induces up-regulation of toll-like receptors (TLR) 4 and 9, as well as inflammation and apoptosis in both human and mouse brain vascular endothelial cells. However, the role of free heme in the depletion of HSC in severe malaria is poorly understood**.** We hypothesize that HSC depletion is a consequence of heme-induced TLR-mediated mechanism(s). We determined the effects of free heme on viability/apoptosis and TLR expression in HSC. Cell viability was assessed by MTT in HSC exposed to heme, apoptosis indices were examined by caspase-3 mRNA expression and TUNEL respectively. The effect of heme exposure on HSC expression of TLRs 1 - 10 was functionally assessed in the presence and absence of TLR specific blockers. Heme significantly decreased cell viability and induced cell apoptosis in both HBVEC and HSC compared to controls ($p<0.05$). Additionally, heme-induced TLR4 was up-regulated in HSC. Our results indicate that 1) free heme contributes significantly to the depletion of HSC and vascular endothelial cells by inducing apoptosis. 2) Free heme could be utilized as a potential biomarker for fatal malaria as it correlates with severity of malaria. 3) Among TLRs, TLR4 played a significant role in the heme-induced pathogenesis of malaria. In conclusion, *Plasmodium* infection activates TLR4 and subsequently induces apoptosis in HSC resulting in decreased numbers of circulating EPC and limited vascular repair in severe malaria pathogenesis.

227

PREVALENCE AND DIVERSITY OF HAEMOPARASITES IN BIRDS AT THE RESERVA NACIONAL ALLPAHUAYO MISHANA (RNAM), IQUITOS, PERU, 2013

Zaira H. Galarce[1], Leonila Ricopa[2], Carmen Reategui[2], Roberson Ramirez[1], Raul Chuquiyauri[3], Joseph M. Vinetz[3], Alfonzo Marzal[4]

[1]Asociacion Benefica Prisma, Iquitos, Peru, [2]Universidad Nacional de la Amazonia Peruana, Iquitos, Peru, [3]Division of Infectious Diseases, Department of Medicine, University of California San Diego, San Diego, CA, United States, [4]Universidad de Extremadura, España, Extremadura, Spain

Birds, like most wild animals, are frequently found to be affected by an enormous range of parasites, both endoparasites and ectoparasites, which represent a potential health risk for both humans and wild animals as well as a threat to the conservation of biodiversity. There is no information from Peruvian Amazon about the existence of these blood parasites, despite having the second richest ornithological diversity worldwide. The lack of research on wild birds in the Peruvian Amazon motivated us to investigate the presence of 3 hemoparasites that are genetically closely related (*Plasmodium, Hemoproteus*, and *Leucocytozoon*) in the Allpahuayo-Mishana National Reserve (AMNR). This is the first study in the Peruvian Amazon focus on identifying the presence of blood parasites in wild birds, and will provide invaluable information needed to assess the risk of emerging diseases that can affect human populations. For this purpose, a series of 10 parallel mist nets were installed in the study area from March to April 2013. A 75ml of blood sample was taken from each bird that was captured to perform a blood smear and a nested PCR amplification of Cytochrome b. A total of 203 birds belonging to 22 families and 62 species were captured. Our preliminary microscopy results revealed that 64 birds belonging to 28 species (31% of the total), were positive for *Plasmodium/Haemoproteus*. Casicus cela was the specie with the highest prevalence of parasites and mixed infections with Microfilaria and *Plasmodium/Haemoproteus*.

228

GENOME-WIDE ANALYSIS OF CHANGES IN BEWO CELLS INDUCED BY CS2 *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES, SCHIZOGONIC PRODUCTS AND CYTOKINES

Winifrida B. Kidima[1], Naveen Bobbili[1], James M. Burns[2], Diane W. Taylor[1]

[1]University of Hawaii at Manoa, Honolulu, HI, United States, [2]Drexel University, Philadelphia, PA, United States

Placental malaria (PM) changes the microenvironment at the intervillous space of the placenta which is essential for fetus growth by exposing the syncytiotrophoblast (ST) to *Plsmodium falciparum* infected erythrocytes (IE), *P. falciparum* schizogonic products (SP) and inflammatory cytokines leading to low birth weight babies. Changes of ST functions occurs during PM, however it is not known what contribute to these changes. We analyzed gene expression changes in the BeWo cell line produced by the binding of IE, IE and parasite SP, and cytokines produced by THP-1 monocytes co-cultured with IE. BeWo cells were syncytialized for 72 hours using 10 μM forskolin in triplicate cultures and exposed to intact CS2 IE, intact CS2 IE plus SP, and conditioned medium (CM) produced by THP-1cells upon exposure to IE and co-cultured for 48 hrs. Gene expression changes were then analyzed by the microarray. Functional annotation of differentially expressed genes (fold change ≥1.5 and adjusted P value ≤0.01) was determined using GO-term context with DAVID version 6.7v and altered pathways were identified using Pathway Miner (BioRag). Conditioned medium containing inflammatory cytokines and chemokines, but not IE alone or IE with SP, significantly altered genes associated with

biological processes and pathways in BeWo cells that are important for fetus and placental growth. The categories of genes enriched (P ≤0.05) among down-regulated genes in BeWo cells treated with CM included, genes linked with vasculogenesis, blood vessel formation and negative regulation of vasoconstriction. Growth pathways significantly altered (P ≤0.05) included insulin, insulin like growth 1, mammalian target of ramphamycin, transforming growth factor beta 1, platelet derived growth factor, epidermal growth factor signaling and prostaglandin synthesis and regulation. Cytokines and chemokine dysregulate pathways and biological processes in BeWo cells that are involved in placental vascular development and remodeling. These results may explain why placental insufficiency occurs as a result of malaria infection.

229

HEMATOPOIETIC STEM/PROGENITOR CELL SOURCES TO GENERATE RETICULOCYTES FOR *PLASMODIUM VIVAX*

Florian Noulin[1], Javed M. Manesia[2], Anna Rosanas-Urgell[1], Annette Erhart[1], Céline Borlon[1], Jan Van den Abbeele[1], Umberto D'Alessandro[3], Catherine M. Verfaillie[2]

[1]Antwerp Institute of Tropical Medicine, Antwerp, Belgium, [2]Stem Cell Institute, Leuven, Belgium, [3]Medical Research Council, Fajura, Gambia

The adaptation of *Plasmodium vivax* to *in vitro* culture remains an important challenge. The preference of *P. vivax* for immature erythrocytes (reticulocytes) constitutes one of the main hurdles. Three different sources of hematopoietic stem/progenitor cells (HSPC) i.e; umbilical cord blood (UCB), bone marrow (BM) and adult peripheral blood (PB) were expanded for 5 days and differentiate for 14 days to obtain reticulocytes. The HSPC population could be efficiently expanded and produce reticulocytes that are equally permissive to *P. vivax*. Interestingly the permissiveness of HSPC-derived reticulocytes was higher than the reticulocyte-enriched blood. Our findings might suggest that *P. vivax* has a preference for immature reticulocytes opening new perspective in the research on *P. vivax* invasion mechanism.

230

TACI IS REQUIRED TO CONTROL PARASITEMIA LEVELS POST PY NL INFECTIONS

Marcela Parra, Xia Liu, Steven Derrick, Amy Yang, Windy Allman, Sheldon Morris, Mustafa Akkoyunlu

Food and Drug Administration, Bethesda, MD, United States

The TACI (transmembrane activator and calcium-modulator and cyclophilin ligand) receptor binds BAFF (B-cell activator factor) and APRIL (a proliferation-inducing ligand), two cytokines critical for B-cell survival and plasma cell generation. To assess the role of TACI in malaria, TACI/KO and the wild type (WT) C57BL/6 mice were infected (i.p) with 1 million *Plasmodium yoelii* (Py) NL parasites and parasitemia was assessed. Results showed that the level of parasitemia was significantly elevated in TACI/KO mice (55.2% at day 28) compared to wild type WT mice (12.4% at day 16), and the parasitemia clearance was substantially delayed in the TACI/KO (28 days) relative to WT control (21 days). Subsequently, anti-Py NL antibodies and BAFF and APRIL concentrations were measured. In addition, to determine the role of B cells in protection, adoptive B-cell transfers were also done. Measurement of serum BAFF and APRIL levels revealed that in TACI/KO mice, the highest levels of BAFF (33.2 ng/ml) and the lowest levels of APRIL (119 ng/ml) were detected at peak parasitemia. Additionally, less anti- Py NL IgG-antibodies were produced by the TACI/KO mice up to Day 22 post infection when compared to WT mice, but the differences in antibody levels vanished between TACI/KO and WT mice at day 28 post infection. Both, WT and TACI/KO mice were immune to a second Py NL challenge with peak parasitemia less than 1% and parasitemia clearance by day 10 following the secondary infection. Importantly, adoptive transfer of "immune" B-cells conferred protection in both WT and TACI/KO mice against Py NL challenge. Conclusion: TACI plays a key role in the control of Py NL infections.

231

PHENOTYPIC CHARACTERIZATION OF THE *PLASMODIUM FALCIPARUM* NOT1 (PF3D7_1103800) GENE KNOCKOUT AND HEAT SHOCK ANALYSIS OF THE PIGGYBAC MUTANT LIBRARY

Phaedra J. Thomas, Jennifer Sedillo, Bharath Balu, Naresh Singh, John H. Adams

University of South Florida, Tampa, FL, United States

Ongoing research in our lab uses genetic approaches to identify new potential targets for therapeutic treatments. The objective of this work is to characterize PF3D7_1103800 and identify other genetic factors in the *piggyBac* mutant library affected by heat shock. The Not1 gene, PF3D7_1103800, is a part of a larger gene regulatory system in eukaryotes known as the CCR4-NOT complex, which is involved in mRNA transcription and degradation. Our research on the asexual stages of the major malaria parasite *Plasmodium falciparum* uses the phenotypic analysis of genetic mutants. The primary phenotypic screens are growth attenuation and changes in virulence factors. My dissertation focuses on the screening for genes involved with stress responses to increased temperature. In the process of a whole genome random transposon mutagenesis of *P. falciparum,* a knockout of *Not1* was isolated. Characterization of protein extracts from mutant and wild type parasites were analyzed by immunoblots with antibodies to EBA-175 and GAP45. The IFA localization of these invasion-related proteins in the *Not1* mutant was compared with wild type patterns. NOT1 and other *piggyBac* mutants were analyzed for altered sensitivity to febrile temperatures during culture through growth analysis with a flow cytometer. The immunoblots display altered expression at mid to late trophozoite stages in the *Not1* mutant when compared to the wild type. The IFAs for the *Not1* mutant reveal patterns similar to that of the *Caf1* mutant, which is also a part of the CCR4-NOT complex and found to be important in parasite egress. Of the more than 40 parasites tested, 2 were significantly increased in growth and 18 were significantly decreased compared to the wild type, including the Not1 knockout. Validation of the *Not1* mutant phenotype should result in delayed growth and invasion of the parasite. Furthermore, exposure of the *piggyBac* mutant library to increases in heat may reveal genetic factors involved with the intraerythrocytic cycle that lead to new drug targets.

232

IRON SUPPLEMENTATION AFTER SEVERE MALARIA IS ASSOCIATED WITH DECREASED HEME OXYGENASE-1 LEVELS ONE MONTH AFTER ILLNESS

Benjamin R. Hanisch, Sarah E. Cusick, Gregory S. Park, Gregory M. Vercellotti, Chandy C. John

University of Minnesota, Minneapolis, MN, United States

Iron treatment in malaria endemic areas can increase the risk of malaria and death. One mechanism by which iron supplementation increases the risk and severity of malaria may be by increasing oxidative stress. To assess changes in oxidative stress after treatment with iron in children with severe malaria, we assessed baseline and 4-week levels of heme oxygenase-1 (HO-1), an enzyme that is upregulated under oxidative stress, in Ugandan children with cerebral malaria (CM) (n= 51), severe malaria anemia (SMA) (n=39) and community children (CC) (n=52). We hypothesized that iron treatment would increase oxidative stress, as measured by HO-1. Upon enrollment, children who were iron deficient (zinc protoporphyrin (ZPP) >=80) either received daily iron therapy immediately (n=62) or 4 weeks later (n=54). All children with SMA and CM and 26 CC had a ZPP>=80, and were randomized to immediate vs. delayed treatment. 26 CC had a ZPP<80 and did not receive iron. Children with CM and SMA had similar baseline HO-1 concentrations, and did not differ in the immediate vs. delayed subgroups. Baseline HO-1 concentrations were lower in CC than in children with CM or SMA (P<0.0001). At 1 month, HO-1 concentrations in children in the CM and SMA delayed iron groups were increased compared to the immediate iron groups (both P<0.05) and the

iron sufficient group (both P<0.001), but HO-1 levels in the iron deficient community control group did not differ between immediate and delayed groups. There was no correlation of HO-1 levels at 1 month in children with CM or SMA who received either immediate or delayed iron treatment with readmission (P>0.05). Contrary to our hypothesis, iron treatment after malaria was associated with significantly lower HO-1 concentrations at 4 weeks. This suggests that iron treatment reduces oxidative stress in iron deficient children after malaria. Future research will explore potential mechanisms for this process.

233

SEQUESTRATION MECHANISMS OF *PLASMODIUM VIVAX*-INFECTED ERYTHROCYTES

Fabio T. Costa[1], Letusa Albrecht[1], Stefanie C. Lopes[1], Andre M. Siqueira[2], Carmen Bezerra-Fernandez[3], Hernando O. del Portillo[3], Irene S. Soares[4], Bruce Russell[5], Laurent Renia[6], Marcus V. Lacerda[2]

[1]University of Campinas, Campinas-SP, Brazil, [2]Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Manaus, Brazil, [3]Barcelona Centre for International Health Research (CRESIB), Barcelona, Spain, [4]University of Sao Paulo, Sao Paulo, Brazil, [5]National University of Singapore, Singapore, Singapore, [6]Singapore Immunology Network, Agency for Science, Technology and Research, Singapore, Singapore

We have previously demonstrated that *Plasmodium vivax*-infected erythrocytes (Pv-iEs) cytoadhere to endothelial cell receptors and form rosettes, a typical feature observed for *P. falciparum*. Rosette is a cytoadhesion phenotype where infected red blood cells can adhere to non-infected red blood cells and in falciparum malaria rosettes are normally associate to poor clinical outcomes and severity and might improve parasite infectivity. Nevertheless, it has recently shown that Pv-iEs harvested from vivax patients form rosettes, however correlation with severity and infectivity was not observed. These observations prompt us to investigate the biological role of rosetting formation of Pv-iEs. Thus, using a panel of 59 *P. vivax* Brazilian isolates, we showed that rosettes are mostly observed at late stages, they are formed mostly by normocytes and parasitemia levels correlate with rosetting rates. Nonetheless, we noticed that autologous plasma improved significantly rosette formation, suggesting a key role of plasma components. Indeed, we found positive correlation between IgM levels and an inverse association of IL6 and IL10 with rosetting rates. Moreover, antibodies towards to VIR proteins disrupt rosettes, which are less likely to be phagocyted *in vitro*. Thus, we hypothesize that rosetting probably acts as a mechanism of immune evasion.

234

AN ADJUSTABLE GAS-MIXING DEVICE TO INCREASE FEASIBILITY OF *IN VITRO* CULTURE OF *PLASMODIUM FALCIPARUM* PARASITES IN THE FIELD

Amy K. Bei[1], Saurabh D. Patel[1], Sarah K. Volkman[1], Ambroise D. Ahouidi[2], Daouda Ndiaye[3], Souleymane Mboup[2], Dyann F. Wirth[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Laboratory of Bacteriology and Virology, Hopital Aristide Le Dantec, Dakar, Senegal, [3]Laboratory of Parasitology and Mycology, Dakar, Senegal

A challenge to conducting high-impact and reproducible studies of the mechanisms of *Plasmodium falciparum* drug resistance, invasion, virulence, and immunity is the lack of robust and sustainable *in vitro* culture in the field. While the technology exists and is routinely utilized in developed countries, various factors–from cost, to supply, to quality–make it hard to implement in malaria endemic countries. Here, we design and rigorously evaluate an adjustable gas-mixing device for the *in vitro* culture of *P. falciparum* parasites in the field to circumvent this challenge. The device accurately replicates the gas concentrations needed to culture laboratory isolates, short-term adapted field isolates, cryopreserved previously nonadapted isolates, as well as to adapt *ex vivo* isolates to *in vitro* culture in the field. We also show an advantage over existing alternatives both in cost and in supply. Furthermore, the adjustable nature of the device makes it an ideal tool for many applications in which varied gas concentrations could be critical to culture success. This adjustable gas-mixing device will dramatically improve the feasibility of *in vitro* culture of *Plasmodium falciparum* parasites in malaria endemic countries given its numerous advantages.

235

MOLECULAR MARKERS OF RADIATION INDUCED ATTENUATION IN INTRAHEPATIC *PLASMODIUM FALCIPARUM* PARASITES

Miranda S. Oakley[1], Hong Zheng[1], Phuong Thao Pham[1], Babita Mahajan[1], Nirbhay Kumar[2], Davidson Sangweme[2], Abhai K. Tripathi[2], Godfree Mlambo[2], Sanjai Kumar[1]

[1]Food and Drug Administration, Bethesda, MD, United States, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Radiation attenuated sporozoite (RAS) vaccination has proved to be a promising approach for malaria vaccine development. While the host requirements for sterile immunity induced by RAS vaccination have been studied, the molecular events that render attenuation to sporozoites in response to radiation remain poorly understood. We performed genome-wide transcriptional profiling of untreated verses radiation-treated intrahepatic parasites on days 3 and 6 post-invasion of human HepG2 hepatic cells by *Plasmodium falciparum* sporozoites of the 3D7 strain to identify parasite gene targets of growth attenuation and enhanced immune protection induced by radiation. Using this approach, we found that 180 parasite genes had significantly altered transcriptional expression in response to radiation on day 3 and 6 post-cultivation in hepatic cells. Among these novel biomarkers, we identified a signature of eight candidate parasite genes that associated with functionally diverse pathways that may regulate radiation induced cell cycle arrest of the parasite within the hepatocyte. In addition, a repertoire of 14 genes associated with protein translation is transcriptionally overexpressed within the parasite by radiation. We hypothesize that this radiation induced enhanced translational activity may be a contributing factor for the sterilizing immunity observed by RAS vaccination by increasing the pool of parasite antigens available for immune presentation either as processed epitopes on the hepatocyte surface or as released antigens from apoptotic infected hepatocytes . These results have significantly increased the repertoire of novel targets for 1) generating genetically attenuated parasite vaccine candidates 2) subunit vaccines against the hepatic stage cycle and 3) biomarkers of safety to define proper attenuation.

236

COMPARATIVE GROWTH ANALYSIS OF MALARIA PARASITE, *PLASMODIUM FALCIPARUM*, USING ^{3}H-HYPOXANTHINE INCORPORATION AND SYBR GREEN FLUORESCENCE

Kyleray R. Katherman, Anastasia Goldys, Deborah B. Champagne, Heather B. Reilly Ayala

George Fox University, Newberg, OR, United States

Differential growth of *Plasmodium falciparum* in red blood cells (RBC) is an essential feature of malaria parasite biology. High growth rate results in increased parasite biomass which is correlated with disease severity in patients. 'Growth' is generally ascertained microscopically or through a surrogate reporting system such as tritium labeled hypoxanthine (^{3}H- Hx). The use of tritium has been the 'gold standard' for measuring parasite growth as it effectively mirrors visually determined parasitemias. This method of labeling requires the parasites to be starved of the essential nucleic acid precursor, hypoxanthine (Hx) for 48 hours in order to facilitate uptake of the isotope. SYBR green, a relatively inexpensive fluorescent dye, has been shown to give similar results to ^{3}H-Hx-incorporation in drug assays, providing an alternative method for ascertaining parasite growth. However, a side-by-side comparison of SYBR green and ^{3}H-Hx-incorporation has yet to be completed in the absence of drug. Here

we provide a head to head comparison of the SYBR green and ^{3}H-Hx-incorporation assays in a set of genetically unique progeny to ascertain if these two methods can be used interchangeably in determining parasite growth. Our data shows a correlation between the data sets indicating that the relative growth rate as measured by both assays is comparable. In addition we use the SYBR green assay to measure differences in parasite growth in Hx starved and unstarved conditions. Preliminary data indicates decreased parasite growth in Hx starved conditions. More interesting is the observation that parasites show variable responses to Hx starvation suggesting a possible genetic mechanism may be involved.

237

ASSESSMENT OF KNOWLEDGE AND ADHERENCE TO THE NATIONAL GUIDELINES FOR MALARIA CASE MANAGEMENT IN PREGNANCY AMONG HEALTHCARE PROVIDERS AND DRUG DISPENSERS IN RURAL, WESTERN KENYA

Christina M. Riley[1], Stephanie Dellicour[2], Peter Ouma[3], Urbanus Kioko[4], Feiko O. ter Kuile[5], Ahmeddin Omar[4], Simon Kariuki[3], Ann M. Buff[6], Meghna Desai[7], Julie R. Gutman[8]

[1]Emory University, Rollins School of Public Health, Atlanta, GA, United States, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]KEMRI/Centers for Disease Control and Prevention Research and Public Health Collaboration, Kisumu, Kenya, [4]Malaria Control Unit, Ministry of Health, Nairobi, Kenya, [5]Liverpool School Of Tropical Medicine, Liverpool, United Kingdom, [6]U.S. President's Malaria Initiative, Nairobi, Kenya, [7]Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Nairobi, Kenya, [8]Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States

Although prompt and effective treatment is a cornerstone of malaria control, information on healthcare provider adherence to malaria treatment guidelines in pregnancy is lacking. Incorrect or sub-optimal treatment can cause adverse consequences to the mother and fetus. We conducted a cross-sectional study from September to November 2013, in all health facilities and randomly selected drug outlets in Siaya County, western Kenya, to assess healthcare provider adherence to and knowledge of national guidelines for treatment of uncomplicated malaria in pregnancy to inform the Kenyan Ministry of Health and relevant stake-holders on knowledge gaps to be targeted. In health facilities, standardized questionnaires were used to interview all healthcare providers and all women of childbearing age who had been assessed for fever. Mystery clients posing as 1st trimester pregnant women or as relatives of women in 3rd trimester collected information from drug outlets. Information on drugs was recorded from prescriptions or after reviewing drugs in patient's possession. None of the drug outlet dispensers, versus 56% of health facility providers, knew the appropriate treatment for 1st trimester patients, while 39% and 87%, respectively, knew the appropriate treatment for 2nd/3rd trimester. Prescription of the correct drug for pregnancy trimester at the correct dosage, was observed in 66% of cases in health facilities and 50% in drug outlets. Prescribing was more often correct in 2nd/3rd trimester than in 1st (65% vs. 32%, p=0.004, and 38% vs. 0%, p<0.001, at health facilities and drug outlets, respectively). Sulfadoxine-pyrimethamine, which is no longer recommended for treatment of acute malaria, was prescribed and available in 4% of cases in health facilities and 23% of simulations in drug outlets (p<0.001). Exposure to artemether-lumefantrine in 1st trimester, which is contraindicated due to its unknown safety, occurred in 13% and 51% of cases in health facilities and drug outlets respectively (p=0.04); none were a result of quinine stock-out. This study highlights knowledge inadequacies and incorrect prescribing practices in the treatment of malaria in pregnancy. These should be addressed through comprehensive trainings and adequate supervision by the Kenya Ministry of Health to improve the quality of patient care and maximize therapeutic outcomes.

238

RANDOMIZED TRIAL TO COMPARE THE SAFETY, TOLERABILITY AND EFFICACY OF BI-MONTHLY INTERMITTENT PREVENTIVE TREATMENT WITH EITHER MEFLOQUINE-ARTESUNATE OR SULFADOXINE-PYRIMETHAMINE PLUS AMODIAQUINE, WITH THE STANDARD REGIMEN OF DAILY PROGUANIL, FOR THE PREVENTION OF MALARIA AND RELATED COMPLICATIONS IN PATIENTS WITH SICKLE-CELL DISEASE IN NIGERIA

Rasaq R. Olaosebikan[1], Samuel K. Ernest[1], Andrea M. Rehman[2], Muna Affara[3], Davis Nwakanma[3], Olugenga Mokuolu[1], Bojang Kalifa[3], Ayodele Ojuawo[1], Brain Greenwood[2], Paul Milligan[2]

[1]University of Ilorin Teaching Hospital, Ilorin, Nigeria, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]UK Medical Research Council, Banjul, Gambia

Effective prophylaxis should be provided for children with sickle-cell disease(SCD) in malaria endemic areas. The aim of this study was to find out if intermittent treatment with mefloquine-artesunate (MQ+AS) could be used in this way. Patients with SCD were randomly assigned one of three intervention groups. Patients in group 1 received a fixed-dose combination of Mefloquine-Artesunate at each bimonthly clinic visit, those in group 2 sulfadoxine-pyrimethamine plus amodiaquine (SP+3AQ) bimonthly whilst patients in group 3 received daily proguanil. Patients were given diaries and are asked to return to the clinic every two months and whenever they were unwell. Three days after each clinic visit, patients or their carer were interviewed to ask about compliance and adverse events. The primary outcome of the trial was tolerability, secondary outcomes were adherence to the regimen, incidence of malaria and the number of outpatients presentations and hospitalizations over 12 months. 270 SCD patients were followed-up from September 2011 to May 2013, 90 in each study group. Adherence to the bimonthly regimens was excellent, (87% received 6 doses of MQ+AS and 84% completed all courses of the drug; 88% received 6 doses of SP+3AQ and 86% completed all courses). In contrast, adherence to the daily proguanil regimen was poor (10% took tablets less than three days in four). Incidence of mild adverse events during three days after each clinic visit, was highest in the MQ+AS group with about 20% of children reporting side effects, the most common of which were nausea, vomiting and abdominal pain, but these symptoms did not affect adherence. Incidence of malaria was low in all groups. The number of malaria cases was 17 (proguanil), 14(SP+3AQ) and 9(MQ+AS), rate ratios compared to proguanil were 0.84 (95%CI 0.4,1.9) for SP+3AQ and 0.55 (95%CI 0.2,1.2) for MQ+AS. The incidence of other illnesses was similar in the groups. Supervised treatments with MQ+AS at the clinic once every 2months is well tolerated and safe and may be more effective in preventing malaria than daily prophylaxis with proguanil. These results justify a larger trial powered to determine efficacy in preventing malaria and malaria-related complications. This trial is registered with ClinicalTrials.gov: NCT01319448 ISRCTN46158146.

239

SAFETY AND EFFICACY OF DIHYDROARTEMISININ-PIPERAQUINE FOR TREATING UNCOMPLICATED FALCIPARUM PREGNANCY-ASSOCIATED MALARIA IN GHANA

Joseph Osarfo[1], Pascal Magnussen[2], Michael Alifrangis[2], Harry Kwami Tagbor[1]

[1]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]Center for Medical Parasitology, Copenhagen, Denmark

Dihydroartemisinin-piperaquine (DHA-PPQ) was approved for treatment of uncomplicated malaria in Ghana in 2008. However, DHA-PPQ is not used in pregnancy due to the paucity of safety data in this population. In light of anticipated DHA-PPQ use by self-medicating pregnant women, we have studied the safety and efficacy of DHA-PPQ compared to artesunate-amodiaquine (ASAQ) in a non-inferiority trial for treatment of uncompliccated malaria in second/ third trimester pregnancies.

Pregnant women who attended antenatal clinics in two districts of the Ashanti Region were screened for falciparum malaria using both a rapid diagnostic test and microscopy. Those testing positive in both were recruited, individually randomized to DHA-PPQ or ASAQ, and followed up actively on days 1, 2, 3, 7, 14, 28 and 42 after the start of treatment, at delivery and 6 weeks post-partum. During this period, assessment of adverse events, assessment of study drug adherence, sampling of blood for haematological and parasitological assessments and data collection on neonatal morbidity and mortality were performed. The primary outcome was parasitological efficacy by day 42. Analysis was primarily per protocol. Uncorrected cumulative parasitological efficacy by day 42 for DHA-PPQ was 91.2% (95% CI: 86.8%, 95.4%) and for ASAQ it was 87.0% (95% CI: 80.6%, 91.7%). By day 28, it was 93.8% (95% CI: 89.3%, 96.9%) for DHA-PPQ and almost 92.0% (95% CI: 86.5%. 95.4%) for ASAQ. There were no reports of hepatic/ renal toxicity or evidence of white blood cell dyscrasia in either study arm. The DHA-PPQ arm had fewer adverse events than the ASAQ arm; vomiting (17% vs 29%; p=0.021), general weakness (40% vs 60%; p<0.001). DHA-PPQ was non-inferior to ASAQ within a margin of 5% and also safe in the second and third trimesters. However, we are cautious in generalizing this conclusion because of the small sample size and our inability to report PCR-corrected efficacy rates. Larger studies are needed to confirm the results of this preliminary study.

240

IN VITRO DRUG TRANSPORT OF ANTIMALARIALS; AMODIAQUINE, MEFLOQUINE AND METHYLENE BLUE

SMDK Ganga Senarathna[1], Madhu Page-Sharp[2], Andrew Crowe[1]

[1]Curtin University and Curtin Health Innovation Research Institute, Bentley, Australia, [2]Curtin University, Bentley, Australia

Identification of P-gp mediated drug-drug interactions that interfere with antimalarial drug absorption would inform optimum drug design combinations in malaria. The study aimed to determine the P-gp substrate and inhibitory activities of amodiaquine (AQ), mefloquine (MQ) and methylene blue (MB). Caco-2 cell lines were grown on 0.6 cm^2 filter inserts at the cell density of 65,000 cells/cm^2. Bidirectional drug transport studies were done on 21-24 day cells with transepithelial electrical resistance > 300 $\Omega.cm^2$. AQ, MQ and MB were assayed via HPLC techniques. Rhodamine 123 (Rh123) quantification was done using fluorescence. MQ at 100 micromolar, pH 7.4 showed an apical-basolateral (A-B) permeability of $11*10^{-6}$ cm/sec and basolateral-apical (B-A) permeability of $12*10^{-6}$ cm/sec. Efflux ratios (ER) for 100 and 10 micromolar MQ suggested diffusion, as they were 1.1 and 1.3 respectively. Other antimalarial drug combinations that included MQ were not altered significantly, nor combinations with the P-gp inhibitor PSC-833. AQ at 100 μM, pH 7.4 showed an A-B permeability of $20*10^{-6}$ cm/sec and B-A permeability of $23*10^{-6}$ cm/sec, thus more permeable than MQ, but still not evidence of active efflux as their efflux ratios were suggestive of diffusion. B-A permeability of AQ did increase when combined with PSC833; however the statistical power of the test was low. P-gp substrate activity of MB was studied at 10 μM, pH 7.4 and showed an ER of 3.4 dropping to 1.6 in combination with 4 micromolar PSC833. Changes in transport rates in both directions were statistically significant. P-gp inhibitory activities of the three drugs were studied by combining drugs with a known P-gp substrate, Rh123. Rh123 efflux was not altered significantly when combined with any of the antimalarials (MQ, AQ or MB). We conclude that MQ and AQ are transported mainly by passive diffusion. MQ diffusion is not altered by artesunate. MB is subjected to P-gp mediated drug efflux. These drugs did not exhibit P-gp inhibitory activity.

241

THE AVAILABILITY AND COST OF ANTIMALARIAL MEDICATIONS IN DRUG OUTLETS IN RURAL SIAYA COUNTY, WESTERN KENYA

Urbanus K. Kioko

Malaria Control Unit, Ministry of Health, Nairobi, Kenya, Nairobi, Kenya

Although antimalarials are free in public health facilities in Kenya, challenges including stock-outs lead to patients seeking treatment in the private sector or directly from drug outlets. Medicine retailers play a major role in the distribution of antimalarials. The Affordable Medicines Facility - malaria program (AMFm) was introduced in June 2010 to make artemisinin combination therapy (ACT) as affordable as less effective alternatives. This study was conducted to determine the availability and cost of rapid diagnostic tests (RDTs) and antimalarials in drug outlets in a high malaria transmission area in rural western Kenya. We conducted a cross-sectional study from September to October 2013 and mapped all drug outlets within the Kenya Medical Research Institute/Centers for Disease Control and Prevention Health and Demographic Surveillance System area in Siaya County. We administered a standardized structured questionnaire to drug outlet personnel to collect data on outlet characteristics and availability, types and prices of RDTs and antimalarials. We identified 181 drug outlets, and 179 agreed to participate. Of these, 13% were registered pharmacies; 25% informal drug outlets; 46% general shops; 13% home-based outlets and 2% other. Only 17 (10%) had RDTs in stock, and 149 (84%) never stocked RDTs. 165 (92%) had ACTs in stock; of these, 98% had artemether-lumefantrine (AL); 13% had dihydroartemisinin-piperaquine (DHA-PPQ); 47% had sulfadoxine-pyrimethamine (SP); 34% had quinine (QN) and 8% had amodiaquine (AQ). Among the QN-stocking outlets, 48% had tablets and 62% had a parenteral formulation. The mean (SD) price in USD of an adult treatment course of AL, DHA-PPQ, QN tablets, SP, AQ was 1.01 (0.38), 4.40 (1.15), 2.24 (1.18), 0.62 (0.31), and 0.42 (0.20) respectively; the mean (SD) price in USD of RDTs was USD 0.92 (0.64). There was no significant difference in mean price of AL with or without the AMFm logo (USD 1.01 and 1.07 respectively, p= 0.45). The majority of drug outlets did not stock RDTs suggesting that testing prior to treatment per the national guidelines is unlikely for patients first seeking treatment in the outlets. The first-line treatment, AL, is widely available across outlet types. SP and AQ are not recommended for treatment but are less expensive than AL; the lower cost might encourage preferential use by patients. Restrictive policies should be enforced to prevent access and sale of ineffective antimalarials.

242

IMPACT OF INTERMITTENT PREVENTIVE TREATMENT WITH DIHYDROARTEMISININ-PIPERAQUINE ON COGNITIVE FUNCTION, SCHOOL ATTENDANCE AND SCHOOL PERFORMANCE IN UGANDAN SCHOOLCHILDREN: A RANDOMIZED PLACEBO-CONTROLLED TRIAL

Joaniter I. Nankabirwa[1], Pauline Amuge[1], Bonnie Wandera[1], Sarah Staedke[2], Simon J. Brooker[2], Moses R. Kamya[1]

[1]Makerere University College of Health Sciences, Kampala, Uganda, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

Intermittent Preventive Treatment (IPT) is a promising option for malaria control in schoolchildren. However, there is still limited evidence of its benefits on cognitive function and educational outcome in this age group in different transmission setting. Therefore, we have investigated the impact of two dosing schedules of IPT with dihydroartemisinin-piperaquine (DP) on cognitive function, school attendance and school performance in Ugandan schoolchildren living in a high malaria transmission setting. We conducted a randomized, double-blind, placebo-controlled trial in 740 schoolchildren aged 6-14 years. Enrolled children were randomized to receive DP once every month (IPTm), once a school term (four treatments over 12 months) (IPTst) or placebo and followed for 18 months. The outcomes included cognitive function which was assessed using the

Ravan's matrices (for abstract reasoning) and the code transmission test (for sustained attention), school performance in the end of year mathematics and English exams and school attendance. Analyses were conducted on an intention to treat basis. Compared to placebo, IPTm marginally reduced the risk of missing school due to being sick (12.5% versus 9.5% p=0.06), especially if the illness was malaria (5.1% versus 0% p=0.05). No difference was observed between IPTst and placebo on school attendance. No impact of IPT compared with placebo was observed on sustained attention (mean difference [MD] IPTm -0.01 p=0.920, and IPTst -0.11 p=0.693), abstract reasoning (MD IPTm 0.09 p=0.726, and IPTst 0.52 p=0.228), results in mathematics exams (MD IPTm -0.8 p=0.418, and IPTst -1.5 p=0.676) or results in English exams (MD IPTm 0.5 p=0.551, and IPTst -1.2 p=0.797). IPTm reduced the risk of missing school due to malaria. However, IPT as implemented in this study (IPTm and IPTst) was not effective in improving the cognitive function or school performance of schoolchildren.

243

ADHERENCE TO CO-FORMULATED AMODIAQUINE-ARTESUNATE VS. ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN FREETOWN, SIERRA LEONE

Kristin Banek[1], Samuel Juana Smith[2], Clare I. Chandler[1], Musa Sillah Kanu[2], Anitta Kamara[2], Daniel Chandramohan[1], Sarah G. Staedke[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]National Malaria Control Programme, Freetown, Sierra Leone

Increasing access to and targeting of artemisinin-based combination therapy (ACT) is a key strategy of malaria control programmes. Approaches, such as co-packaging ACTs into blister packs, have been shown to improve adherence. While co-packaging facilitates correct treatment dosing, it does not reduce the quantity or frequency of tablet intake. To overcome theses limitations, several ACTs are now produced as co-formulated tablets. Although there have been a number of studies looking at adherence to ACTs, the majority have focused on adhernce to artemether-lumefantrine (AL). The aim of this study was to measure the level of caregiver adherence to co-formulated amodiaquine-artesunate (AQAS) compared to AL in Sierra Leone. This open-labelled randomised controlled study was conducted from September 2013 to January 2014 in two public health centres in urban Freetown. Patients 6-59 months presenting for fever at the health facility were randomized to receive either AQAS or AL at the time of prescription. Adherence was assessed using self-report of medication intake and package inspection. Exit interviews of caretakers were conducted at the health facility and follow-up interviews at their home on day four post-prescription. Of 1,950 patients screened, 795 were excluded due to: inappropriate age (29.6%), residence outside of the catchment area (12.3%), prior participation in the study (6.6%), no fever or history of fever (9.1%), and evidence of severe disease (2.0%). Of the 1,155 patients enrolled, 90% completed study follow-up, and 859 (87.5%) received an ACT. Preliminary results from one study site show that there was no difference in adherence to AQAS (96.4%) compared to AL (97.1%) (p=0.56). Reasons for non-adherence were similar for both treatment arms (forgot the last dose or took dose incorrectly). Vomiting was the most cited reason for missing a treatment dose for both groups. Data analysis for the second site is currently on-going. Full results, including comparison of caregiver adherence at both study sites and factors influencing adherence, will be presented.

244

POPULATION PHARMACOKINETICS OF ORALLY ADMINISTERED MEFLOQUINE IN HEALTHY VOLUNTEERS AND PATIENTS WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

Piero L. Olliaro[1], Stephanie E. Reuter[2], Richard N. Upton[2], Allan M. Evans[2], Visweswaran Navaratnam[3]

[1]World Health Organization, Geneva, Switzerland, [2]University of South Australia, Adelaide, Australia, [3]Universiti Sains Malaysia/Taylors University, Penang/Selangor, Malaysia

The determination of dosing regimens for the treatment of malaria is largely empirical and thus a better understanding of the pharmacokinetic/pharmacodynamic properties of anti-malarial agents, such as mefloquine, is required to assess the adequacy of current treatment regimens and identify sources of sub-optimal dosing which could select for drug-resistant parasites. Mefloquine pharmacokinetics were assessed in 24 healthy adults and 43 patients with *P. falciparum* malaria. Population pharmacokinetic modelling was conducted using NONMEM. A two-compartmental model with a single transit compartment and first-order elimination from the central compartment most adequately described mefloquine concentration-time data. The model incorporated population parameter variability for clearance (CL/F), central volume of distribution (VC/F) and absorption rate constant (KA), and identified, in addition to body weight, malaria infection as a covariate of VC/F (but not CL/F). Simulations predict that falciparum malaria infection is associated with a shorter elimination half-life (407 c.f. 566hr) and time above MIC (766 c.f. 893hr). This is first population pharmacokinetic study to show falciparum malaria to influence mefloquine disposition. Protein binding, anaemia and other factors may contribute to differences between healthy individuals and patients. As VC/F is related to the earlier portion of the concentration-time profiles, which occurs during acute malaria, and CL/F is more related to the terminal phase during convalescence after treatment, this may explain why malaria was found to be a covariate for VC/F but not CL/F. These findings highlight the importance of patient characteristics when determining optimal dosing regimens for use in clinical practice; more studies will be needed in different populations.

245

ANEMIA IN ACUTE AND CONVALESCENT UNCOMPLICATED FALCIPARUM MALARIA TREATED WITH ARTESUNATE-AMODIAQUINE AND COMPARATORS IN SUB-SAHARAN AFRICA

Piero L. Olliaro[1], Julien Zwang[2]

[1]World Health Organization, Geneva, Switzerland, [2]Drugs for Neglected Diseases initiative, Geneva, Switzerland

Anemia is both a complication of malaria and a potential drug adverse reaction. Recovering from malaria should coincide with increased hemoglobin (Hb) levels, but the process is confounded by poorly-understood parasite, host and drug-related factors. Delayed hemolytic anemia was recently reported after parenteral artemisinin for severe malaria. We analysed 5189 patients with acute uncomplicated falciparum malaria from 8 studies conducted at 14 sites in 9 Sub-Saharan Africa countries during 2002-2009. Median age was 4 years (range 6m - 86y). 64% of the patients were 10 yo. 2380 patients were treated with artesunate-amodiaquine (ASAQ, 45.8%), 2809 with comparators (54.2%). In ASAQ groups, high parasitaemia was associated with low Hb both at baseline (r=-0.287) and post-treatment at D3, at D7, D14, and D28 (r=-0.165, -0.483, -0.161, -0.382, respectively, p=0.001 for all, Spearman test). Patients before ASAQ treatment had a mean Hb=10.9 g/dL (sd 2.20). Mean changes between D0-D3 were -0.48 g/dL (-4.0%); D0-D7 -0.45 g/dL (-3.2%); D0-D14 +0.54 g/dL (+7.5%); D0-D28 +0.77 g/dL (+9.8%). By multivariate analysis with random effects on the site (9594 Hb records) Hb increased over time (r=0.04, p=0.001) and in older patients (r=0.06, p=0.001) and decreased with higher parasitaemia (r=-0.39, p=0.001). By

multivariate survival analysis, patients with higher parasitaemia (adjusted hazard ratio, AHR=1.32, 95%CI 1.19-1.46, p=0.001), and children <6 yo (AHR=1.91, 95%CI 1.55-2.34, p=0.001) and anemia (<10 g/dL) before treatment (AHR=1.26, 95%CI 1.09-1.46, p=0.002) were at higher risk for parasite recurrence. On D0 32.6% (502/1541) patients were anemic, 39.6% (610/1541)on D7,16.2% (249/1541) on D28. Between D7 and 28, 2.0% (19/931) became anemic, 37.7% (230/610) remained anemic and 62.3% (380/610) became normal. Similar trends were observed with other combination treatments. Anemia and high parasitaemia concur to increase malaria treatment failure. Hb increases by D14 after initial fall. Delayed anemia did not occur in uncomplicated malaria.

246

SCALE UP OF INTEGRATED HOME-BASED MANAGEMENT IN SENEGAL

Seynabou Gaye[1], Julie Thwing[2], Fatou Ba[1], Alioune B. Gueye[1], Moussa Ndour[1], Ibrahima Diallo[1], Medoune Ndiop[1], Mady Ba[1]

[1]Senegal National Malaria Control Program, Dakar, Senegal, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States

Since 2008, the Senegal National Malaria Control Program (NMCP) has implemented home-based management of malaria (PECADOM) using rapid diagnostic tests (RDTs) and artemisinin-based combination therapy (ACTs). In 2012, the NMCP and partners piloted integrated home-based management, including case management of diarrhea with zinc and oral rehydration salts (ORS) and pneumonia with antibiotics (in children under 5 years) as well as malaria in all age groups. Encouraging results prompted the scale-up of integrated home-based management in the southeastern regions of Tambacounda and Kedougou, which suffer from high all-cause under 5 mortality. In 2013, 428 villages in Tambacounda and Kedougou selected a home-based care provider (DSDOM). They received four days of classroom instruction on diagnosis and treatment of diarrhea, pneumonia, and malaria, followed by 15 days of practical experience at the health post nearest the village, supervised by the head nurse. Each provider received a drug box, timer, vest and hat, case register, RDTs, ACTs, co-trimoxazole, ORS, and zinc. Health districts received a budget to provide monthly supervision by the nurse and quarterly supervision by the district health management team. From June to December 2013, DSDOMs saw 12,979 patients: 5,705 patients under 5 years and 7,274 patients 5 years and older. Of these, 10,704 (82%) had fever and 98% were tested with an RDT; 7,310 (70%) had positive tests and 7,652 were treated with an ACT. Of children under 5, 2074 (36%) were diagnosed with malaria, 1,041 (18%) were diagnosed with diarrhea, 53 (5%) were treated with ORS alone, 35 (3%) were treated with zinc alone, and 953 (92%) received both. There were 656 (11%) children under 5 diagnosed with pneumonia and treated with co-trimoxazole. Of the remaining 578 patients, 575 (10%) were diagnosed with upper respiratory infection, and symptomatic relief was recommended. Of the 3,217 patients (25%) referred to the health posts (all ages), 2,791 (22%) had a negative RDT, 30 (0.2%) were less than two months, 25 were pregnant women (0.2%), and 359 (3%) had severe disease. In 2014, integrated PECADOM will be scaled up to an additional nine regions, and DSDOMs will be trained to identify and refer acute malnutrition among children under 5 years. These efforts should contribute substantially to the achievement of Millennium Development Goals 4 and 6, the reduction of under 5 mortality and the fight against malaria and other diseases.

247

CHANGE IN MALARIA FALCIPARUM TREATMENT IN A MUNICIPALITY OF ACRE STATE, BRAZIL

Eduardo Saad[1], Paola Marchesini[1], Oscar Lapouble[2], Izanelda Magalhães[3], Ana Carolina Faria Santelli[1]

[1]Ministry of Health, Brasília, Brazil, [2]Brasilia University, Brasília, Brazil, [3]State Secretary of Health of Acre, Rio Branco, Brazil

Brazil has around 300.000 malaria cases/year which represents about 60% of cases in Americas. In 2012, Acre state showed a 37% increase in malaria cases compared to 2011.Since 2006 Acre state has been using artesunate+mefloquine (ASMQ) for *P. falciparum* treatment, started as a efficacy study and adopted as treatment as soon as the drug was registered in Brazil. According World Health Organization recommendations to not use mefloquine in high transmission areas due to its longer half-life and possible development of resistance, The malaria advisory committee has suggested in 2011 to change the treatment and in 2012, treatment has been changed to artemether+lumefantrine (AL). All others Amazonia regions were already using al since 2006.The aim of the present study was to evaluate if the change in treatment regimens has contributed to increase cases in Rodrigues Alves municipality in 2013. An observational cross sectional study was perfomed based on primary data and were used as a measure of Odds ratio association to estimate relative risk. Study population was patients treated in health units or by field health workers in routine visits in Rodrigues Alves, with positive thick blood smear for *Plasmodium falciparum*. Data were analysed with Epiinfo 3.5.3. A total of 210 questionnaires were analysed. Respondents were male in 54% and the median age was 15 years (1-80). The slide cure verification on day 7 (d7) was performed in 191 (60%) patients. Treatment with ASMQ has been used in 99 (47%) patients. Three (3%) have not been cured. 111 (53%) have been treated with AL and one (0.9%) has not been cured. The odds ratio was 0.29 and IC95% 0.02-2.84. The results show that individuals treated with AL had 0.29 times more probability to have been cured when compared to subjects treated with ASMQ. However, this difference was not significant statistically, and the protection factor was possible related to a spurious association. In this way it suggests a similar efficacy between the two drugs. On the other hand, possibly there was a reduction of the mefloquine half-life effect to prevent new infections. Monitoring resistance surveillance to antimalarial drugs has prominent role to contain or eliminate the resistant parasites before they spread to higher transmission areas what will put in serious risk the recent progress in malaria control. It is important to create new strategies to detect early decrease of effectiveness of these drugs.

248

REPEATED SPOROZOITE EXPOSURES WITH TRIMETHOPRIM-SULFAMETHOXAZOLE (TMP-SMX) PROPHYLAXIS INDUCE HETEROLOGOUS PROTECTION AND EFFECTOR/EFFECTOR MEMORY T-CELL RESPONSES IN RODENTS

Charlotte V. Hobbs, Jillian Neal, Solomon Conteh, Charles Anderson, Patrick E. Duffy

National Institutes of Health/National Institute of Allergy and Infectious Diseases, Rockville, MD, United States

Repeated experimental malaria exposures in individuals receiving antimalarial prophylaxis can induce long-lasting sterile immunity, a strategy known as Chemoprophylaxis Vaccination or "CVac". We previously showed that TMP-SMX given at prophylactic doses can arrest liver stage development of malaria parasites, and that TMP-SMX prophylaxis during repeated malaria exposures induces long-lived sterile CD8 T-cell dependent immunity in mice. We set out to assess this protection against heterologous challenge, and to characterize the CD8 T cell responses observed. Mice receiving prophylactic TMP-SMX were inoculated with *Plasmodium yoelii 17 XNL* sporozoites once a month for 3 months, after which TMP-SMX was discontinued (TMP-SMX Cvac). One to three months later, mice were challenged with *P. yoelii P265 BY or P. berghei*

sporozoites. Splenic and hepatic lymphocytes harvested from unchallenged mice were phenotyped using flow-cytometry. TMP-SMX CVac induced sterile heterologous protection against *P. yoelii P265 BY* (p=0.00009) and *P. berghei* (p<0.0001) at 1 and 3 months, equivalent to the level of protection against homologous challenge that we previously reported. CD8 T cells identified using H-2K^{d} restricted epitope of CS Protein had Effector/Effector Memory Phenotypes at 1 and 3 months (p<0.05) with enhanced responses in the liver. In conclusion, TMP-SMX CVac induces protection against heterologous sporozoite challenge for up to 3 months in mice, characterized by CD8 T cell-Effector/Effector Memory responses, which parallels responses induced by irradiated sporozoite vaccination. Because HIV and malaria geographically overlap, we must understand how the use of TMP-SMX in HIV management impacts malaria infection and immunity in children.

249

PHARMACOKINETICS AND *EX VIVO* ANTIMALARIAL ACTIVITY OF ARTEMISININ-PIPERAQUINE VERSUS ARTESUNATE-AMODIAQUINE IN HEALTHY VOLUNTEERS

Nguyen N. Quang[1], Nguyen T. Chinh[1], Chu X. Anh[1], Nguyen X. Thanh[2], Geoffrey Birrell[3], Marina Chavchich[3], Dennis Shanks[3], **Michael D. Edstein**[3]

[1]Department of Infectious Diseases, Military Hospital 108, Hanoi, Vietnam, [2]Military Institute of Hygiene and Epidemiology, Hanoi, Vietnam, [3]Department of Defence, Brisbane, Australia

Reduced susceptibility to artesunate and artemisinin-based combination therapies (ACTs) such as dihydroartemisin-piperaquine has been reported in western Cambodia and south Vietnam. This is of immense concern as ACTs are currently recommended worldwide for first-line treatment of uncomplicated *Plasmodium falciparum* malaria. In Vietnam, dihydroartemisinin-piperaquine is the ACT of choice for the treatment of falciparum malaria, with little knowledge of the efficacy and tolerability of alternative ACTs. Recently, we reported Artequick (artemisinin-piperaquine) and Coarsucam (artesunate-amodiaquine) to be highly efficacious (PCR-corrected cure rate >98%) and well tolerated in Vietnamese patients with falciparum malaria in south-central Vietnam. Because there is limited pharmacokinetics (PK) data on these two ACTs, we evaluated the PK of artemisinin-piperaquine and artesunate-amodiaquine in two groups of 22 healthy Vietnamese subjects. Group 1 volunteers were administered daily a dose of two tablets of Artequick (each tablet contains 62.5 mg artemisinin and 375 mg piperaquine) for 3 days. Group 2 volunteers received a daily dose of two tablets of Coarsucam (each tablet contains 100 mg artesunate and 270 mg amodiaquine) for 3 days. Serial blood samples were collected up to 42 days and 28 days after the last dose of Artequick and Coarsucam, respectively. Plasma samples were assayed by LC/MS/MS for estimating the PK properties of the partner drugs for each ACT. Furthermore, we carried out a head-to-head comparison of the *ex vivo* antimalarial activity (pharmacodynamics-PD) of the two ACTs using the volunteers plasma samples after drug administration to determine which ACT possessed greater blood schizontocidal activity, *in vitro*. The PK-PD relationship of the two ACTs will be presented and discussed, including their implications for the treatment of malaria.

250

EXPLOITING EVOLUTION TO SUPPRESS THE EMERGENCE AND SPREAD OF DRUG RESISTANCE IN MALARIA

Leila S. Ross[1], Onkar M. Singh[2], Paul Rowland[2], Francisco Javier Gamo[3], Maria José Lafuente-Monasterio[3], Amanda K. Lukens[4], Dyann F. Wirth[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]GlaxoSmithKline, Stevenage, United Kingdom, [3]GlaxoSmithKline, Tres Cantos, Spain, [4]The Broad Institute, Cambridge, MA, United States

Rising drug resistance threatens to make malaria untreatable, necessitating both the discovery of new antimalarial agents and the development of strategies to identify and suppress the emergence and spread of drug resistance. Drug resistance can rapidly compromise the effective useful lifetime of antimalarial agents. However, drug resistance emerges in the context of a population and is limited by evolutionary fitness costs. We focused on in-development dihydroorotate dehydrogenase (DHODH) inhibitors. We identified eleven point mutations and gene amplification in the PfDHODH target, as well as several unknown resistance mechanisms. These mutant resistant parasites were often hypersensitive to other PfDHODH inhibitors, which immediately suggested a novel combination therapy approach to preventing resistance. Indeed, a combination of wild-type and mutant-type selective inhibitors led to resistance far less often than treatment with either drug alone. The effects of point mutations in PfDHODH were corroborated with purified recombinant proteins. Comparative growth assays demonstrated that two mutant parasites were less fit than their wild-type parent, and the purified protein of those mutants showed a decrease in catalytic efficiency, thereby suggesting a reason for the diminished fitness. Co-crystallography of PfDHODH with three inhibitors suggested that hydrophobic interactions are important for drug binding and selectivity, and that mutations in the active site tunnel have more serious catalytic and fitness consequences than mutations in the active site lid. Fitness limitations result in a small number of mutational escape pathways being heavily favored in *Plasmodium*. These escape pathways can be anticipated and blocked, and doing so increases the efficacy of an individual patient´s treatment and protects the useful lifetime of novel therapeutics.

251

MEFLOQUINE VERSUS SULFADOXINE-PYRIMETHAMINE FOR INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY (IPTP): A MULTIPLE-OUTCOME ANALYSIS ON EFFICACY AND TOLERABILITY

Valerie Briand[1], Sylvie Escolano[2], Valérie Journot[3], Achille Massougbodji[4], Michel Cot[1], Pascale Tubert-Bitter[2]

[1]UMR216, Research Institute for Development, Université Paris Descartes, Sorbonne Paris cité, Paris, France, [2]Institut national de la santé et de la recherche médicale, Centre for research in Epidemiology and Population Health, U1018, Villejuif, France, [3]Institut national de la santé et de la recherche médicale, U897-Epidemiologie-Biostatistique, Bordeaux, France, [4]Faculté des Sciences de la Santé, Centre d'Étude et de Recherche sur le Paludisme Associé à la Grossesse et l'Enfance, Cotonou, Benin

Evaluation of alternative drugs to replace sulfadoxine-pyrimethamine (SP) for intermittent preventive treatment (IPTp) is urgently needed because of the spread of resistance of parasites to the drug, which raises the question of the short-term efficacy of IPTp-SP to prevent malaria in pregnancy. Very few options are currently available, of which none fulfil all the ideal properties for IPTp. Therefore, alternatives need to be evaluated on basis of a benefit-risk ratio. Mefloquine (MQ) is one of the options that have already been evaluated for IPTp. Here, we reanalysed data from the first Beninese trial on IPTp-MQ using a multiple-outcome approach, which allowed the joint assessment of efficacy and tolerability. The trial was conducted in Ouidah, South Benin, and involved 1600 pregnant women, who were randomised in two groups (IPTp with MQ, 15mg/kg, versus IPTp with SP (1500-75mg) administered twice during pregnancy). For the present analysis, the overall superiority of one drug to the other was defined as: superiority on low-birth-weight (birth weight < 2500 grams), placental malaria or maternal anaemia (Hb < 10g/dL), non-inferiority on all efficacy outcomes and non-inferiority on tolerability defined as severe adverse events (AE) - either cutaneous or neuro-psychiatric - or low compliance with the treatment (i.e., reluctance to receive the second IPTp dose because of AE at the first dose or refusal to receive the second dose whatever the reason). MQ was found to be overall superior to SP (global P value=0,004). By applying different strategies to deal with missing data and/or including stillbirths, spontaneous abortions and multiple pregnancies in the analyses provided similar results. Whatever

the final decision on the use of MQ for IPTp, such a statistical approach outweighing the benefits/disadvantages of a large prevention strategy should systematically be taken into account by institutional policy makers.

252

CYP 2D6 METABOLISM IS ESSENTIAL FOR THE ANTIMALARIAL ACTIVITY OF TAFENOQUINE AND NPC-1161B

Jason C. Sousa[1], Sean R. Marcsisin[1], Gregory A. Reichard[1], Diana Caridha[1], Qiang Zheng[1], Norma Roncal[1], Ronan McNulty[1], Julio Careagabarja[1], Richard J. Sciotti[1], Jason W. Bennett[1], Victor E. Zottig[1], Gregory Deye[1], Qigui Li[1], Lisa Read[1], Mark Hickman[1], N. P. Dhammika Nanayakkara[2], Larry A. Walker[2], Bryan Smith[1], Victor Melendez[1], Brandon S. Pybus[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]University of Mississippi, Oxford, MS, United States

Tafenoquine (TQ) is an 8-aminoquinoline (8AQ) that has been tested in several Phase II and Phase III clinical studies and is currently in late stage development as an anti-malarial prophylactic agent. NPC-1161B is a promising 8AQ in late preclinical development. It has been reported that the 8AQ drug primaquine requires metabolic activation by CYP 2D6 for efficacy in humans and in mice, highlighting the importance of pharmacogenomics in the target population when administering primaquine. A logical follow-up study was to determine whether CYP 2D activation is required for other compounds in the 8AQ structural class. In the present study, the anti-malarial activities of NPC-1161B and TQ were assessed against luciferase expressing *Plasmodium berghei* in CYP 2D knock-out mice in comparison with normal C57BL/6 mice (WT) and with humanized/CYP 2D6 knock-in mice by monitoring luminescence with an *in vivo* imaging system. These experiments were designed to determine the direct effects of CYP 2D metabolic activation on the anti-malarial efficacy of NPC-1161B and TQ. NPC-1161B and TQ exhibited no anti-malarial activity in CYP 2D knock-out mice when dosed at their ED_{100} values (1 mg/kg and 3 mg/kg, respectively) established in WT mice. TQ anti-malarial activity was partially restored in humanized/CYP 2D6 knock-in mice when tested at two times its ED_{100}. The results reported here strongly suggest that metabolism of NPC-1161B and TQ by the CYP 2D enzyme class is essential for their anti-malarial activity. Furthermore, these results may provide a possible explanation for therapeutic failures for patients who do not respond to 8AQ treatment for relapsing malaria. Because CYP 2D6 is highly polymorphic, variable expression of this enzyme in humans represents a significant pharmacogenomics liability for 8AQs which require CYP 2D metabolic activation for efficacy, particularly for large-scale prophylaxis and eradication campaigns.

253

A CONSTANT RATE INTRAVASCULAR INFUSION MODEL FOR THE EVALUATION OF INCREASES IN HEMATOCRIT AFTER ARTEMISININ-BASED COMBINATION TREATMENTS OF ACUTE FALCIPARUM MALARIA IN CHILDREN

Kazeem Akano[1], Adejumoke I. Ayede[2], Akintunde Sowunmi[1]

[1]University of Ibadan, Ibadan, Nigeria, [2]University College Hospital, Ibadan, Nigeria

A constant rate intravascular infusion model, which employed the principles of constant rate intravenous infusion of drugs, was used to evaluate the kinetics of the increases in hematocrit after artesunate-amodiaquine (AA) or artemether-lumefantrine (AL) treatments of acute, uncomplicated falciparum malaria in 112 children. The model assumed baseline hematocrit was zero and a constant rate increase in hematocrit from baseline after treatment, and involved semi-logarithm plots of the difference between hematocrit at plateau and that at earlier times, against the corresponding times. Hematocrit reached a plateau in a median time of 28 days after treatment started. Mean plateau hematocrit was 6.75% (95%CI 6-7.5) and was similar for AA- and AL- treated children [6.92% (95%CI 6.01-7.83), n = 81 *v* 6.32% (95%CI 4.92-7.7), n=31, P 0.45]. Times to plateau were significantly shorter and plateau hematocrit significantly lower in non-anemic compared to anemic children. Declines from plateau were monoexponential with mean half time of 2.49 days (95%CI 2.1-2.79) and were similar in AA and AL-treated children [2.42 days (95%CI 2.07-2.77) *v* 2.68 days (95%CI 2.03-3.3), P = 0.46]. Hematocrit half times were significantly shorter in anemic compared to non-anemic children [2.09 days (95%CI 1.77-2.41, n 59) v 2.94 days (95%CI 2.41-3.46, n 53), P = 0.006) indicating hemopoietic responses may have been 'accelerated' following successful treatment in the former. The constant rate intravascular infusion model permits evaluation of increases in hematocrit following antimalarial treatments and may be used in observational and clinical studies in uncomplicated falciparum malaria.

254

COMBINING COMMUNITY CASE MANAGEMENT OF MALARIAL AND SEASONAL MALARIA CHEMOPREVENTION FOR CHILDREN LESS THAN 10 YEARS IN SENEGAL: FEASIBILITY, IMPACT ON MALARIA AND ANEMIA

Roger C. Tine[1], Babacar Faye[1], Cheikh T. Ndour[2], Badara Cissé[1], Matt Cairns[3], Magatte Ndiaye[1], Jean Louis Ndiaye[1], Pascal Magnussen[4], Ib Bygbjerg[4], Brian Greenwood[3], Oumar Gaye[1]

[1]Université Cheikh Anta Diop, Dakar, Senegal, [2]Centre Hospitalier Universitaire de Fann, Dakar, Senegal, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom, [4]University of Copenhagen, Copenhagen, Denmark

Malaria and anemia are commonly associated in tropical regions. Scaling up antimalarial interventions as advocated by the WHO, will contribute to further reduce malaria burden and may induce a significant reduction in the global anemia burden. Malaria control in many epidemiological situations such as in Senegal will require a multi-intervention strategy with the use of combination of antimalarial interventions. Studies are needed, to document the feasibility as well as the impact on malaria and anemia of combining several antimalarial interventions. Relevant studies are also needed to assess potential anemia determinants and risk factors while scaling up antimalarial interventions, for an effective and integrated anemia control. This project aimed to assess the potential benefit of adding seasonal malaria chemoprevention (SMC) to community case management of malaria (CCMm) and to investigate factors associated with anemia among <10 years. The study objectives were covered by undertaking: i) a cluster randomised trial during witch eight communities were randomised to receive either CCMm combined with SMC (intervention arm) or CCMm alone (control arm), (ii) a case control study with anaemic and non.aneamic children. The overall adjusted rate ratio for incidence of malaria attacks in intervention and control communities was 0.15, indicating a protective effect of CCMm+SMC of 85% (95% CI:39.9%-96.3%, p=0.01). The case control study, demonstrated that the main factors significantly associated with anemia in these communities were: malaria parasitaemia (aOR=5.23, 95% CI[1.1-28.48]), sickle cell disorders (aOR=2.89, 95% CI[1,32-6.34]), alpha-thalassemia (aOR=1.82, 95% CI[1.2-3.35]), stunting (aOR=3.37, 95% CI[1.93-5.88], age ranged from 2 to 4 years (aOR=0.13, 95% CI[0.05-0.31]) and age > 5 years (aOR=0.03, 95% CI[0.01-0.08]). The combination of CCMm and SMC will result in a significant reduction of malaria and anemia burden. However, to achieve an effective control of anemia, integrated approaches taking into account other aetiologies of anemia need to be developed.

255

RISK FACTORS FOR POOR QUALITY ARTEMISININ CONTAINING ANTIMALARIALS IN TANZANIA'S PRIVATE SECTOR - RESULTS FROM A NATIONALLY REPRESENTATIVE OUTLET SURVEY

Rebecca Thomson[1], Caroline Lynch[1], Harparkash Kaur[1], Boniface Johanes[2], Charles Festo[2], Admirabilis Kalolella[2], Albert van Wyk[1], Naiela Malik[1], Ifey Fadeyi[1], James H. Long[1], Shunmay Yeung[1], David Schellenberg[1], Isabel Swamidoss[3], Mike D. Green[3], Prabha Dwivedi[4], Facundo M. Fernandez[4], Mark Taylor[1], Katia Bruxvoort[1], S. Patrick Kachur[3], Catherine Goodman[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Georgia Institute of Technology, Atlanta, GA, United States

Artemisinin-containing antimalarials (ACAs) are acknowledged to be the most effective treatment for uncomplicated malaria. There have been reports that in some areas up to a third of antimalarials are "fake", especially in the private sector, but few studies have been conducted in a representative fashion. Poor quality (falsified, substandard or degraded) antimalarials can lead to increased mortality and morbidity and contribute to resistance. In Tanzania the private sector is commonly used as a source of treatment for fever. We purchased ACAs from drug retailers throughout Tanzania mainland and measured the quality of each sample. A nationally representative survey was conducted in 2010 as part of the Independent Evaluation of the Affordable Medicines Facility-malaria. Every drug store, pharmacy and general retailer in 49 wards was visited and a sample of every ACA in stock was purchased. The active pharmaceutical ingredient (API) of the artemisinin and partner component was measured using high performance liquid chromatography and mass spectrometry, followed by multivariate analysis to determine risk factors for poor quality. Of the 1,737 ACAs purchased and analysed, 87.1% were in tablet form and a quarter were WHO prequalified products. All samples contained the artemisinin derivative (ART), with 4.1% being outside the acceptable 85-115% API range. Based on multivariate analysis of the ART component, ACAs manufactured in Europe were 4.02 times more likely to be of poor quality (p=0.01), while WHO prequalified ACAs had 0.05 times the odds of being poor quality (p=0.01). When combined with the partner component, 12.1% of samples were of poor quality, while granular ACAs had 8.3 times the odds of being poor quality (p=0.04), and WHO prequalified ACAs had 0.07 times the odds (p=0.002). Weak medicine regulatory systems have been observed in a range of low and middle income countries, reflecting lack of financial resources, manpower and capacity. However, these results indicate that important improvements in quality can be achieved by ensuring that only products meeting WHO pre-qualification are registered and permitted onto the market.

256

PLASMODIUM FALCIPARUM MALARIA-ASSOCIATED ANEMIA IN UNDER FIVE-YEAR-OLD NIGERIAN CHILDREN: BEFORE AND AFTER ARTEMISININ-BASED COMBINATION TREATMENTS

Titilope Okuboyejo[1], Chimere Agomo[2], Godwin Ntodom[3], Stephen Oguche[4], Henrietta U. Okafor[5], Ismaila Watila[6], Philip Agomo[2], William Ogala[7], Olajide Banjo[3], Kazeem Akano[8], Bayo Fatunmbi[9], Akintunde Sowunmi[8]

[1]Covenant University, Ota, Nigeria, [2]Nigeria Institute of Medical Research, Yaba, Lagos, Nigeria, [3]National Malaria Control Programme, Federal Ministry of Health, Abuja, Nigeria, [4]Department of Paediatrics, University of Jos, Jos, Plateau State, Nigeria, [5]Department of Pediatrics, Institute of Child Health, University of Nigeria Teaching Hospital, Enugu, Nigeria, [6]Department of Paediatrics, Specialists Hospital, Maiduguri, Borno State., Nigeria, [7]Department of Paediatrics, Ahmadu Bello University, Zaria, Kaduna State, Nigeria, [8]Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria, [9]World Health Organization, Regional Office, Manila, Philippines

The risk factors for, and recovery from anemia in acute falciparum malaria were characterized in 672 <5year-olds from five geographical areas of Nigeria before and after artemether-lumefantrine or artesunate-amodiaquine treatments. Of these, 294 (43.8%, 95%CI 40.0-47.6) presented with anemia (hematocrit <30%). Before treatment, an age <3 years and duration of illness >3 days were independent risk factors for anemia, and anemia at enrolment and parasitemia ≥100,000/μL after treatment. Malaria-attributable fall in hematocrit was 6.4% (95%CI 6.06-6.84). Mean time to recovery from anemia was 12.7d (95%CI 11.9-13.4), was similar with both treatments, and correlated positively with enrolment parasitemia (P=0.012). Fall in hematocrit per 1000 asexual parasites cleared from peripheral blood after treatment was significantly lower at higher compared with lower parasitemias (P<0.0001). Kinetics of the deficit in hematocrit (from 30%) after treatment began were estimated by a non-compartment model. Declines in hematocrit deficit were monoexponential, with a mean elimination half-life of 0.37d (95%CI 0.35-0.38). Deficit in hematocrit half-lives and efficacy in anemic children were similar for both treatments. Artemether-lumefantrine and artesunate-amodiaquine, influence the risk of anemia after treatment, conserve hematocrit at high parasitemias, and may influence recovery from *Plasmodium falciparum* malaria-associated non-severe anemia in Nigerian children.

257

ARTEMETHER-LUMEFANTRINE PHARMACOKINETICS AND PHARMACODYNAMICS IN VERY YOUNG CHILDREN TREATED FOR UNCOMPLICATED MALARIA IN UGANDA

Eskouhie Tchaparian[1], Emmanuel Arinaitwe[2], Nancy Sambol[1], Shelley McCormack[1], Victor Bigira[2], Darren Creek[3], Humphrey Wanzira[2], Abel Kakuru[2], Mary Muhindo[2], Daniel Blessborn[4], Jordan Tappero[5], Yngve Bergqvist[4], Francesca Aweeka[1], **Sunil Parikh**[6]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Makerere University, Kampala, Uganda, [3]Monash University, Parkville, Australia, [4]Dalarna University, Borlange, Sweden, [5]Centers for Disease Control and Prevention, Atlanta, GA, United States, [6]Yale School of Public Health, New Haven, CT, United States

Artemether-lumefantrine (AL) is the most widely utilized treatment for malaria. Pharmacokinetic (PK) and pharmacodynamic (PD) data are limited in the youngest children, but concern exists over inadequate exposure in young children. Our objective was to describe the population PK/PD of lumefantrine (LR) in children 6 to 24 months of age. Capillary blood samples were collected on filter paper (n=1027) over 21 days during 249 episodes of malaria occurring in 105 children. Samples were assayed using solid-phase extraction followed by HPLC. Population PK analysis of LR with

the software Monolix® (v.4.3) employed a 2-compartment open model with first-order absorption and allometric scaling for CL, Q, V1 and V2. Cox proportional hazards regression, stratified by use of trimethoprim-sulfamethoxazole (TS) prophylaxis, was conducted to explore relationships of LR levels on days 7 and 14 with recurrent parasitemia to days 28 and 42. Point estimates for the typical patient in this population were 173 L for V1, 0.94 L/h for Q, 5,740 L for V2, and 0.021 L/h for CL. The influence of hematocrit on V1 was the only covariate identified as significantly ($p \leq 0.005$) affecting PK, with a positive correlation. Multivariate regression revealed that, in those children not taking TS, higher day 7 LR capillary concentrations were significantly associated with lower cumulative risk of recurrent parasitemia by days 28 and 42 (HR 0.7, $p<0.001$ for both). Children with detectable LR on day 14 (n=15/129) had no recurrent parasitemia by day 28. Additionally, while exposure was not significantly affected by TS usage in the population PK model, day 7 LR levels were significantly higher in children on TS compared to those not on TS (median level 252 vs 209 ng/mL). Our results describe a 2-compartment PK model for LR disposition in children with a significant covariate effect of hematocrit on V1. LR exposure was significantly associated with recurrent parasitemia, and TS significantly impacted that relationship. Additional analyses are underway to inform treatment strategies in the youngest age group.

258

RITONAVIR-BOOSTED LOPINAVIR (LPV/R) ANTIRETROVIRAL THERAPY ASSOCIATED WITH LOWER CONCENTRATIONS OF DESETHYLAMODIAQUINE IN MALARIA-UNINFECTED HIV-POSITIVE PEOPLE TREATED WITH ARTESUNATE-AMODIAQUINE

Victor Mwapasa[1], Fraction Dzinjalamala[1], Jane Mallewa[1], Mavuto Mukaka[1], Saye Khoo[2], Robert Robert[3], David Lalloo[4]

[1]Malawi College of Medicine, Blantyre, Malawi, [2]University of Liverpool, Liverpool, United Kingdom, [3]Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi, [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

In sub-Saharan Africa, most HIV-infected (HIV+) individuals on antiretroviral therapy (ART) are exposed to malaria. When infected with malaria they require treatment with artesunate-amodiaquine (ASAQ). Few studies have examined the pharmacokinetics (PK) and safety of ASAQ in HIV+ individuals taking ARTs containing Nevirapine (NVP) or ritonavir-boosted Lopinavir (LPV/r). We conducted an open label clinical trial to compare the maximum concentration (Cmax) and area under concentration-time curve (AUC) of amodiaquine (AQ) and desethylamodiaquine (DESAQ) in antiretroviral naive HIV+ individuals and those taking NVP and LPV/r-based ART. In step 1 of the trial, malaria uninfected adults (n=6/ART group) received half the standard dose of ASAQ (1 tablet of 100/270mg each) at times 0, 24 and 48hrs. In Step 2, another group of malaria uninfected adults (n=25/ART group) received a standard dose of ASAQ (2 tablets of 100/270mg each). We performed data-rich PK blood sampling and assessed treatment emergent hematological and biochemistry abnormalities. In Steps 1 and 2, AQ concentrations were well below the HPLC assay lower limit of quantitation (25ng/mL). From step 1 to Step 2, DESAQ AUC significantly increased 7-fold from 5,724 ng.hr/mL (95%CI: 3,877-10,100) to 40,400 ng.hr/mL (95%CI: 36,285-43,175), $p<0.000001$. In Step 1, there were no significant differences in DESAQ Cmax and AUC among participants in the LPVr-, EFV and ART-naive groups. In Step 2, compared with the ART-naive group, participants in the LPV/r-ART group had lower DESAQ Cmax (267, [95%CI: 178-375] vs 412, [95%CI: 324-678] ng/mL, $p<0.0001$) and AUC (27,893, [95%CI: 17,276-41,383] vs 48,172, [95%CI: 35,402-59,336]) ng.hr/mL, $p<0.0001$). However, no significant differences in Cmax and AUC were observed between the NVP-based and ARV-naïve groups. In step 2, cases of transaminitis (n=2) and neutropenia were commonly observed in the NVP-ASAQ and ART-naïve groups but not in the LPV/r group. Thus, LPV/r-ART reduces DESAQ plasma concentration and may be associated with reduced efficacy of ASAQ. However, higher DESAQ levels may be associated with liver and hematological toxicities.

259

HIGH RESOLUTION MELTING (HRM) ANALYSIS HIGHLIGHTS MALARIA MISDIAGNOSIS AMONG MICROSCOPICALLY NEGATIVE FEBRILE PATIENTS IN WESTERN KENYA, AND HAS IMPLICATIONS FOR BETTER DRUG USE

Purity N. Kipanga[1], David Omondi[2], Paul O. Mireji[3], Patrick Sawa[4], Dan Masiga[5], Jandouwe Villinger[1]

[1]International Centre of Insect Physiology and Ecology and Egerton University, Nairobi, Kenya, [2]Department of Medical Biosciences, University of Western Cape, Bellville, South Africa, Cape Town, South Africa, [3]Yale University School of Public Health, New Haven, CT, United States, [4]International Centre of Insect Physiology and Ecology, Mbita, Kenya, [5]International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya

Microscopy and rapid diagnostic tests (RDTs) are common tools for diagnosis of malaria, but are deficient in detecting low *Plasmodium* parasitemia, often leading to inappropriate medical prescriptions and patient care based on misdiagnoses of clinical presentations. We developed and applied a molecular diagnostic tool (nPCR-HRM) that combines the sensitivity and specificity of nested PCR (nPCR) and direct PCR-high resolution melting analysis (dPCR-HRM) to screen blood samples from febrile patients for low-parasitemia malaria in a rural malaria endemic setting.Blood samples (n=197) were collected in two islands of Lake Victoria, Kenya, from febrile patients without *Plasmodium* detectable by microscopy or RDTs. 18S rRNA gene sequences were amplified from extracted DNA by nPCR-HRM, nPCR, and dPCR-HRM to detect and differentiate *Plasmodium* parasites. Data on administration of antimalarials were also collected. The coupled nPCR-HRM assays detected *Plasmodium* parasitemia in 62 (31·5%, 95% CI 25·0-38·0%) of the samples, among which 53 (26·9%, 95% CI 20·7-33·1) had *P. falciparum*. However, nPCR and dPCR-HRM detected *Plasmodium* parasitemia in only 20 (10·2%, 95% CI 5·9-14·4) and 39 (19·8%, 95% CI 14·2-25·4) of the samples respectively. Among low-parasitemia infections determined by nPCR-HRM, only 42 (67·7%) patients were treated with antimalarials, whereas 110 (81·5%) patients not infected with *Plasmodium* parasites were treated with antimalarials. Our findings demonstrate the limitations of differential diagnostics of febrile illness in rural malaria endemic settings that confound epidemiological estimates of malaria, and lead to inadvertent overprescription of antimalarial drugs to non-malaria febrile patients and under-prescription of antimalarial drugs to low-parasitemia malaria patients. nPCR-HRM enhances low-parasitemia malaria diagnosis and can potentially surmount the deficiencies of microscopy and RDT based results in determining malaria parasitemia, and evaluating malaria epidemiology.

260

PRESUMPTIVE DIAGNOSIS MALARIA TREATMENT IN FEBRILE CHILDREN IN PARTS OF SOUTHEASTERN NIGERIA

Uchechukwu M. Chukwuocha[1], Chinyere Okoro[2], Ikechukwu N. Dozie[1], Chinyere N. Ukaga[3], Geoffery C. Nwakwuo[1]

[1]Federal University of Technology, Owerri, Nigeria, [2]Federal Medical Center, Owerri, Nigeria, [3]Imo State University, Owerri, Nigeria

Malaria diagnosis in Nigeria has largely been done clinically; based to a larger extent on recognition of symptoms. Parasitological confirmation is a key component of case management of malaria. Clinical diagnosis has poor accuracy and leads to over diagnosis of malaria and poor management of non-malaria febrile illnesses and wastage of antimalarial drugs. Parasitological diagnosis using microscopy was performed among 560 febrile children, 0-12years attending pediatric clinics in Federal Medical Center Owerri and some primary health care centers in Imo State, South Eastern Nigeria after obtaining Ethical clearance. Their blood

samples were collected prior to treatment and were treated for malaria according to the IMCI guidelines and the standard routine practice of the clinic. These children were separated into two age groups: group I (0-≤5) and II (>5-12) for data analysis purpose. Out of 560 children (0-12 years) enrolled, 156(27.9%) were actually positive for malaria parasites, while 404(72.1%) were slide negative. Only *Plasmodium falciparum* was found in the positive samples. Children's age was significantly related to the prevalence of uncomplicated malaria (p=0.000) and a high determinant in explaining 6.4% of the variance in the prevalence of uncomplicated malaria (F=37.915 and p=0.000). Group II (>5-12) had higher parasite prevalence of 44% compared to 20% prevalence found in group I (0≤5) although group I had higher parasitaemia (density count). Only children between 0-≤5 years received antimalarials in compliance with the IMCI guidelines. This gave an overtreatment of 83.1% in children 0-≤5 years based on blood smear microscopy confirmation. The findings highlight the need for the scaling-up of parasitological confirmation of all patients suspected to stand overtreatment with the ACTs. Improving the diagnostic system for effective health care delivery in Nigeria and other poor endemic areas will not only provide a good platform for malaria treatment/monitoring but also reduce rapid onset of drug resistance, wastage of antimalarials and adequate management of other febrile illnesses in children.

261

LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR MALARIA DIAGNOSIS TARGETING THE APICOPLAST GENOME

Eniyou C. Oriero[1], Jan Jacobs[2], Jean-Pierre Van geertuyden[3], Davis C. Nwakanma[1], Umberto D'Alessandro[1]

[1]Medical Research Council Unit, The Gambia, Fajara, Gambia, [2]Insitute of Tropical Medicine, Antwerpen, Belgium, [3]International Health Unit, University of Antwerpen, Antwerpen, Belgium

Malaria still remains a global health burden with an estimated 627,000 malaria deaths worldwide in 2012. The shortcomings of current diagnostic methods are a concern, and the malERA Consultative Group on Diagnoses and Diagnostics has called for improved malaria diagnostic methods. Current malaria diagnostic methods do not rapidly and accurately detect asymptomatic infections which contribute significantly to transmission. As malaria declines and large numbers of samples need to be screened to target intervention measures appropriately, high throughput amplification of nucleic acids via polymerase chain reaction (PCR) become more relied on, however, they are not easily field deployable. Isothermal amplification methods have been developed to address the major shortcomings of PCR methods. Different targets are being amplified, the most common being the conserved small subunit ribosomal RNA 18S locus. In this study, we report the development and optimization of the apicoplast genome of *Plasmodium falciparum* as a target for molecular diagnosis of malaria using a loop-mediated isothermal amplification (LAMP) assay. *P. falciparum* sequences from 15 Gambian isolates and 8 laboratory clones were aligned against the PlasmoDB reference sequence (emb|X95275.2|). Primers were designed from a highly conserved region of the consensus sequence, approx. 1.5kb segment of the gene coding for a ribosomal RNA protein (AP|0010:rRNA); and validated *in silico*. The assay was optimized for temperature, primer, dNTPs and $MgCl_2$ concentration. 184 archived DNA samples from West Africa and S.E Asia were analyzed against a reference PCR method. End point determination was by naked eye and agarose gel electrophoresis after staining with SYBR Green I®. Preliminary results show Sensitivity of 100.00% (95% CI: 93.88 % to 100.00 %), Specificity of 83.78 % (95% CI: 67.98 % to 93.77 %), Positive Predictive Value of 90.77 % (95% CI: 80.97 % to 96.51%), Negative Predictive Value of 100.00 % (95% CI: 88.68 % to 100.00 %) and Kappa index of 0.86 (95% CI: 0.76 to 0.97). Based on the preliminary results, the apicoplast genome shows to be a suitable target for malaria diagnosis, with diagnostic parameters comparable to the reference PCR method. Being easily field-adaptable, without need for a thermocycling equipment, this assay could facilitate targeted interventions towards malaria control and elimination.

262

CHALLENGES OF MALARIA DIAGNOSIS IN PEDIATRIC PATIENTS AT A NIGERIAN HOSPITAL

Olusimbo K. Ige[1], Regina Oladokun[2]

[1]Malaria Action Program for States, Ibadan, Nigeria, [2]University College Hospital, Ibadan, Nigeria

This study compares the performance of routine malaria diagnostic tests, and explores the challenges of malaria diagnosis in paediatric patients in an endemic setting in South West Nigeria. This was a Cross sectional study conducted at the children's outpatient and emergency units of the University College Hospital, Ibadan, Nigeria. Patients seen between May and August, 2013 were enrolled in the study. The records of all 532 children aged six months to 12 years who received treatment for an acute febrile illness at the hospital during the study period were reviewed. The proportion of children classified as having malaria by clinical diagnosis, Rapid Diagnostic Test (RDT) and blood smear microscopy were compared. Factors associated with test positivity were explored using multivariate analysis. By clinical diagnosis 45.2% of children were diagnosed as having malaria, 37.6% tested positive to malaria parasite on RDT and 19.3% had positive blood smears on microscopy. Logistic regression showed that with RDTs, younger children were less often found to be positive than older children [OR: 0.594 (0.401-0.879)]. A similar lower probability of positivity was found for younger children on microscopy [OR0.624 (0.391-0.996)]. Positive smears were however recorded 3.9 times more often for those who gave a history of fever compared to those who did not [OR: 3.882 (1.154-13.057)]. The true malaria morbidity among these paediatric patients remains questionable due to the differences in the results produced by the different diagnostic methods. The clinical implication of RDT-positive but microscopy-negative samples may be grave if microscopy results are erroneous. Quality control systems and surveillance of routine malaria diagnostics are imperative to limit misdiagnosis of malaria in this endemic setting.

263

EVALUATING THE PERFORMANCE OF ID-FLUORESCENT IN SITU HYBRIDIZATION IN DETECTING MALARIA PARASITES IN WESTERN KENYA

Joseph O. Osoga, Elizabeth Wanja, John N. Waitumbi, Bernard O. Guya

Kenya Medical Research Institute, Kisumu, Kenya

Diagnosis of malaria parasites requires highly sensitive and specific diagnostic tools that may help rationalize antimalarial drug use in Kenya. In many cases, the identification of malaria parasites concerns their salient features that can be utilized to distinguish between species and subspecies. Conventional techniques including serology and microscopy (Giemsa) have limitations making it difficult to meet these requirements. Currently, detecting very low density infection has been done with alternative methods including nucleic acid amplification tests (NAAT) such as Polymerase Chain Reaction (PCR); Loop mediated Isothermal amplification (LAMP) and Fluorescent *In* Situ Hybridization (FISH). These nucleic acid techniques generally involve the use of DNA and RNA probes and have been shown to be very sensitive and specific. However, these techniques are not easily accessible and relatively expensive. Data to-date shows that *Plasmodium* FISH assays for detection of *Plasmodium* parasites and speciation of *P. falciparum* and *P. vivax* have high sensitivity and specificity and are easy to perform. The FISH assays detect both asexual and sexual stages of the malaria parasites. This current study proposes to evaluate the performance of *Plasmodium* FISH assays for the diagnosis of *P.falciparum* malaria infections, more specifically *P. falciparum* gametocytes and low parasitemia in patients. The technique will be evaluated against

microscopy and RT-PCR. Malaria blood films (MBF) will be obtained from clinical and cultured *P. falciparum* samples. The data collected will be analyzed by use of Chi-square for comparisons. This study will provide an alternative malaria diagnostic technique which is robust, sensitive, specific, inexpensive and reliable.

264

SEVERE MALARIA CASE MANAGEMENT PRACTICES IN SELECTED STATES IN NIGERIA: NEED FOR URGENT INTERVENTION

Yetunde Oke, Banji Ipadeola, Bolatitio Aiyenigba, Grace Nwankwo, Justice Adaji, Olatunde Olotu, Aniefiok Akpasa, Abba Umar

Malaria Action Program for States, Abuja, Nigeria

Severe malaria is a life-threatening medical condition that requires emergency interventions including prompt and effective treatment to prevent death (WHO 2000). The AQUAMAT study showed a relative reduction in mortality of 22.5% with use of parenteral Artesunate compared to quinine in the management of severe P.*falciparum* malaria and the national policy on malaria diagnosis and treatment has been revised based on this evidence. The goal of the study was to determine baseline capacity and management practices for severe malaria in selected facilities in Nigeria with the aim of designing interventions to address specific gaps identified. A cross-sectional study design was used to assess twenty-four health facilities in three states (Benue, Kogi and Oyo States). Data on capacity of health care providers; three months malaria services provided at different service delivery points; and medical supplies were collected. Double data entry method was used for data entry into SPSS software programme and analysis done with STATA version 10.0 Total number of malaria cases reported in the three states over the three months preceding the assessment (May, June and July 2013) was 18, 695 and diagnosis of severe malaria was made in 8.6% of the total malaria cases. Out of the severe malaria cases, about 76% of cases were discharged and mortality was recorded in about 2% of the cases. However, providers in most of the facilities managed severe malaria with injectable artemether (46%), quinine (37%) and artesunate (29%). Majority of the health facilities (96%) practiced parasite-based diagnosis of malaria but 29% monitored the parasite clearance of patients with severe malaria. Seventy percent of the facilities did not have basic supplies for supportive management of severe malaria. In addition, 67% of the health facilities experienced stock out of parenteral artesunate in the previous three months. Case management of severe malaria is still sub-optimal in study sites and to reduce the mortality attributable to malaria, effort must be intensified toward health system strengthening with emphasis on capacity building of health care providers, medical commodity security and improvement in supportive management of severe malaria.

265

MALARIA DIAGNOSIS SERVICE AVAILABILITY - MAPPING OF PRIVATE SECTOR SERVICE DELIVERY OUTLETS IN 7 PMI/ MAPS SUPPORTED STATES, NIGERIA

Abiodun Ojo[1], Bolatito Aiyenigba[2], Sonny Johnbull Ogboi[3], Adamu Onu[4], Olabanji Ipadeola[1], Muhammad Salihu[2], Kolawole Maxwell[5], Ebenezer Baba[5], Abba Umar[2]

[1]Malaria Consortium/MAPS, Abuja, Nigeria, [2]FHI360/MAPS, Abuja, Nigeria, [3]University of Camerino, Camerino, Italy, [4]Garki Specialist Hospital, Abuja, Nigeria, [5]Malaria Consortium, Abuja, Nigeria

The private sector's role in health services in Nigeria has increased in recent times, and provides approximately 60% of health services. The MAPS project supports the implementation of parasitological confirmation of malaria cases in public health facilities in selected states. A mapping exercise was carried out to identify the private facilities providing malaria diagnostic services and determine the gaps in existing malaria diagnostic capacity with the aim of designing a strategy for improvement. Data were obtained from questionnaires administered to facility owners and location data collected by handheld GPS device. Overall, 394 private health facilities in urban areas of 7 states were mapped; Hospital/Clinics (79.7%) and Stand-alone Laboratories (20.3%). Majority (28.9%) of personnel were Nurses/Midwives, followed by Auxiliary Nurses (21.9%); Medical Specialist (14.3%) and Medical Officers (10.1%); others were CHEWs (8.1%), Medical Lab Scientist (6.3%), Lab Technicians (5.7%), Lab Assistants (3.1%); and CHOs (1.6%). Only 97 (24.6%) health workers had received training on malaria diagnosis; 43.5% of facilities offered both malaria microscopy and rapid diagnostic test (mRDT) while 17.7% and 38.8% offered only mRDT and microscopy respectively. Quality of microscopy was sub-optimal because of poorly trained personnel, re-cycling of slides and sub-optimal reagents. Overall prevalence of clinically diagnosed malaria in the outpatients was 70.3%, and malaria slide positivity rate was 78.8% using available 3-months outpatients/laboratory statistics. A good pool of human resources exists in the private sector and their capacity needs to be built on malaria diagnosis especially mRDT for non-laboratory staff. Quality of malaria microscopy could be improved with re-training of laboratory scientists and increased access to slides and good reagents. Policy framework that provides access to quality malaria diagnostic materials and creates an enabling environment is needed to support the massive scale-up of public campaigns against malaria control.

266

SUCCESS OR FAILURE OF CRITICAL STEPS IN COMMUNITY CASE MANAGEMENT OF MALARIA WITH RAPID DIAGNOSTIC TESTS

Petra Mens[1], Esmee Ruizendaal[1], Suzan Dierckx[2], Koen Peeters[2], Franco Pagnoni[3], Henk Schallig[1]

[1]Royal Tropical Institute, Amsterdam, Netherlands, [2]Institute of Tropical Medicine, Antwerp, Belgium, [3]Global Malaria Programme, Geneva, Switzerland

Community case management of malaria (CCMm) by community health workers (CHWs) aims to increase access to correct and timely treatment. Currently the World Health Organization recommends to treat only confirmed malaria cases, rather than to treat presumptively. In general, CCMm with rapid diagnostic tests (RDTs) is considered a good strategy, but no in-depth review of the steps determining its success or failures has been performed. We describe an in-depth review of existing literature to provide a comprehensive overview of the success or failure of critical steps in CCMm with RDTs to identify why certain programs of studies have better results with CCM than others and to identify areas for further research. Our study showed that CHWs performed RDTs correctly, although specificity levels were variable (between 83.2 and 97.9%). Also safety issues such as reusing lancets were seen in certain studies and warrant attention. CHWs showed high adherence to test results but treating patients with a negative RDT for malaria remained an issue in several areas (ranged between 0.2 and 58%). Uptake and acceptance by the community was high, but negative-tested patients did not always follow up referral advice. Information on CHW motivation was limited and needs to be further studied. In general CHWs were not satisfied with the financial compensation. However, when asked about the most motivating aspect of their work, community respect and spiritual outcome were mentioned the most. Stock outs occurred in 4 out of 5 studies that reported on these. It is generaly believed that CCM with RDT also reduced morbidity and mortality compared to CCM without RDT this could not be concluded form this study. Interestingly the preception of the pateints on morbidity showed that CCM with RDT is worse than without RDT. In addition the actual cost benefit of the intervention could not be assessed. Although several studies have looked at it the data is too heterogenic to make a firm conclusion. In conclusion, we showed that trained CHWs can deliver quality care for malaria using RDTs. However, lower RDT specificity could lead to misdiagnoses of non-malarial diseases. Other threats for CCMm are non-adherence to negative test results and low referral

completion. Integrated CCM where the CHW has more treatment options may solve this. Repeated training is key in the successful execution and subsequent treatment by CHWs.

267

TESTING, TREATING AND TRACKING MALARIA IN A LOW RESOURCE SETTING: IMPROVED CASE MANAGEMENT THROUGH EMPOWERMENT OF A MEDICINES AND THERAPEUTICS COMMITTEE AT ENYIRESI GOVERNMENT HOSPITAL IN ATIWA DISTRICT, GHANA

Francis K. Annan[1], Nii O. Mensah[1], MalariaCare[2]

[1]Enyiresi Government Hospital, Anyinam, Ghana, [2]Washington, DC, United States

The World Health Organization initiative, T3: Test. Treat. Track, urges malaria-endemic countries and donors to move towards universal access to diagnostic testing and antimalarial treatment, and to build stronger malaria surveillance systems. Health providers in Ghana have practiced presumptive diagnosis and treatment of malaria for many years. Provider adherence to malaria test results is only around 50%. To operationalize the T3 initiative and achieve universal diagnosis and treatment, there is a need to improve provider adherence. Enyiresi Government Hospital, a district health facility, switched from presumptive diagnosis to treating patients based on malaria test results through strong leadership, training, and inter-departmental collaboration. Hospital leaders empowered the medicines and therapeutics committee (MTC) to implement a policy to treat suspected malaria cases only when a positive diagnosis is received from the laboratory. The MTC developed a peer review mechanism and motivated staff across departments including the outpatient department (OPD), consulting room, laboratory, and pharmacy. As a result, the hospital improved testing of suspected malaria cases from 24% in 2010 to 100% in 2012. During this time, the annual number of diagnosed outpatient department (OPD) cases of malaria declined from 10,243 (25% of total OPD visits) in 2010 to 1,707 (5% of total OPD visits) in 2012. Because of ongoing stewardship by the MTC, the hospital began monitoring the number of malaria cases treated and their outcomes and investigations for other causes of fever were redoubled in malaria test negative cases. Consequently, they noted that malaria morbidity and mortality dropped significantly across all age groups. Resources once used for treating clinically diagnosed malaria were refocused on other diagnoses and the community increased trust in medical care at the facility. Successful implementation of the T3 initiative required a strong leadership commitment, collaboration among departments, effective training, and a steady supply of malaria commodities.

268

PERSISTENCE OF *PLASMODIUM FALCIPARUM* DIAGNOSTIC ANTIGENS AFTER TREATMENT WITH ARTEMISININS: ASSOCIATION WITH PARASITE STAGE AND MECHANISM OF CLEARANCE

Charles J. Woodrow[1], Elizabeth A. Ashley[1], Kasia Stepniewska[1], Carole Fogg[2], Marion Barends[3], Roger Twesigye[4], James Kiguli[4], Carolyn Nabasumba[4], Vincent Batwala[4], Patrice Piola[2], Philippe Guerin[2], Lily Keereecharoen[3], Carit ler Moo[3], Anchalee Jaidee[3], Khin M. Lwin[3], Rose McGready[3], Kesinee Chotivanich[1], Hugh Kingston[5], Arjen Dondorp[1], Nicholas White[1], Francois Nosten[3]

[1]Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, [2]Epicentre, Kampala, Uganda, [3]Shoklo Malaria Research Unit, Mae Sot, Thailand, [4]Mbarara University of Science and Technology, Mbarara, Uganda, [5]Menzies School of Health Research, Darwin, Australia

The most sensitive rapid diagnostic tests of *Plasmodium falciparum* are based on detection of histidine-rich protein 2 (HRP2), but HRP2 persists in the blood after treatment and cannot be used to assess treatment response. We measured antigen levels over a 4-week period in ACT-treated hyperparasitaemic patients with *P. falciparum* in Uganda and Thailand (prior to the emergence of artemisinin resistance). Antigen clearance was quantified by calculation of area-under-curve (AUC) after normalization to baseline antigen level. Whole blood HRP2 levels showed a wide range of AUCs: Uganda median 415 d·% (range 48-2588); Thailand 590 d·% (33-1534). The primary determinant of AUC was admission stage; in patients presenting with >75% tiny/small rings (32/40 in Uganda, 24/38 in Thailand), median HRP2 AUCs were respectively 6.4 and 10.7-fold higher than cases with later stages (p=0.0005, <0.0001). Later stages at baseline strongly predicted rapid HRP2 clearance (AUROC = 0.89 for the combined dataset). LDH clearance was relatively rapid and narrower in terms of AUC range: Uganda median 53 d·% (range 19-205); Thailand 76 d·% (25-141). The stage effect was also reduced with median AUCs in early ring infections only 2.0 and 1.9-fold-higher than with late stages (p=0.1665, 0.0002). All seven infections in Thailand showing long parasite clearance half-lives (>4.5h) had late rings at presentation, but paradoxically rapid HRP2 clearance. These data offer a clear explanation for the persistence of HRP2 in whole blood following artemisinin therapy. We propose that in patients who present with mostly young rings at baseline (the majority), parasites are removed by pitting in the spleen and once-infected erythrocytes return to the circulation where they provide the source for persistently high levels of HRP2 and ongoing positivity of HRP2-based RDTs. In contrast, when patients present with large rings/trophozoites, the spleen is unable to pit these larger parasite forms, the entire infected cell is removed by immune clearance or sequestration, and HRP2 clearance is rapid. Consistent with this model, in these less common infections, parasite clearance can be paradoxically slow.

269

PERFORMANCE OF THREE CARESTART™ MALARIA RAPID DIAGNOSTIC TESTS AFTER REDUCTION IN MALARIA PREVALENCE IN BAGAMOYO, TANZANIA

Billy E. Ngasala

MUHAS, Dar es Salaam, United Republic of Tanzania

This study aimed at testing the diagnostic performance of three malaria rapid diagnostic tests (RDTs) (CareStart™ Malaria HRP-2, pLDH [Pf-pLDH/pan-pLDH] and HRP-2/pLDH (Pf/pan) Combo Test) with reference to light microscopy for the diagnosis of falciparum malaria in Tanzania. Blood samples were collected from 627 patients suspected to have malaria at th Miswe, and Yombo (Bagamoyo District) primary health facilities from October 2013 to December 2013. The samples were examined immediately by light microscopy and the CareStart™RDTs at the site, second reading of blood slides was done at reference laboratory. Statistical analysis was performed using Stata version 11. Overall 31 of 381 (8.1%) malaria suspected cases were detected by microscopy compared to 5 of 78(6.4%) by CareStart™ Combo kit, 8 of 80 (10.0%) by both PLDH kit and 18 of 223(8.1) by HRP-2 RDT kit. CareStart™Combo RDT kit's sensitivity and specificity for the diagnosis of malaria were 100.0% (47.8%-100%, 95% CI) and 98.6% (92.6%-100%) respectively, compared to standard microscopy. The sensitivity and specificity of CareStart™ PLDH and HRP-2 were found to be 87.5% (47.3%- 99.7%) and 100 %(81.5%-100%), respectively. The CareStart™Combo RDT had positive predictive value of 83.3% (35.9%-99.6%) compared to 50.0% (23.0%- 77.0%) for CareStart™ PLDH and 62.1% (42.3%-79.3%) for HRP-2 RDTs. All the three CareStart™ RDTs had the high negative predictive values of >=98.5% (91.8%-100%, 95% CI). In conclusion, the performances of CareStart™ RDTs had variation (CareStart™ Malaria HRP-2 and HRP-2/pLDH (Pf/pan) Combo Test) maintained high accuracy compared to pLDH [Pf-pLDH/pan-pLDH] RDT.

270

MOLMALQA: A MOLECULAR MALARIA EXTERNAL QUALITY ASSURANCE NETWORK TO SUPPORT CLINICAL TRIALS

Sean C. Murphy[1], Cornelus C. Hermsen[2], Alexander D. Douglas[3], Nick J. Edwards[3], Ines Petersen[4], Gary Fahle[5], Matthew Adams[6], Andrea A. Berry[6], Zachary P. Billman[1], Sarah C. Gilbert[3], Matthew B. Laurens[6], Odile Leroy[4], Kristen E. Lyke[6], James S. McCarthy[7], Christopher V. Plowe[6], Rebecca Rockett[7], Annette M. Seilie[1], Theo Sloots[7], Kathleen A. Strauss[6], Karina Tellen[2], Adrian V. Hill[3], Robert W. Sauerwein[2]

[1]University of Washington Medical Center, Seattle, WA, United States, [2]Radboud University Medical Center, Nijmegen, Netherlands, [3]Oxford University, Oxford, United Kingdom, [4]European Vaccine Initiative, Heidelberg, Germany, [5]National Institutes of Health, Bethesda, MD, United States, [6]University of Maryland, Baltimore, MD, United States, [7]QIMR Berghofer Medical Research Institute, Herston, Australia

Nucleic acid (NA) tests for malaria parasites are important diagnostic endpoints in many clinical trials of malaria vaccine and drug candidates. While many NA tests are described, there is no formalized external quality assurance (EQA) program that provides site qualification and ongoing EQA for the diverse assays in use. We recruited a network of centers performing controlled human malaria infection (CHMI) clinical trials to participate in an EQA effort. Here, we report on initial and ongoing EQA work. Each clinical site received blinded *Plasmodium falciparum*-positive and -negative samples of known parasite density that were prepared by the coordinating laboratory according to needs of each recipient site. Sites tested the samples and reported the data to the coordinating center. All sites in the first round of this effort demonstrated 100% specificity, achieved limits of detection consistent with each laboratory's expectations and demonstrated as-expected levels of within-laboratory precision (<10% coefficient of variation). Reported parasite densities generally agreed with expected values (<0.5 $\log_{10}$ parasites/mL). Where deviations from expected values were observed, root cause analyses were performed. In one laboratory, such analyses revealed reasons for observed quantitative differences and led to assay recalibration at that site. The MolMalQA effort is recruiting additional participating sites and is developing other reagents and standards to share with the malaria community. These efforts may eventually establish a dedicated long-term EQA program in support of high-quality molecular malaria diagnostics.

271

TEST AND TREAT PEDIATRIC FEVERS: META-ANALYSIS OF 15 NATIONAL SURVEYS ASSESSING THE EFFECT OF MALARIA DIAGNOSTIC TESTING ON DRUGS USED BY FEBRILE CHILDREN UNDER FIVE IN DIFFERENT MALARIA RISK CONTEXTS

Emily W. Johansson[1], Peter W. Gething[2], Helena Hildenwall[3], Bonnie Mappin[2], Max Petzold[4], Stefan S. Peterson[1], Katarina E. Selling[1]

[1]Uppsala University, Uppsala, Sweden, [2]University of Oxford, Oxford, United Kingdom, [3]Karolinska Institutet, Stockholm, Sweden, [4]University of Gothenburg, Gothenburg, Sweden

In 2010, the World Health Organization revised treatment guidelines to recommend testing all suspected malaria cases prior to treatment. Studies from health facilities demonstrate reduced anti-malarial prescriptions after test introduction, and in some studies, increased antibiotic prescriptions. Many of these studies also indicate widespread non-adherence to test results. Yet, there is no evidence on how testing affects drugs used to treat pediatric fevers at the population level, nor if this effect differs by malaria risk, source of care or child's age. 15 DHS/MIS in 2009-2013 were included that collected fever prevalence, care-seeking, diagnostic test and drug use, and geocoded survey clusters to link malaria risk estimates to datasets (n=16,831 febrile under-fives taken for care). Mixed-effects logistic regression models quantified the influence of testing on three outcomes (any anti-malarial, ACT and any antibiotic use) after adjusting for data clustering and confounding socioeconomic covariates. Preliminary results suggest large country variability in malaria testing's effect on treatment patterns. In Uganda, tested pediatric fevers had 1.36 higher odds of antibiotic use (AOR: 1.36, 95% CI: 1.08-1.70) compared to those not tested, and no significant difference in any anti-malarial (AOR: 1.25, 95% CI: 0.97-1.62) or ACT use (AOR: 0.84, 95% CI: 0.67-1.07). Conversely, in Tanzania, tested pediatric fevers had significantly reduced odds of antibiotic use (AOR: 0.48, 95% CI: 0.31-0.74) and significantly higher odds of using any anti-malarial (AOR: 3.68, 95% CI: 2.38-5.68) and ACT (AOR: 1.95, 95% CI: 1.33-2.88). Further analyses will examine this association in all studied countries individually and in the pooled dataset. Results will be stratified separately by malaria risk, source of care and child's age. Findings could inform future research to better understand reasons for variability in effect across settings or population groups (Final results will be available by June 2014).

272

AN ENHANCED CHEMILUMINESCENCE SLOT BLOT ASSAY FOR DETECTION OF MOSQUITO STAGE *PLASMODIUM FALCIPARUM* PARASITE

Bryan Grabias[1], Sanjai Kumar[1], Hong Zheng[1], Bingbing Deng[2], Babita Mahajan[1], Yukiko Kozakai[1], Kazutoyo Miura[2], Carole Long[2]

[1]Laboratory of Emerging Pathogens, Division of Emerging and Transfusion Transmitted Diseases, Kensington, MD, United States, [2]Laboratory of Malaria and Vector Research, Rockville, MD, United States

Robust, quantitative detection of malarial antigens in preclinical and clinical phases of development as well as surveillance and epidemiological studies, particularly in the mosquito host, necessitates the development of immunoassays with superior sensitivity and reproducibility. In this work, we have developed and characterized a research-grade, quantitative enhanced chemiluminescence-based Slot Blot assay (ECL-SB) for detection of *Plasmodium falciparum* circumsporozoite protein produced in recombinant form (r*Pf*CSP) in *E. coli* and as native protein from Oocysts (*PfOocyst*) developing in the midguts of *P. falciparum*-infected *Anopheles stephensi* mosquitoes. The ECL-SB detects as little as 1.25 pg of r*Pf*CSP (linear range of quantitation 2.5-20 pg; $R^2 = 0.9505$). We also find that the earliest detectable expression of native *Pf*CSP in *Pf*Oocyst by ECL-SB occurs on day 7 post infected blood meal. The ECL-SB was able to detect approximately as few as 0.5 day 8 *PfOocysts* (linear quantitation range 1-4, $R^2 = 0.9795$) and determined that one *PfOocyst* expressed approximately 2.0 pg (0.5-3 pg) of native *Pf*CSP, suggesting that this detection limit agrees with the assay's sensitivity limit for purified r*Pf*CSP. The ECL-SB is highly reproducible; the Coefficient of Variation (CV) for inter-assay variability for r*Pf*CSP and native *Pf*CSP were 1.74% and 1.32%, respectively. The CVs for intra-assay variability performed on three days for r*Pf*CSP were 2.41%, 0.82% and 2% and for native *Pf*CSP 1.52%, 0.57%, and 1.86%, respectively. The ECL-SB also matched microscopic analysis of *P. falciparum* prevalence based upon *Pf*CSP expression on day 8 *Pf*Oocysts in two independent feeding assays, yielding estimates of 83.3% and 75% compared to microscopy's 85.7% and 80%, respectively. Thus, based on its performance characteristics, ECL-SB could be valuable not only in vaccine development and manufacturing, but also to measure the parasite prevalence in mosquito populations and in studies of transmission-blocking activities in endemic areas.

273

QUALITY INSPIRED PROJECT - A KEY TO ACHIEVING RESULTS WITH MALARIA INTERVENTIONS

Grace P. Qorro

Jhpiego, Dar es Salaam, United Republic of Tanzania

With an aim to accelerate malaria case management, Tanzania Ministry of Health and Social Welfare (MoHSW) is strengthening its pre-service education program to ensure graduates have the right knowledge and

skills to diagnose and treat malaria. Investment in pre-service education lessens the burden on in-service training since those entering the workforce will have the knowledge and skills they need to provide. Jhpiego, through MAISHA (Mothers And Infants Safe, Healthy and Alive) program, provided technical assistance to the MoHSW to help develop a pre-service malaria case management-updates Learning Resource Package (LRP), which includes: Facilitator's Manual, Participant's Manual, Activity Worksheets and Training Modules addenda. The LRP was developed based on national malaria policy, guidelines and in-service training materials; it is taught using job aids, power point presentations, video demonstration and numerous case scenarios which reflect what actually happens in real life situations at service delivery points. The LRP aims at reinforcing appropriate practices for care of malaria patients and management of commodities with emphasis on parasite-based diagnosis and compliance to results, proper recording and reporting; and management of malaria in special situations and groups. The training package is well organized with laboratory and medical supplies which gives each participant an opportunity for hands-on activity to acquire and strengthen their skills. Checklists to guide Quality Assurance/Quality Improvement (QA/QI) processes have been included in these training materials. The project successfully provided competence-based orientation on malaria case management updates to 210 medical instructors. Annually, it reaches more than 4,000 students from eight Zonal Health Resource Centers and 480 students from Medical Universities. There is a need to incorporate the addenda developed into these training modules for easy use. In the near future, clinical skills-mentorship will be conducted in selected schools using the nationally approved QA/QI checklists.

274

CLINICAL SIGNS AND SYMPTOMS CANNOT RELIABLY PREDICT *PLASMODIUM FALCIPARUM* MALARIA INFECTION IN PREGNANT WOMEN LIVING IN AN AREA OF HIGH SEASONAL TRANSMISSION

Marc C. Tahita[1], Halidou Tinto[1], Joris Menten[2], Jean-Bosco Ouedraogo[3], Robert T. Guiguemde[4], Jean-Pierre Van Geertruyden[5], Annette Erhart[6], Umberto D'Alessandro[7]

[1]Clinical Research Unit of Nanoro/IRSS-DRO, Ouagadougou, Burkina Faso, [2]Institute of Tropical Medicine, Antwerpen, Belgium, [3]IRSS-DRO, Bobo-Dioulasso, Burkina Faso, [4]Laboratory of Parasitology and Entomology, Centre Muraz, Bobo-Dioulasso, Burkina Faso, [5]International Health Unit, University of Antwerp, Antwerpen, Belgium, [6]Department of Public Health, Institute of Tropical Medicine, Antwerpen, Belgium, [7]Disease Control and Elimination Theme, Medical Research Council Unit, Fajara, Gambia

Malaria in pregnancy is a major public health problem in endemic countries. Though the signs and symptoms of malaria among pregnant women have been already described, clinical presentation may vary according to intensity of transmission and local perceptions. Therefore, determining common signs and symptoms among pregnant women with a malaria infection may be extremely useful to identify those in need of further investigation by rapid diagnostic test or microscopy. Six hundred pregnant women attending the maternity clinic of Nanoro District Hospital, Burkina Faso were recruited, 200 with suspected clinical malaria and 400 as controls. Cases were matched with controls by gestational age and parity. Signs and symptoms were collected and a blood sample taken for rapid diagnostic test, microscopy and haemoglobin measurement. A multivariate model was used to assess the predictive value of signs and symptoms for malaria infection. The overall prevalence of malaria was 42.6% (256/600) while anaemia was found in 60.8% (365/600) of the women. Nearly half (49%) of the cases and 39.5% of the controls had a malaria infection (p = 0.03). The most common signs and symptoms among the cases were fever (36%,72/200), history of fever (29%,58/200) and headache (52%,104/200). The positive predictive value for fever was 53% (95%CI:41-64), history of fever 58% (95%CI:37-63) and headache 51% (95%CI:41-61). Signs and symptoms suggestive of malaria are frequent among pregnant women living in areas of intense transmission. Common malaria symptoms are not strong predictors of infection. For a better management of malaria in pregnancy, active screening to detect and treat malaria infection early should be performed on all pregnant women attending a health facility.

275

PREVALENCE AND PREDICTORS OF MALARIA MISDIAGNOSIS IN HEALTHCARE SEEKING CHILDREN UNDER SIX MONTHS OF AGE IN UGANDA

Martin K. Mbonye[1], Sarah Nakioba[2], Sarah M. Burnett[3], Marcia R. Weaver[4], Jean-Pierre Van Geertruyden[1]

[1]University of Antwerp, Antwerp, Belgium, [2]Save the Children, Kampala, Uganda, [3]Accordia Global Health Foundation, Washington, DC, United States, [4]University of Washington, Seattle, WA, United States

There is limited information on malaria prevalence and diagnosis among infants under six months of age. Little is known regarding how clinicians in limited resource settings diagnose malaria in this age group, given different and overlapping clinical manifestations and known protection against infection such as maternal antibodies. We identified the malaria prevalence and diagnosis practices, and predictors of malaria misdiagnosis in outpatients. We utilised data on individual outpatients under six months from 36 primary health care facilities in Uganda from January to December 2010. Multivariate logistic regression models were used to identify clinical and operational factors associated with misdiagnosis of malaria. Of the 23,587 young infant outpatients, 10,426 (44.2%) were diagnosed with malaria of which only 3,189 (30.6%) were laboratory confirmed. Of the 3,884 patients with a negative diagnostic test result for malaria, 1,213 (31.2%) were misdiagnosed with malaria. The odds of misdiagnosis were higher if the patient was one (aOR 3.64, 95%CI 1.26, 10.5), three (aOR 4.47, 95%CI 1.36, 14.7), four (aOR 6.34, 95%CI 2.03, 19.7) or five (aOR 6.86, 95%CI 1.95, 24.1) months old; had presence of clinical fever (aOR 2.64, 95%CI 1.33, 5.26); visited the health facility during a week when multivitamins were out of stock (aOR 1.62, 95%CI 1.12, 2.33) or visited a health facility with medium to high malaria endemicity (aOR 1.47, 95%CI 1.01, 2.16). The odds of misdiagnosis were lower when the patient was also diagnosed with cough/cold (aOR 0.32, 95%CI 0.19, 0.53) or urinary tract infection (aOR 0.16, 95%CI 0.03, 0.76). Triage status, clinic reattendance, health provider training, availability of antimalarial, antibiotic and oral rehydration drugs, and diagnosis with anaemia, pneumonia and diarrhoea did not have an effect on odds of misdiagnosis. In conclusion, we found high rates of malaria prevalence and misdiagnosis in young infants. Clinicians were less likely to trust malaria test results when the infant was older or had fever or when health facility had medium to high malaria endemicity or lacked multivitamins. Clinicians were more likely to trust malaria test results when other diagnoses were made. These findings can inform future interventions to improve the quality of malaria care in infants.

276

QUALITY IMPROVEMENT OF MALARIA DIAGNOSTICS WITH RAPID DIAGNOSTIC TESTS WITH THE DEKI READER IN TANZANIAN MILITARY HEALTH FACILITIES

Akili K. Kalinga[1], Christopher Mswanya[2], Charles Mwanziva[2], George Amoo[3], Lucky Temu[4], Sarah Chiduo[4], Lalaine Anova[5], Eyako Wurapa[5], David Schnabel[5], Colin Ohrt[5], Ian Fine[6], Geeta Bhat[6], Adam Mwabulanga[2]

[1]National Institute for Medical Research, Dar es Salaam, United Republic of Tanzania, [2]Tanzania Peoples Defense Force, Dar es Salaam, United Republic of Tanzania, [3]Amethyst Technologies LLC, Baltimore, MD, United States, [4]Walter Reed Malaria Programme-Tanzania, Dar es Salaam, United Republic of Tanzania, [5]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [6]Fio Corporation, Toronto, ON, Canada

Malaria is the major cause of mortality and morbidity in Tanzania, where over 90% of the population is at risk for infection. Malaria diagnosis remains problematic, especially in resource-challenged settings. The use

of malaria Rapid Diagnostic Tests (mRDTs) has become a useful alternative to microscopy for diagnostic purposes, however its quality control (QC) is needed to ensure accurate diagnosis. In Tanzania we have successfully used the Deki Reader (DR) as a means of addressing diagnostics quality improvement issues through remote monitoring. The DR is a rugged, mobile *in vitro* diagnostic device which interprets commercially available RDTs. The DR automatically captures and securely transfers patient diagnostic data, along with the RDT image, to a centralized database over mobile phone networks. All results from the field are accessible from anywhere in the world in real-time by logging into a password protected web portal. Since 2012 DRs have been deployed at five camps overseen by the Tanzania People's Defence Forces (TPDF). Malaria RDT data and images were monitored remotely for quality assurance on a regular basis. When quality issues were detected monitors communicated with users at the testing facilities to investigate and troubleshoot problems. In this ongoing study, the DR was instrumental in remotely identifying false positive and negative mRDT interpretations made by users. Errors were due to either misinterpretation of mRDT weak lines or improper RDT preparation. Initially, approximately 5.5% of all mRDTs processed were incorrectly interpreted by users. After 6 months of remote QC monitoring and interaction with users, the problems decreased to 2.5%. Similarly, RDT preparation problems decreased from 2.3% to 0.1%. In summary, the use of DR reduced quality issues with RDTs, decreased cost of physical site visits, improved the malaria surveillance mechanism and improved patient care. The DR has proven to be a valuable tool for quality assurance of mRDT use and may be useful in other similar settings for malaria control// elimination efforts.

277

MALARIA DIAGNOSTIC AND MONITORING IN REMOTE RIVERINE VILLAGES IN THE AMAZON BASIN DURING ITINERANT MEDICAL MISSIONS

Jorge L. Maguiña[1], Johanna Hernandez[2], Juan F. Sanchez[1], Luis Loayza[3], Ronald Ramirez[4], Carmen Lucas[1], Salomon Durand[5], G. Christian Baldeviano[1], Andres G. Lescano[1]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Dirección Regional de Salud Loreto, Iquitos, Peru, [3]Marina de Guerra del Peru, Lima, Peru, [4]Union Biblica del Peru – VINETrust Peru, Iquitos, Peru, [5]U.S. Naval Medical Research Unit - 6, Iquitos, Peru

Malaria affects primarily people living in remote tropical settings. In the Amazon Basin, malaria is concentrated in the riverine villages with limited access to healthcare and diagnostic capacities. Therefore, innovative strategies are needed to deliver diagnostics and treatment to these communities. We describe the contributions of two itinerant riverine medical missions to malaria control. Malaria diagnostics and treatment was provided at two riverine medical missions: 1) the Amazon Hope ship lead by the VineTrust since 2002, and 2) the Rio Napo ship of the Peruvian Navy, which started trips in the Napo River during 2013, as part of multiple medical and social services. NAMRU-6 supports the diagnoses and molecular characterization of cases providing equipment, materials and training. Blood smears and filter paper samples were collected from all suspect cases for malaria diagnosis by microscopy and PCR, respectively. The sensitivity and specificity of microscopy were calculated using PCR as the gold standard. Diagnosis was provided in 20 trips of the Amazon Hope ship in the Marañon, Amazonas, Ucayali, Puinahua and Tigre river basins. A total of 793 febrile cases were tested by microscopy, and 80 (10%) were malaria positive. Three quarters were due to *Plasmodium vivax*. The mean age was 23 years old and 60% were men. The Rio Napo Ship conducted three trips between July and December 2013, and tested 156 febrile cases for malaria. Microscopy diagnoses identified 46 (29%) positives, 26% due to *P. vivax* and 3% due to *P. falciparum*, respectively. The prevalence of *P. vivax* and *P. falciparum* by PCR was 28% and 5%, respectively. Compared to PCR, the sensitivity of microscopy for *P. vivax* was 89% and for *P. falciparum* was 57%, while the specificity for both species was 98%. Itinerant medical ships can extend malaria diagnostic capacities and surveillance to remote, hard to reach areas. Participation and cost-effectiveness can be improved providing training, basic diagnostic resources and other medical and social services.

278

PECADOM PLUS: AN ACTIVE, COMMUNITY-BASED APPROACH TO MALARIA DETECTION AND TREATMENT

Youssoupha Ndiaye[1], **Anne Linn**[2], Seynabou Gaye[1], Ian Henesse[3], Patrick Linn[2], Karine Nordstrom[2], Isaac A. Manga[4], Mady Bâ[1], Chris Hedrick[2]

[1]Ministry of Health and Social Action, Dakar, Senegal, [2]Peace Corps Services, Dakar, Senegal, [3]Emory University, Atlanta, GA, United States, [4]University Cheikh Anta Diop, Dakar, Senegal

The region of Kedougou has the highest prevalence of malaria in Senegal, and geographic, educational and financial barriers often impede rural populations from accessing care. A home-based care model (PECADOM) has been implemented in Senegal since 2008, addressing many of these barriers to care and care seeking. Volunteer, home-based care providers (DSDOMs) perform rapid diagnostic tests (RDTs) for malaria and administer treatment for cases of uncomplicated malaria, referring complicated cases. However, this passive model relies on patients seeking care from DSDOMs. To reduce barriers to treatment seeking, Peace Corps and the Saraya Health District have piloted an active case finding model in which DSDOMs were paid to conduct weekly sweeps of every household in their village to actively seek patients with symptoms of malaria. RDTs were undertaken on those with symptoms, with free treatment provided for positive cases. The model was implemented in fifteen villages from July through November 2013, the period of highest malaria transmission. Fifteen comparison villages were chosen among villages with the original, passive PECADOM model, and three sweeps were conducted in these villages to estimate and compare prevalence of malaria at the beginning, midpoint and end of the active model pilot. At baseline, point prevalence of symptomatic malaria confirmed by RDT for the total population was 1.1% in both sets of villages (p=.79). During the midline comparison sweeps, the point prevalence was 2.5 times higher (p=.007) in the comparison villages (2.9%) than in the intervention villages (1.1%). At endline, the point prevalence in comparison villages (2.5%) was nearly 16 times higher (p=.003) than in the intervention villages (.16%), where only 6 cases of symptomatic malaria were found. This pilot study shows that this model of active weekly sweeps by community health workers is both feasible and effective in increasing access to care and reducing malaria prevalence. Due to these promising results, PECADOM Plus will be scaled up to the entire region of Kedougou in 2014.

279

QUALITY IMPROVEMENT AND NOVEL USE OF MALARIA DIAGNOSTICS IN VIETNAM

Awalludin Sutamihardja[1], Phuc T. Nguyen[2], Dung K. Vu[2], Tuyet T. Vu[2], Huong T. Kieu[2], Yen T. Nguyen[2], Van T. Nguyen[2], Dung T. Duong[2], Hang B. Hoang[2], Tuan V. Pham[2], Phu H. Nguyen[2], Xa X. Nguyen[2], Anh Q. Nguyen[2], Hieu M. Truong[2], Bruce Lee[1], Gerard C. Kelly[1], Nicholas Martin[3], Hien Nguyen[4], Duong T. Tran[2], Thang D. Ngo[2], Colin Ohrt[1]

[1]Malaria Elimination Initiative, GHG, University of California San Francisco, San Francisco, CA, United States, [2]Department of Epidemiology, NIMPE, Ha Noi, Vietnam, [3]Naval Medical Research Centre - Asia, Singapore, Singapore, [4]U.S. Embassy, Ha Noi, Vietnam

The National Malaria Control and Elimination Program in Vietnam is currently planning to eliminate malaria by 2030. They are currently conducting baseline assessments and developing methods for malaria diagnostic quality improvement. Quality malaria diagnostics are essential for malaria elimination efforts. False negative diagnostic results in both symptomatic and asymptomatic infected individuals will increase the malaria transmission reservoir. False positive results (malaria is not really

present) will result in misapplication of limited resources for malaria elimination operation. NIMPE in collaboration with UCSF are developing a quality improvement (QI) program for malaria diagnostics to expedite malaria elimination. In this program, for both case management and screening, the necessary and most cost-effective approaches for baseline assessments and QI will be developed and implemented with the objective of minimizing false negative results. Malaria microscopy is currently used as standard of care for malaria diagnostic in Vietnam. For microscopy, we will assess the effectiveness and cost effectiveness of the cross-checking system currently in place. In a sample of sites, we will assess the accuracy of reporting at each level and estimate how many cases are not being reported. We will assess the competency of microscopists and identify the most cost effective ways to improve competence. We will inspect a sample of laboratories and make suggestions for improvement. Rapid diagnostic tests have been introduced in areas with high malaria endemicity and remote areas with difficulty in access to health service. Similar activities will be conducted at a sample of these sites. For case confirmation/foci investigation, the simplest, fastest and least expensive approaches will be developed to confirm positive microscopy. The initial results from this effort will be available in August 2014 for presentation. These results will be compared and contrasted with our recent experiences with diagnostics QI in Tanzania.

280

TRANSFORMING ARMY DIAGNOSTICS LABORATORY INTO A RESEARCH READY FACILITY, IMPROVEMENT IN QUALITY ASSURANCE; RUVU JKT CASE STUDY

George Amoo[1], C. Ohrt[2], D. Schnabel[2], L. Anova[3], C. Mwanziva[4], A. Sutarmihardja[5], S. Chiduo[5], S. Mgata[5], L. Temu[5], M. Hickman[2], A. Kalinga[3], K. Wurapa[2], J. Mwabulanga[4], J. Balsinger[2], K. Brown[1], A. Felton[1]

[1]Amethyst Technologies LLC, Baltimore, MD, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Clinical Research Management (ctr), Silver Spring, MD, United States, [4]Tanzania People Defense Forces, Dar es Salaam, United Republic of Tanzania, [5]Walter Reed Program, Dar es Salaam, United Republic of Tanzania

Malaria continues to be one of the diseases affecting many U.S. and Coalition personnel deployed in Sub Sahara Africa. It is responsible for over 100 million reported cases annually and 1-2 million deaths, especially in children. Diagnosis of malaria is based on clinical symptoms which are non-specific, leading to false diagnosis and over use of anti-malarial drugs, increasing the potential of drug resistance, as well as the number of malaria cases. Within Tanzania microscopy continues to be a challenge with the Tanzania People's Defense Force (TPDF) and Tanzania National Service Program (JKT) medical services. Amethyst Technologies LLC (ATL) with its partners developed a comprehensive malaria microscopy and Rapid Diagnostic Testing assessment program to capture information for strengthening of the quality of Malaria Diagnostics at Ruvu JKT as a potential research site. The need to develop quality malaria diagnosis at Ruvu JKT was critical in getting the site laboratory ready for malaria research. Information on infrastructure, safety, human resources, training, and diagnosis was obtained in order to establish the minimum guidelines and requirements for quality malaria microscopy. The assessment came up with a quality management plan to provide on-going quality improvement of malaria microscopy and enabled the research team to estimate the amount of malaria transmission. We will present data obtained through continues training, onsite corrective actions supporting activities and discuss how these data were used to develop our Quality Management Program at Ruvu JKT for research readiness.

281

MODULAR DESIGN OF ANTIPARASITIC CHEMOTHERAPEUTICS USING PRENYLATION AS NEW TOOL IN DRUG DISCOVERY

Exequiel O. Porta, Guillermo R. Labadie

Rosario Chemistry Institute - Argentinean National Research Council, Rosario, Argentina

One of seven people in the world is affected by Neglected Tropical Diseases. Treatments of these diseases are insufficient and inefficient and require constant development of new drugs and strategies. Within the universe of small organic molecules, the isoprenoids are the most important and numerous families of metabolites. In this regard, it is useful to focus on the design, synthesis and studies of molecular probes that interfere with different biosynthetic routes, particularly the isoprene and sterol pathways. The implemented strategies used modern medicinal chemistry approaches like diversity-oriented synthesis, parallel solution library preparation, conventional multistep synthesis, midthroughput screening, bioinformatics and computational chemistry, allowed to generate more than 100 new compounds. The generated libraries were tested against the parasites responsible of the malaria (*Plasmodium falciparum* chloroquine sensitive and resistant strains), visceral leishmaniasis (*Leishmania donovani*), HAT (*Trypanosoma brucei)* and Chagas´ disease (*T. cruzi*). As result of our effort we have found very promising new hits to develop drugs against malaria and other diseases. Our strategy also included action mechanism studies of the lead compounds. To do that, fluorescent tagged isoprenes were synthesized to be incorporated on the lead structure to perform cellular localization by fluorescent microscopy.

282

EVALUATION OF THE PROPHYLACTIC ACTIVITY OF ORAL 8-AMINOQUINOLINE DERIVATIVES IN C57BL/6 MICE USING AN *IN VIVO* IMAGING SYSTEM

Qigui Li, Lisa H. Xie, Diana Caridha, Qiang Zeng, Norma Roncal, Jing Zhang, Ping Zhang, Ronan McNulty, Michael Harris, Lisa Read, Mark Hickman

Walter Reed Army Institute of Research, Silver Spring, MD, United States

As anti-malarial drug resistance escalates, new safe and effective medications are necessary to prevent and treat malaria infections. The US Army is developing 8-aminoquinoline (8-AQ) analogues with improvements in hemolytic toxicity, which is expected to be safer in preventing malaria in deployed military personnel. To compare the prophylactic efficacy of these compounds, a transgenic *P. berghei* parasite expressing the bioluminescent reporter protein luciferase was utilized to visualize and quantify parasite development by using a real-time *in vivo* imaging system (IVIS) at 24, 48, and 72 hours post infection. Two standard compounds of 8-AQ, tafenoquine (TQ) and primaquine (PQ) were treated with single or multiple regimens. As an additional endpoint, blood stage parasitemia was assessed periodically over the next 30 days by flow cytometry. C57BL/6 Albino mice were infected intravenously with 50,000 sporozoites by tail vein and treated orally with three multiple doses of TQ and PQ at their minimal curative doses of 5 and 25 mg/kg, respectively. The outcome of this treatment showed no bioluminescence liver signal and no blood stage parasitemia was observed, suggesting both drugs showed 100% causal activity at the doses tested. Single dose oral treatment with 5 mg TQ or 25 mg of PQ, however, yielded different results as only TQ treatment resulted in causal prophylaxis in *P. berghei* sporozoite-infected mice. TQ is highly effective for causal prophylaxis in mice at a minimal curative single oral dose of 5 mg/kg, which is a five-fold improvement in potency versus PQ. We also tested these drugs in a more sensitive C57BL/6 wild-type strain (black) where mice were infected with only 10,000 sporozoites and treated orally with three multiple doses of TQ at 3 mg/kg or with PQ dosed orally at 15 mg/kg. None of the mice demonstrated a bioluminescence liver signal and no blood stage parasitaemia was observed suggesting both drugs showed 100% causal

activity at the doses tested. Accordingly, we conclude TQ is highly effective for causal prophylaxis in C57BL/6 wild-type mice at a minimal curative single oral dose of 3 mg/kg, which is also a five-fold improvement in potency versus PQ where the minimal curative dose required is 15 mg/kg. Both stains are working well to screen the prophylactic activity of oral 8-aminoquinoline derivatives in liver-stage, but the C57BL/6 wild-type mice are more sensitive than C57BL/6 albino animals.

283

LONG-TERM PROPHYLAXIS AND PHARMACOKINETIC EVALUATION OF A SINGLE INJECTION OF INTRAMUSCULAR DECOQUINATE IN MICE INFECTED WITH *PLASMODIUM BERGHEI* SPOROZOITES

Qigui Li, Lisa H. Xie, Diana Caridha, Qiang Zeng, Jing Zhang, Ping Zhang, Chau Vuong, Brittney Potter, Jason Sousa, Sean Marcsisin, Lisa Read, Mark Hickman

Walter Reed Army Institute of Research, Silver Spring, MD, United States

This study was conducted to evaluate the prophylactic efficacy and pharmacokinetics of an intramuscular (IM) depot formulation of decoquinate (DQ) into mice infected with *Plsmodium berghei* sporozoites. Due to the poor bioavailability of oral DQ, a novel drug dispersion technique was utilized to create DQ microparticles suspended in an oily vehicle to retard drug release suitable for long-term malaria prophylaxis. To evaluate the depot formulation, pharmacokinetic studies in normal animals and antimalarial efficacy in liver-stage malaria mice were conducted following single IM-DQ injections at 60, 120 and 240 mg/kg for 2, 4, 6, or 8 weeks prior to infection with *P. berghei* sporozoites. The liver stage efficacy evaluation was monitored by using an *in vivo* imaging system (IVIS). Efficacy results showed that 100% of animals were protected from *P. berghei* infection after injection with a single dose of depot DQ at 120 and 240 mg/kg administered 8 weeks prior to sporozoite inoculation. The 120 mg/kg IM dose is the minimal prophylactic dose required to obtain causal prophylaxis of malaria sufficient for a period of 8 weeks. In addition, a significant increase in the elimination half-life of the depot DQ (1,484 hrs) was achieved compared to that of orally dosed plain DQ (8.3 hrs). The depot IM formulation with slow-release mode provided release of very low and constant drug concentrations in the plasma and liver resulting in a minimal inhibition concentration (MIC) at 5-6 ng/ml in plasma and 70-90 ng/g in the liver. General safety and injection site tolerability were also assessed longitudinally to examine any issues with drug toxicity that may potentially preclude the use of this formulation in man. Modest body weight changes were found after IM dosing not only in the animals treated with IM-DQ, but also in mice treated with the vehicle control which may be a consequence of animal handling. Depot DQ injections up to a single intramuscular dose of 4,800 mg/kg, 40-fold higher than the dose required for treatment, were well tolerated in mice and final evaluations of toxicity will be conducted through histopathological monitoring. Further development of a depot IM-DQ formulation for malaria prophylaxis will be needed to deliver even longer periods of malaria prophylaxis protection beyond the 8 weeks achieved in this study.

284

EXPERIMENTAL STUDY OF THE RELATIONSHIP BETWEEN *PLASMODIUM* GAMETOCYTE DENSITY AND INFECTION SUCCESS IN MOSQUITOES; IMPLICATIONS FOR THE EVALUATION OF MALARIA TRANSMISSION-BLOCKING INTERVENTIONS

Dari F. Da[1], Thomas S. Churcher[2], Serge R. Yerbanga[1], Bienvenue K. Yaméogo[1], Ibrahim Sangaré[1], Jean Bosco Ouédraogo[1], Robert E. Sinden[2], Andrew M. Blagborough[2], Anna Cohuet[3]

[1]Institut de Recherche en Sciences de la Santé (IRSS/DRO), Bobo-Dioulasso, Burkina Faso, [2]Imperial College, London, United Kingdom, [3]Institut de Recherche pour le Dévéloppement (IRD/MIVEGEC), Montpellier, France

The evaluation of *Plasmodium* transmission blocking interventions (TBIs) such as vaccines or drugs to control malaria widely uses mosquito membrane-feeding assays. In these experiments, the intensity of *Plasmodium* infection within the anopheline vector is expected to affect the measured efficacy of the molecules to block transmission. Gametocyte density in the host blood is a broad determinant of the infection success in the mosquito; however, uncertain estimates of parasite densities and intrinsic characteristics of the infected blood can induce variability. To control this variation in TBI evaluation, a feasible method is to dilute infectious blood samples, ensuring that gametocyte numbers are directly comparable between parallel samples. To examine this in detail, we describe the effect of diluting *Plasmodium* gametocyte infected blood and the subsequent impact on mosquito infectivity and TBI efficacy, for two parasite-vector combinations: *Plasmodium falciparum/Anopheles gambiae* and *Plasmodium berghei/Anopheles stephensi*. We additionally examined how blood dilution influences the observed blocking activity of mAb 13.1 against Pbs28 of *Plasmodium berghei*. In the natural species combination (*P. falciparum/An. Gambiae),* blood dilution revealed a positive linear relationships between gametocyte density and oocyst load. Similar relationships was observed in the *P. berghei / Anopheles stephensi* system when using heat inactivated blood as a solvent, whereas diluting infected mice blood with fresh uninfected blood substantially increases infectiousness. This suggests that highly infected mice blood contains inhibitory factors or reduced blood moieties, which impedes infection and may lead to misinterpretation of TBI evaluation assays. In the lab-based system, the transmission blocking activity of mAb 13.1 was confirmed to be density-dependant. This data highlights the need to perform evaluations of TBI candidates carefully, at gametocyte densities and infection intensities that are broadly translatable.

285

ANTIMALARIAL ACTIVITY OF NOVEL HARMINE-DERIVED HEAT SHOCK PROTEIN 90 INHIBITORS

Abebe G. Bayih[1], Asongna Folefoc[1], Scott C. Eagon[2], Marc Anderson[2], Dylan R. Pillai[1]

[1]University of Calgary, Calgary, AB, Canada, [2]San Francisco State University, San Francisco, CA, United States

The emergence of multi-drug resistant *Plasmodium falciparum* strains poses a serious challenge in the control of malaria and could have catastrophic outcomes. This necessitates development of effective, safe and affordable new drugs. Previous studies have shown that the natural beta-carboline alkaloid compound, harmine, is a promising antimalarial targeting the *P. falciparum* heat shock-90 (PfHsp90) enzyme. In this study, we have developed a microwave-assisted synthetic route to generate analogs of harmine and have tested their antimalarial effect both *in vitro* and *in vivo*. Out of 45 harmine-derived compounds tested, two (17A and 21A) bound to PfHsp90 with EC50 values of 12.2 ± 2.3, and 23.1 ± 8.8μM, respectively. These compounds also inhibited *P. falciparum* W2 with IC50 values of 1.3 ± 0.027 and 1.4 ± 0.07μM, respectively. *In vitro* cytotoxicity assay showed that both compounds are not toxic. The efficacy of these compounds *in vivo* was assessed using *P. berghei* infection of BALB/c mice. Three daily injections of infected mice with 100mg/kg of

each of 17A and 21A showed significant reduction in parasitemia as compared to the vehicle control. Compounds 17A and 21A resulted in reduction of parasitemia by 51.5% and 56.1%, respectively. Mice treated with 17A and 21A showed a median survival time of 11 and 14 days, respectively while the vehicle control mice showed a median survival time of only 8.5 days. Log-rank test indicated that the survival of mice treated with 21A was significantly higher than vehicle control mice. In summary, we have identified a novel, non-toxic harmine derivative able to bind PfHsp90, inhibit *P. falciparum* W2 *in vitro* at micromolar concentrations and reduce parasitemia in the *P. berghei* mouse model. 21A also improved survival of infected mice, a trait not observed in the parent molecule. This could be attributed to the changes made on harmine backbone. Further dose-ranging studies of 21A are required coupled with development of focused libraries based on the current lead structure.

286

DEVELOPMENT OF ANTIMALARIALS WITH SERCAP PROLIFE FROM DOS DERIVED COMPOUNDS

Nobutaka Kato, Eamon Comer, Jennifer Beaudoin, Bertrand Braibant, Sebastian Le Quement, Carol Mulrooney, Jeremy Duvall, Michael Foley, Benito Munoz, Stuart Schreiber, Christina Scherer, Christina Scherer

The Broad Institute, Cambridge, MA, United States

In the last decade, hundreds of small molecules with previously unknown antimalarial activities were identified, but the drug discovery and development pipeline still lacks compounds with non- erythrocyte stage activity and target diversities. We screened and identified a novel series of compounds derived from Diversity-Oriented Synthesis (DOS) with potent activity against blood-stage *Plasmodium falciparum*. Importantly, the compounds are equipotent against a panel of *Plasmodium* field isolates with resistance to clinically relevant therapeutics, suggesting that these compounds have a novel mechanism of action compared to current therapeutics. The compounds are active in *in vitro* assays for liver-stage parasites (*P. berghei*) and late-stage (IV & V) gametocytes (*P. falciparum*). Intriguingly, the compounds also show *in vitro* activity against the liver stage (small forms) of P. cynomolgi. Finally, compounds in this series showed excellent *in vivo* efficacy in a *P. berghei* mouse model. This profile is consistent with the target product profile envisioned for Single Exposure Radical Cure and Prophylaxis (SERCaP) for treatment of uncomplicated malaria.

287

DEVELOPMENT OF A NOVEL PRECLINICAL ANTIMALARIAL CANDIDATE

Ian Gilbert[1], Kevin Read[1], Beatriz Baragana[1], Neil R. Norcross[1], Alan H. Fairlamb[1], Irene Hallyburton[1], Suzanne Norval[1], Maria Osuna-Cabello[1], David Gray[1], Paul Willis[2], Lidiya Bebrevska[2], Simon Campbell[2], Peter Siegl[2], Susan Charman[3], Dennis Kyle[4], Robert Sinden[5], Michael Delves[5], Sergio Whittlin[6], Elizabeth Winzeler[7], Carol Sibley[8], David Fidock[9], Marcus Lee[9], Mariana de Almeida[9], Julian Rayner[10]

[1]University of Dundee, Dundee, United Kingdom, [2]Medicines for Malaria Venture, Geneva, Switzerland, [3]Monash University, Melbourne, Australia, [4]University of South Florida, Tampa, FL, United States, [5]Imperial College, London, United Kingdom, [6]Swiss Tropical and Public Health Institute, Basel, Switzerland, [7]University of California San Diego, CA, United States, [8]University of Washington, Seattle, WA, United States, [9]Columbia University, New York, NY, United States, [10]Sanger Institute, Cambridge, United Kingdom

Malaria is a devastating disease, leading to several hundred million clinical cases and over 600,000 deaths each year. There is an urgent need to develop new drugs to treat this disease, to counter resistance to current drugs and to expand the range of clinical indications which can be tackled. This includes the need for single-dose treatment, transmission blocking, chemoprevention and treatment for relapsing malaria. Here we report the discovery and development of a potential new antimalarial agent. The starting point for this project was a phenotypic screen carried out against *Plasmodium falciparum* at the University of Dundee, UK. Several series were identified and one of these was optimized to a compound which fulfilled the Medicines for Malaria Venture criteria for a late lead compound. This compound was extensively profiled in a large number of assays and has now been progressed into preclinical development with the aim of entering human clinical trials. This pre-clinical candidate shows promise as a possible single-dose treatment in combination with another antimalarial and demonstrates both transmission blocking and chemoprevention potential.

288

PROGRESS TOWARDS MALARIA ERADICATION: NEW SCREENING ASSAYS AND NEW COMPOUNDS WITH ANTI-GAMETOCYTE ACTIVITY

Sarah D'Alessandro[1], Nicoletta Basilico[1], Silvia Parapini[1], Yolanda Corbett[1], Paola Misiano[1], Grazia Camarda[2], Giulia Siciliano[2], Luca Cevenini[3], Elisa Michelini[3], Aldo Roda[3], Pietro Alano[2], **Donatella Taramelli**[1]

[1]University of Milan, Milan, Italy, [2]Istituto Superiore di Sanità, Rome, Italy, [3]University of Bologna, Bologna, Italy

Drugs able to inhibit *Plasmodium falciparum* gametocytes and thus malaria transmission are strongly needed to achieve the goals of the malaria elimination/eradication agenda. Here, we describe two microtiter screening assays, based on different viability markers and read-outs: the pLDH assay, based on the spectroscopic detection of the gametocyte lactate dehydrogenase (pLDH) activity, and the LUC assay, performed using a parasite strain which expresses a novel luciferase enzyme (LUC1-G) under the control of a promoter specific for gametocytes. The assays have been validated and show good Z' factor and signal to background ratio. Both assays have been used to screen the "MMV-Malaria box" library. A primary screening at a single dose of 3.7 µM was performed with the pLDH assay, measuring parasite viability at two different time points: 72h post-treatment (72 h assay) and 144 h. In the latter case, the compounds were removed from the cultures, which were incubated for further 72h (72+72h assay). The dual checkpoint assay allows the identification of gametocytocidal compounds with fast versus slow speed of action. Seven out of 400 Malaria box compounds inhibited gametocyte viability by more than 50% already after the first 72h incubation; fifteen compounds showed an activity higher than 75% at 144h. Based on the results of the primary screening, thirty-six compounds with activity higher than 50% at 144h were selected for dose-response experiments and IC50 determination in both assays. Seven compounds showed an IC50 lower than 1 µM with both methods. A good correlation between the IC50 results with the two assays was obtained. In conclusion, the pLDH and LUC assays can be used as fast and cheap screening methods for the identification of novel gametocitocydal compounds and to investigate their mode of action (fast versus slow). The evaluation of potential transmission blocking agents selected from other libraries or newly synthesized is on going and results will be ready in the Fall of 2014.

289

PHOSPHOLIPASE A2 INHIBITION IN *PLASMODIUM FALCIPARUM*: A POTENTIAL NOVEL ANTIMALARIAL STRATEGY

Vasiliki Pappa[1], Vidya Mony[1], Kyu Rhee[2], Johanna Daily[1]

[1]Albert Einstein College of Medicine, Bronx, NY, United States, [2]Weill Cornell Medical College, New York, NY, United States

Plasmodium falciparum causes millions of infection worldwide, including severe disease, mainly in children residing in sub Saharan Africa. Currently, artemisinin based combination therapies (ACT) are the treatment of choice for the majority of malaria endemic countries. However, resistance

to artemisinin has been reported, which may impact the future efficacy of ACTs. Thus, it is imperative to identify new drug candidates with antimalarial activity. We present data for a potentially novel antimalarial drug target. Phospholipases A2 (PLA2) hydrolyze phospholipids to lysophospholipids, fatty acids, and arachidonic acid, a precursor to leukotrienes and prostaglandin production, which are mediators of inflammation. This enzyme family includes secretory (sPLA2), calcium independent (iPLA2) and cytosolic PLA2 (cPLA2) members that carry out a range of functions, including antimicrobial activity, signaling and metabolism. We identified a putative PLA2 encoded in the *P. falciparum* genome. The open reading frame includes the GXSXG and DXG/A conserved catalytic motifs and its predicted molecular weight of 78kDa is consistent with either the iPLA2 or cPLA2 classification. To determine if PLA2 inhibition of *P. falciparum* alters parasite growth we carried out *in vitro* drug susceptibility assays against a panel of PLA2 inhibitors. We have found that AACOCF3, which inhibits cPLA2 has a 50% of inhibitory concentration (IC50) in multiple *P. falciparum* strains including, 3D7 (10.8 μM), Dd2 (15.2 μM) and HB3 (7.4 μM). PLA2 inhibitors against sPLA2 or against iPLA2, did not inhibit parasite growth. Detailed microcopy studies demonstrate that the antimalarial effect of AACOCF3 takes place during the trophozoite stage of the parasite's life cycle. To determine the target pathway of pfPLA2 we will carry out metabolic labelling with 13C-choline, in the presence and absence of AACOCF3 in infected and uninfected erythrocytes. This data will describe a previously unexplored aspect of parasite biology and may inform a novel antimalarial strategy

290

PHYTOMEDICINES FROM *WARBURGIA UGANDENSIS* AND *ZANTHOXYLUM USAMBARENSE* AGAINST *PLASMODIUM KNOWLESI*

Lydiah H. Kutima[1], Patrick S. Were[1], Hastings Ozwara[2], Waudo Walyambillah[1]

[1]Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya, [2]Institute of Primate Research, Nairobi, Kenya

Malaria affects about 500 million people annually with a mortality of up to 2.7 million. The high cost of effective treatment hampers malaria control. In Africa, about 75% of the population either does not have access to or cannot afford conventional medicine and therefore traditional medicine for malaria treatment is an alternative, as reported previously. *Warburgia ugandensis* Sprague (Canellaceae) and *Zanthoxylum usambarense* (Engl.) Kokwaro (Rutaceae) are commonly used as traditional medicine by many communities in Kenya (Njoroge and Bussmann, 2006). Both plants grow in both highland and lowland areas especially in forests around Nairobi, Masaai Mara, Samburu, Southwest of Mt. Kenya and Kakamega, as reported previously. Hot and cold decoctions from leaves and stem barks of *Warburgia ugandensis* are culturally used to treat tooth decay, asthma and bronchitis while *Zanthoxylum usambarense* is used to treat malaria, upper respiratory tract infections, tooth decay and sore gums. Phytochemical studies on *Zanthoxylum usambarense* revealed that it contains alkaloids of tetrahydroprotoberberine type, as reported previously. Moreover, its *in vitro* antimalarial activity against *Plasmodium falciparum* has been reported.The aim of this study was to determine the anti-plasmodial activities of extracts from *Warburgia ugandensis* and *Zanthoxylum usambarense* against the fifth human malaria parasite,*Plasmodium knowlesi*. *Plasmodium knowlesi* is widely distributed in parts of Kenya, Malaysia, the Philippines, Myanmar and Thailand, and can also be fatal, presenting an urgent need for more focused investigations on its control. Eight plant extracts were screened for *in vitro* anti-plasmodial activity *against Plasmodium knowlesi*, in a 96-well plate incubated at 37°C on a RPMI culture medium supplemented with Baboon serum. Inhibitory concentrations (IC_{50}) values of between 3.14 and 75 μg/ml, up to 69% chemosuppression of parasites growth and over 80% survivorship of treated mice were observed. The two medicinal plants, *Warburgia ugandensis* and *Zanthoxylum usambarense* possess bioactive compounds against malaria parasites and could be exploited for further development into malaria therapy.

291

LEAD OPTIMIZATION OF BROAD-SPECTRUM ANTIMALARIAL ACRIDONES

Jane X. Kelly[1], Rosie Dodean[1], Yuexin Li[1], Christina Nolan[2], Qigui Li[2], Sean Marcsisin[2], Raul Olmeda[2], Thulan Long[2], Heather Gaona[2], Diana Caridha[2], Lisa Xie[2], Isaac Forquer[1], Rolf Winter[1], Dave Hinrichs[1], Stephanie Huezo[3], Roland Cooper[3], Mike Riscoe[1], Victor Melendez[2], Lisa Read[2]

[1]Portland Veterans Affairs Medical Center, Portland, OR, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Dominican University of California San Rafael, CA, United States

We have previously reported the discovery of a novel antimalarial acridone chemotype that displays efficacy against sporozoite-induced *Plasmodium* infection in addition to efficacy against blood stage parasites. We have been successful in producing extremely potent new lead candidates with pico molar IC_{50} values against MDR resistant parasites, as well as full protection of liver stage infection at comparable dosage with primaquine. Details of the design, chemistry, structure-activity relationships (SAR), safety, metabolic studies, and mechanism of action will be presented.

292

TARGET IDENTIFICATION EFFORTS OF NOVEL ANTIMALARIALS FROM DOS LIBRARY

Tomoyo (Sakata) Kato[1], Nobutaka Kato[2], Eamon Comer[2], Amanda K. Lukens[2], Eli Moss[2], Victoria Doroski[3], Daniel E. Neafsey[2], Christina Scherer[2], Stuart L. Schreiber[2], Elizabeth A. Winzeler[3], Dyann F. Wirth[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, MA, United States, [3]University of California San Diego, San Diego, CA, United States

Given that resistances to all drugs in clinical use have arisen, discovery of new anti-malarial small molecules that act on novel targets and/or exhibit novel mechanisms of action is urgent necessity. Using blood stage *Plasmodium falciparum*, we screened approximately 100,000 small molecules of a diversity-oriented synthesis (DOS) library, which were designed to provide more structural complexity than traditional compound library. Among these unique compounds not related to existing pharmacophores, BRD3842 and BRD3444 were selected as lead candidates based on their good potency at not only blood stage but also liver and gametocyte stages and *in vivo* efficacy using *P.berghei*. BRD3842 has a spirocyclic carboline structure with one sterocenter, showing a characteristic biphasic dose response curve in blood stage assay (EC50s: 60nM & 700nM); the enantiomer is not active. When 100nM was applied, BRD3842 inhibited parasite growth at schizont stage and reduced production of next generation, while the compound at higher dose (5μM) arrested the parasites at the ring/early troph stage. BRD3444 has an azetidine-fused eight membered ring structure with three stereocenters, two of which are critical to its activity based on SSAR analysis. The EC50 for blood stage is 15nM, and the compound are potent to all stages including early ring stage that artemisinin is not very effective. In order to identify their targets and mechanism of action by genomic approach, we performed resistant selection by exposing parasites with the compounds for short period intermittently. Resistant lines to each compound were evolved in two (BRD3842) or three (BRD3444) independent flasks, exhibiting 3.5- and 10-fold shifts and 3.4-, 13- and 67-fold shifts in EC_{50} values correspondingly. Multiple clones were then isolated from each selection, and whole genome was sequenced and analyzed to identify non-synonymous SNPs. Further studies including copy number variations analysis, target validation, drug phenotype analysis will be discussed.

293

PROGRESS IN THE PRECLINICAL DEVELOPMENT OF ANTIMALARIAL DM1157

David H. Peyton[1], Sandra Shotwell[2]

[1]Portland State University, Portland, OR, United States, [2]DesignMedix, Inc., Portland, OR, United States

We are developing a class of hybrid molecules made from a chloroquine-like moiety and a chemosentisizer (reversal agent) against chloroquine-resistance (CQR). These molecules have been shown to have low-nanomolar *in vitro* IC_{50} values against all strains of malaria tested so far, whether chloroquine-sensitive or -resistant, often surpassing the activity of even chloroquine against chloroquine-sensitive strains of *P. falciparum*. Here we report on the selection of a lead compound, DM1157, and its progress in the preclinical development pathway. This update will cover results from *in vitro* evaluations (including off-target activity), rodent toxicology and pharmacokinetics, and the initial second-species work. Also, aspects of the scale-up chemistry for GMP-production will be discussed.

294

TARGET IDENTIFICATION OF TWO CHEMICALLY DIVERSE DRUG-LIKE COMPOUNDS USING CHEMOGENOMICS

Pamela A. Magistrado[1], Amanda K. Lukens[1], Victoria C. Corey[2], Maria Linares Gomez[3], Francisco Javier Gamo-Benito[3], Roger Wiegand[4], Elizabeth A. Winzeler[5], Dyann F. Wirth[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Biomedical Sciences Graduate Program, San Diego, CA, United States, [3]Diseases of the Developing World Medicines Development Campus, Madrid, Spain, [4]The Broad Institute of MIT and Harvard, Cambridge, MA, United States, [5]Department of Pediatrics, San Diego, CA, United States

We aim to identify the mode of action of the most prominent compounds from whole-cell *Plasmodium falciparum* screens of chemically diverse libraries. We have used a chemogenomic approach whereby resistant parasites are generated by *in vitro* selection under drug pressure and whole genome sequencing is employed to identify the genetic basis of resistance. Independent selections acquiring mutations in the same gene or pathway indicate candidate determinants of the resistance phenotype. Study compounds were selected based on availability, purity, potency in a multi-drug resistant strain, and lack of known mechanisms of action such as mitochondrial function and folate biosynthesis. To further eliminate overlap with known targets, we performed cross-resistance testing against drug resistant lines with well-characterized mutations in dihydroorotate dehydrogenase, heat shock protein 90, prolyl tRNA synthetase and PfATPase4. Two chemically distinct drug-like compounds, MMV006767 and MMV007564, did not exhibit cross-resistance in any of the assays employed indicating they inhibit novel targets. To gain more insight into the compounds' mechanism of action, MMV006767- and MMV007564-resistant lines were generated *in vitro* by intermittent and continuous selection methods. Resistant parasites emerged with 3- to 12-fold EC_{50} shifts. Whole genome sequencing revealed that MMV007564-resistant lines from 3 independent selections have novel mutations in the Pf cyclic-amine resistance locus (*Pfcarl*). PfCARL, a conserved protein with unknown function, has recently been shown to be the target of imidazolopiperazines – a class of compounds distinct from MMV007564. Given their diverse chemotypes, these results suggest that PfCARL can act as a common resistance mechanism in the parasite. Our findings may also reveal new structure-activity relationship for GNF156 – an imidazolopiperazine currently in Phase II clinical trials. Further characterization of the mode of action of MMV007564 as it relates to PfCARL and whole genome sequencing for MMV006767-resistant lines are underway.

295

INHIBITORS OF UBIQUITIN E3 LIGASE AS POTENTIAL NEW ANTIMALARIAL DRUG LEADS

Jagrati Jain[1], Rajnish Sahu[2], Larry A. Walker[1], Babu L. Tekwani[1]

[1]National Center for Natural Product Research, Department of Pharmacology, University, MS, United States, [2]National Center for Natural Product Research, University, MS, United States

Malaria is a deadly disease caused by parasites of the genus *Plasmodium* and transmitted by female *Anopheles* mosquito. Malaria is curable if diagnosed early and treated appropriately. According to a recent WHO estimate, every year 219 million cases (range 154–289 million) and 660 000 deaths (range 490 000–836 000) occur every due to malaria. As the parasite has developed resistance for the most of the currently used antimalarial drugs, there is an urgent need to identify new molecular targets and to find new leads against those molecular targets. The ubiquitin/proteasome pathway is the principal system for degradation of proteins in eukaryotic. Ubiquitin is a highly conserved 76 amino acid polypeptide that covalently attaches to target proteins through the concerted action of three classes of enzymes: the ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin-protein ligase (E3). The endoplasmic reticulum associated degradation (ERAD) system of *Plasmodium falciparum* is essential for the survival and maintenance of the parasite. The ERAD system is composed mainly of three proteins: ubiquitin-activating E1 enzyme (UBA1), ubiquitin-conjugating E2 enzyme (UBC), and *Plasmodium* ubiquitin E3 ligase (HRD1). The protein encoded by Pf14_0215, a putative E3 ubiquitin ligase, has been localized to reticular structures in the trophozoite and schizont stages of the parasite. In the late schizont stage PF14_0215 proteins reside within globular structures surrounding each budding merozoite. PF14_0215 E3 ubiquitin ligase resides in the ER membrane and is essential for the parasite survival. We screened a set of selected standard E3 ubiquitin ligase inhibitors *in vitro* against *Plasmodium falciparum* cultures. Among these, JNJ- 26854165 and HLI 373 showed significant activity against both D6 and W2 strains of *P. falciparum*. The compounds were simultaneously tested against Vero cells for mammalian cell cytotoxicity. The active compounds JNJ-26854165 and HLI 373 were significantly less toxic to mammalian cells as compared to *Plasmodium*. Thus, these lead compounds showed a good selectivity as antimalarials. Further investigations on the E3 ligase inhibitors promise new understanding of the importance of E3 Ligase functions in the malaria parasite, and may provide new classes of antimalarial drug leads.

296

MAPPING OUT OF ANTIMALARIAL DRUGS ON STOCK AT THE MARKET IN A RURAL DISTRICTS OF GHANA

Alexander A. Nartey[1], Evelyn K. Ansah[2], Patricia Akweongo[3], Gloria A. Nartey[1], Mary A. Pomaa[1], Doris Sarpong[1], Clement Narh[1], Margaret Gyapong[1]

[1]Dodowa Health Research Centre, Accra, Ghana, [2]Research and Development Division, Accra, Ghana, [3]School of Public Health, University of Ghana, Accra, Ghana

Antimalarial drugs are a very important component of any policy for effective reduction of morbidity and mortality related to the malaria disease. The availability of efficacious and high quality antimalarials and their correct use can mitigate the risk of morbidity and mortality among the people of sub-Saharan Africa who have the highest risk of contracting and dying from malaria. Chemical (medicine) shops are major source of care for most developing countries where anti-malarial drugs can be purchase at the counter. The paper seeks to identify the different kinds of anti-malarial drugs on the market for malaria treatment in a rural district in Ghana. A structured questionnaire was used during two seasons (peak and low malaria transmission seasons) to collect information on anti-malarial drugs from all 58 chemical shops within the Dangme West district now (Shai Osudoku and Ningo Prampram districts). Pictures of the anti-

malarial drugs were taken, the active ingredients, and also the source of the drugs documented. GIS locations of the shops were also recorded to ascertain the proximity of the shops to households in the communities. Majority (72.0%) of the chemical and pharmacy shop owners are males. Only 7.0% of the shops are pharmacy while the remainder is licensed chemical shops. The total numbers of antimalarial drugs counted were forty nine (49). Among the stock, 4.2% were quinine, 31.9% of them were monotherapies such as artemether, Amodiaquine, Artesunate etc. Altogether, 59.4% of the artemisinin combination therapies (ACTs) were artemether + Lumefantrine, 25.0% were Artesunate + Amodiaquine. Other antimalarials observed were 9.4% Sulfadoxine + Pyrimethamine and 3.1% of of Artesunate + Sulfamethoxypyrazine + Pyrimethamine. About 47% of the anti-malarial drugs were pediatric formulations. GIS mapping shows that majority of the households are within a periphery of 5km to a chemical shop. The national antimalarial drug policy recommends the use of ACTs for malaria treatment however; all sorts of anti-malarial drugs which are not ACTs are in stock at the chemical shops in Ghana. Chemical shops are closer to households and play a very important role in the treatment of malaria hence there is the need to train chemical sellers to stock and administer the recommended antimalarials.

297

AN EFFICIENT PRIMARY HUMAN HEPATOCYTE CULTURE PLATFORM FOR ASSESSING DRUG AND VACCINE EFFICACY AGAINST *PLASMODIUM* PARASITES

Steven P. Maher

University of South Florida, Tampa, FL, United States

Malaria remains one of the world's most resilient public health problems, fighting the spread of this disease has been hampered due to a poor understanding of the parasite's unique biology and inadequate *in vitro* culture protocols for the liver stages of *Plasmodium falciparum* and *P. vivax*, two causative agents of malaria. Here we describe a novel dual-chambered microfluidic device designed to serve as an improved liver stage culture platform. Devices are manufactured from polydimethylsiloxane and feature a PDMS membrane with 10 µm pores separating two microfluidic channels. Primary human hepatocytes are concentrated and injected into a collagen-coated device channel to initiate culture. By incorporating *in vivo*-like cell confinement within an *in vitro* system, hepatocyte architecture is maintained; leading to production of albumin, clotting factor IX, and bile salts over three weeks of continuous culture. Optimized infection conditions with as few as 10,000 parasites yield several developing liver forms within each device. Arrays of devices, each containing dilutions of drugs or immune sera, form an inhibition of invasion and development assay useful for assessing the efficacy of novel compounds and vaccine targets. Future studies include high-resolution imaging of time-lapsed intracellular parasite development to better understand the parasite's liver forms and low-to-medium throughput drug screening.

298

IN-VITRO SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* TO EIGHT ANTIMALARIAL DRUGS IN CAMBODIA, 2012-2013

Dalin Dek[1], Vorleak Try[1], Sokunthea Sreng[1], Seila Suon[1], Sivanna Mao[2], Chantha Sopha[3], Baramey Sam[4], Meng C. Chuor[1], Jennifer M. Anderson[5], Chanaki Amaratunga[5], Pharath Lim[5], Rick M. Fairhurst[5]

[1]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [2]Sampov Meas Referral Hospital, Pursat, Cambodia, [3]Makara 16 Referral Hospital, Preah Vihear, Cambodia, [4]Ratanakiri Referral Hospital, Ratanakiri, Cambodia, [5]National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States

Artemisinin (ART)-resistant *Plasmodium falciparum* was first reported in western Cambodia in 2009 and now threatens the efficacy of all ART-based combination therapies (ACTs). While ART resistance is established in western Cambodia, there is no firm evidence of piperaquine (PPQ) resistance there. To monitor for resistance to PPQ and other antimalarials, we conducted a clinical efficacy study of dihydroartemisinin-piperaquine (DHA-PPQ) for the treatment of uncomplicated *P. falciparum* malaria and measured in-vitro drug susceptibilities for parasites in 2012-2013 from Pursat, Preah Vihear and Ratanakiri in western, northern and eastern Cambodia, respectively. Using a SYBR Green I fluorescence assay, we calculated the in-vitro IC_{50}s of 197 parasites to eight antimalarials: chloroquine (CQ), mefloquine (MQ), quinine (QN), PPQ, artesunate (ATS), dihydroartemisinin (DHA), pyronaridine (PYN), and atovaquone (ATV). Geometric mean IC50s (GMIC50s) for CQ, QN, PPQ, and DHA are significantly higher in Pursat and Preah Vihear than in Ratanakiri ($p \leq 0.005$). In contrast, GMIC50s for MQ are significantly lower in Pursat than in Preah Vihear and Ratanakiri, and have decreased in Pursat over the past 2 years. Significant positive correlations between $GMIC_{50}$s for PPQ and those for CQ, QN, ATS, and DHA were observed. These data suggest that the recent replacement of ATS-MQ with DHA-PPQ as first-line treatment for malaria in Cambodia may be selecting for PPQ resistance and against MQ resistance.

299

EMERGING PIPERAQUINE RESISTANCE AND RISING MEFLOQUINE SUSCEPTIBILITY LINKED TO *PLASMODIUM FALCIPARUM* MULTIDRUG RESISTANCE GENE 1 (PFMDR1) GENOTYPES IN ISOLATES COLLECTED FROM CAMBODIA IN 2008-2013

Suwanna Chaorattanakawee[1], David Saunders[1], Nitima Chanarat[1], Kritsanai Yingyuen[1], Paktiya Teja-isavadharm[1], Panita Gosi[1], Piyaporn Sai-gnam[1], Soklyda Chann[1], Youry Se[1], Darapiseth Sea[1], Nillawan Buathong[1], Sabaithip Sriwicha[1], Siratchana Sundrakes[1], Jessica Manning[1], Delia Bethell[1], Char Meng Chuor[2], Chanthap Lon[1], Charlotte Lanteri[1]

[1]Armed Forces Research Insititute of Medical Sciences, Bangkok, Thailand, [2]The National Center for Parasitology, Entomology and Malaria Control (CNM), Phnom Penh, Cambodia

Growing reports of clinical failures with the current first-line artemisinin combination therapy (ACT) in Cambodia, dihydroartemisinin-piperaquine (DHA-PPQ), are alarming. We conducted *ex vivo* drug susceptibility testing of *Plasmodium falciparum* isolates to help inform public health policy in selecting an alternative ACT to replace DHA-PPQ in Cambodia. We performed histidine-rich protein-2 (HRP-2) ELISA *ex vivo* testing in a panel of antimalarials (DHA, artesunate, mefloquine, chloroquine, piperaquine, lumefantrine, quinine, and atovaquone) and molecular marker genotyping of 753 isolates collected in 2008-2013 from western, northern, and southern Cambodia. We found temporal and regional trends in *ex vivo* susceptibility and pfmdr1 amplification results corresponding with the 2010 national malaria treatment guideline change replacing artesunate-mefloquine (AS-MQ) with DHA-PPQ. Northern isolates during 2010-2013 had a significant reduction in PPQ susceptibility with geomean IC50s more than doubling (12.8 to 29.6 nM), whereas MQ IC50s concomitantly decreased from 67.1 to 26 nM. Isolates with multiple pfmdr1 copies and the 184F mutation had significantly reduced susceptibility to DHA, AS, MQ, quinine, lumefantrine, and atovaquone, whereas isolates with a single pfmdr1 copy plus 184F mutation showed reduced susceptibility to chloroquine and PPQ. The frequency of northern isolates with pfmdr1 amplification declined over 2009-2013, corresponding with diminishing MQ use. We found a high level of chloroquine (CQ)-resistance, as indicated by all evaluable isolates being of the *P. falciparum* CQ resistance transporter (pfcrt) gene CVIET mutant haplotype. Nearly 20% of enrolled patients had plasma active against *P. falciparum* blood stages in an *ex vivo* bioassay indicating prior malaria drug use and correlating well with patient-reported malaria history. Overall, our findings suggest PPQ resistance is emerging, while MQ sensitivity is increasing and linked with pfmdr1 deamplification, and support reintroduction of artesunate-MQ in areas in Cambodia where DHA-PPQ is failing.

300

POPULATION GENETICS OF *PLASMODIUM FALCIPARUM* SURVIVING ARTEMISININ TREATMENT IN CHILDREN TAKING PART IN AN EFFICACY CLINICAL TRIAL IN KISUMU COUNTY, WESTERN KENYA

Lorna J. Chebon, Bidii Ngalah, Edwin Kamau, Luiser Ingasia

U.S. Army Medical Research Unit-Kenya/Walter Reed Project, Kisumu, Kenya

The genetic basis for artemisinin resistance is beginning to be understood with most data coming from Southeast Asia. In order to establish how widely applicable the identified genetic markers are in predicting artemisinin resistance, comparable data is needed from other regions where artemisinin is the drug of choice for treating malaria. This study aims to establish whether similar molecular mechanisms are driving artemisinin resistance in western Kenya as those seen in Southeast Asia. A total of 75 samples from a recently completed *in vivo* efficacy study and archived samples (before ACT introduction) were used. We measured six hourly parasite counts in the patients to obtain clearance rates. DNA purified was genotyped with respect to twelve polymorphic microsatellite markers to determine multiplicity-of-infections in order to identify samples containing >1 parasite clone. Ninety one single nucleotide polymorphisms distributed across the *P. falciparum* genome were genotyped using the Sequenom platform as previously published. Effect of parasite genotype on clearance rates will be assessed by correlating the 91-locus genotypes to parasite clearance half-lives. Heritability was assessed by comparing variance of parasite clearance within and between clonally identical parasites recovered in >2 subjects and phylogenetic analysis carried out to determine parasite relatedness. Parasite clearance half-life with a geometric mean of 2.6 h (1.19-4.70) was reported with the slower clearance patients showing >3.05h slope half-life. Of the 91 SNPs assessed, 85% (78) gave robust genotype data which were correlated with parasite clearance half-life to show a positive correlation. Of the 75 samples genotyped, 26 had single clone infections to be included in heritability analysis. Genetically indistinguishable parasites (multilocus parasite genotypes) infecting >1 person will be identified. Genetic profiles of the parasites at baseline and those from *in vivo* efficacy study will be analyzed; phylogenetic analysis. Tracking of genetically determined artemisinin resistance in *P. falciparum* is critical in monitoring emergence/ spread of Artemisinin resistant parasites in Kenya. Findings will inform authorities to develop containment strategies as artemisinin resistance emerges in Kenya.

301

FACTORS IN TROPICAL RURAL AREA STUDY SITES THAT CAN EFFECT DRIED BLOOD SPOT DRUG ASSAYS IN THERAPEUTIC ANTI-MALARIAL DRUG MONITORING

Karnrawee Kaewkhao, Joel Tarning, Nicholas J. White, Daniel Blessbron

Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand

Dried blood spots (DBS) as a sampling technique has been in use for decades. It is now increasingly used in preclinical and clinical studies, especially in antimalarial drug studies. DBS require a small volume of blood, and it is a simple and cheap technique to collect blood samples. It is particularly useful in rural areas with no laboratory facilities for sample processing, storage and shipping of blood samples. DBS offer an additional advantage in pediatric studies, where it is ethically challenging and difficult to collect large volumes of blood. However, the high humidity in tropical regions will significantly extend the drying time of blood spots on filter paper and could have an impact on the sample integrity. The storage conditions at the study site and conditions during transportation can also affect the integrity of the samples. These are some examples of pre-analytical issues that are difficult to control in rural areas. At the analytical laboratory, the samples enter a controlled environment and storage conditions are often investigated to minimize drug loss as part of analytical method development. We will demonstrate how environmental conditions during drying and storage can affect the outcome of antimalarial drug measurements and what precautions that needs to be in place to minimize these errors. If used properly, DBS sampling is a useful technique for therapeutic drug monitoring in rural areas and has many advantages over the conventional venous blood sampling technique.

302

IMPACT OF SEASONAL MALARIA CHEMOPREVENTION ON THE MOLECULARS MARKERS OF RESISTANCE AND THE DEVELOPMENT OF ANTI MALARIA IMMUNITY IN SENEGAL

Aminata C. Lo[1], Babacar Faye[1], El hadji Ba[2], Cheikh Sokhna[2], Badara Cisse[1], Paul Milligan[3], Colin Sutherland[3], Oumar Gaye[1]

[1]University Cheikh Anta DIOP of Dakar/Senegal, Dakar, Senegal, [2]Institue de recherche pour le developpement of Dakar/Senegal, Dakar, Senegal, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom

The Chemoprevention of seasonal malaria with AQ + SP was adopted in areas with seasonal malaria transmission. The emergence and spread of antimalarial drug resistance poses a serious public health problem. Prophylactic or therapeutic failures LEAD re-emergence of malaria accompanied by an increase in transmission, morbidity and mortality especially among children. The spread of resistance to SP but also to AQ resulting in mutations located on pfdhfr, pfdhps, pfcrt and pfmdr1 genes could affect the effectiveness of this strategy. A child born of immune mothers are protected during the first months of his life, by passive transfer of maternal immunity. The child is exposed to infection and begins to develop his own immunity, a process that will take several years before becomes effective. Hence the need to check whether SMC will have an impact on immunity in children. As well as objectives of this thesis we i) examine the prevalence of molecular markers of resistance of *Plasmodium falciparum* to SP and AQ and ii) evaluate the impact of seasonal malaria chemoprevention on the acquisition of antibodies directed against malaria. To do this, blood samples on filter paper were made during cross-sectional surveys conducted after the intervention in three health districts in central Senegal in 2008, 2009 and 2010. We determined the mutation associated with resistance to SP (pfdhfr and pfdhps) and AQ (pfcrt and pfmdr1) by PCR and secondly measuring the production of antibodies against antigens of MSP1 and AMA1 of *P. falciparum*. The results show about the prevalence of molecular markers of resistance that the triple mutation pfdhfr were high between 2008 and 2010. The quintuple mutation pfdhfr / pfdhps were not observed either in control areas or in SMC area after three years of implementation. However, the absolute prevalence of resistance markers was lower in areas with SMC. Mutations pfdhfr 164L and 540E pfdhps were not found. Regarding immunity, it was found that children living in control areas produced more antibodies (MSP1 and AMA1) than those in areas with strategy. So it seems that the SMC retards the development of immunity in these children. In view of our results, it is likely that the SMC has no impact on the prevalence of molecular markers of resistance to SP and AQ but appears to delay the acquisition of anti malarial immunity in children undergoing strategy.

303

POLYMORPHISMS OF *PFMDR1*, *PFCRT* AND *PFNHE1* MS4760 GENES IS ASSOCIATED WITH LOW VITRO QUININE SENSITIVITY IN KENYAN ISOLATES

Nancy J. Cheruiyot

Maseno University, Kisumu, Kenya

In combination with antibiotics, quinine is recommended as the second-line treatment for uncomplicated malaria, first-line treatment for severe malaria and for treatment of malaria in the first trimester of pregnancy. Quinine (QN) has been shown to have high clinical failures and yet the mechanisms of action and resistance are not been fully elucidated. However, parasite QN reduced sensitivity is linked to polymorphisms in multiple genes including multidrug resistance 1 (*Pfmdr1*), chloroquine-

resistance transporter (*Pfcrt*), multidrug resistance protein (*Pfmrp*) and the sodium/hydrogen exchanger gene (*Pfnhe1*). In this study, we investigated the association between *in vitro* quinine susceptibility with genetic polymorphisms in *Pfmdr1*codons 86 and 184, *Pfcrt* codon 76*,* and *Pfnhe1* ms4760 in field isolates from western (lowland and highlands) and coastal regions of Kenya. *In vitro* activity was assessed as the drug concentration that inhibits 50% of parasite growth (IC_{50}). DNA was extracted and polymorphisms in *Pfmdr1*, *Pfcrt* and *Pfnhe1* genes were determined by sequencing. Associations between the *in vitro* QN sensitivity [phenotypic] and the polymorphisms of the *Pfmdr1*, *Pfcrt* and *Pfnhe1* gene [genotypic] were established. Data revealed there was significant association between polymorphisms in *Pfmdr1*-86Y, 184F and *Pfcrt*-76T with quinine susceptibility ($p = 0.0001$). 82% of parasites resistant to quinine carried mutant alleles at these codons (*Pfmdr1*-86Y, 184F and *Pfcrt*-76T) whereas 74% of parasites susceptible to quinine carried the wildtype allele. Fifteen different profiles of ms4760 in *Pfnhe1* gene were observed, five of these had not been described. In addition, quinine IC_{50} of parasites with *Pfnhe1* ms4760 3 DNNND repeats was significantly higher compared to those with 1 or 2 repeats ($p = 0.033$ and $p = 0.0043$ respectively). The validity of these genetic markers is probably only relevant in the context of the genetic background of the isolates. Clinical efficacy studies are required to confirm the validity of these markers and the importance of parasite genetic background.

304

USE OF MOBILE PHONE TECHNOLOGY FOR REAL-TIME REPORTING OF FEVER CASES AND MALARIA TREATMENT FAILURE IN AREAS OF DECLINING MALARIA TRANSMISSION IN TANGA, NORTHEASTERN TANZANIA: A PILOT STUDY

Filbert Francis[1], Deus Ishengoma[1], Acleus Rutta[1], Bruno Mmbando[1], Mwele Malecela[2], Kasturi Haldar[3], Martha Lemnge[1], Edwin Michael[3]

[1]National Institute for Medical Research Tanzania, Tanga, United Republic of Tanzania, [2]National Institute for Medical Research Tanzania, Dar es salaam, United Republic of Tanzania, [3]Notre Dame University, Notre Dame, IN, United States

Early detection and management of febrile illnesses need to be improved for communities to benefit from the declining trends of malaria. Capturing events on malaria, drug resistance or associated disease in real-time, and pipelining them to appropriate clinics and/or laboratories for proper management is essential for patient care and disease control. Currently mobile phone technology is common in developing world and most developing countries are using mobile phone technology to collect and transferhealth information and services to remote populations. This study is assessing the applicability of mobile phone technology in facilitating detection and management of fever cases by village health workers (VHWs) in north-eastern Tanzania. This is a prospective pilot study involving three villages in Muheza district (Mamboleo, Magoda and Mpapayu) and one dispensary of Magoda. VHWs are suing mobile phone technology in surveillance of febrile episodes (axillary temperature ≥37.5^{0}C or/and history of fever past 24hours) through active (ACD) and passive case detection (PCD). Cases found positive by malaria rapid diagnostic test (mRDT) are given first dose of artemether/lumefantrine (ALu) at the dispensary. VHWs visit each patient at home to supervise intake of remaining doses. In May 2013, the population was estimated to be 2934 individuals in 678 households. Between 15th November 2013 and 15th March 2014, a total of 528 febrile cases aged 6.5 months -94 year were attended. Most of the cases (82.3%) were detected through PCD; 21.0% were positive by mRDT and 19.3% by microscopy. Of those with malaria, 89.5% had completed 7 days of follow-up and 10.5% were recurrent infections. No severe cases requiring a referral were reported. In conclusion, preliminary findings show that mobile phone based data collection tools have been successfully used in surveillance and timely reporting of fever episodes and monitoring of drug utilization through VHWs.

305

DOXYCYCLINE REDUCED *IN VITRO* SUSCEPTIBILITY IN *PLASMODIUM FALCIPARUM* KENYAN FIELD ISOLATES IS ASSOCIATED WITH *PFTETQ* KYNNNN SEQUENCE POLYMORPHISM

Angela A. Omondi

Maseno University, Kisumu, Kenya

Doxycycline is widely used for malaria prophylaxis by international tourists visiting malaria endemic countries. Its efficacy profile as an antimalarial in Kenya is not well defined due to limited available information regarding reference *Plasmodium falciparum* doxycycline molecular markers. We determined doxycycline *in vitro* susceptibility profiles in Kenyan isolates and further assessed their association with *Plasmodium falciparum metabolite drug transporter* and *Plasmodium falciparum GTPase tetQ protein gene* polymorphisms from 2010 to 2013.*In vitro* susceptibility testing using SYBR green I dye was employed for determination of doxycycline 50% inhibitory concentrations. While quantitative real-time polymerase chain reaction (qPCR) was used to determine copy number variation in 96 *P.falciparum* Kenyan isolates. Direct sequencing was used for determination of the number of KYNNNN motif repeats within the *PftetQ protein gene*. There was a marked reduction in median doxycycline 50% inhibitory concentrations from 18,956nM [15,234-50,141] in 2010 to 3445nM [1,744-12,775] in 2013, indicative of increasing susceptibility to the drug. However 15% of the isolates with doxycycline IC_{50} above the resistance threshold of 35,000nM, had a higher odds of having <3 KYNNNN motif repeats polymorphism of *Plasmodium falciparum GTPase tetQ protein gene* relative to those with IC_{50} < 35,000nM was (Odds ratio [OR], 15 [95% confidence interval (CI), 3.0-74.3]; P < 0.0002). While the odds of having increased copy number of both *Plasmodium falciparum GTPase tetQ protein* gene and *Plasmodium falciparum metabolite drug transporter* gene was (odds ratio [OR], 0.4100, 95% confidence interval (CI) [0.04-3.7]; P= 0.65) and OR, 0.205 [95% CI, 0.02-1.6; P=0.172] respectively for the 96 samples. *PftetQ* KYNNNN polymorphism is associated with a reduced doxycycline susceptibility phenotype in Kenyan isolates.

306

ANALYSIS OF CANDIDATE GENETIC LOCI ASSOCIATED WITH DELAYED PARASITE CLEARANCE FOLLOWING TREATMENT WITH ARTEMISININ BASED COMBINATION THERAPY IN *PLASMODIUM FALCIPARUM* ISOLATES FROM WESTERN KENYA

Ben Andagalu[1], Ngala Bidii[2], Luiser Ingasia[1], Angela Omondi[1], Hosea Akala[1], Bernhards Ogutu[1], Edwin Kamau[1]

[1]KEMRI Walter Reed Project, Kisumu, Kenya, [2]KEMRI Walter Reed Project/ JKUAT, Kisumu, Kenya

In a recent study conducted in South East Asia (SEA), regions on chromosomes 10, 13 and 14 of *Plasmodium falciparum* were identified that showed association with slow parasite clearance rates (CRs) when subjects were treated with artemisinin based combination therapy (ACT). We conducted a study to analyze chromosome 10, 13 and 14 regions as recently published to establish the genetic baseline of *P. falciparum* in western Kenya. Historical samples collected through surveillance studies from 1995-2013 and samples from an ACT efficacy clinical study conducted in western Kenya in 2013-2014 were analyzed. Parasite clearance rates for subjects in the ACT efficacy trial were calculated using the WWARN online parasite clearance estimator. All subjects from the ACT trials achieved parasite clearance within 42 hours of treatment, with a median CR constant of 0.27(range 0.15 - 0.58). More than 60% of the SNPs that were showed to be association with slow CR in SEA were present in samples from western Kenya. Interestingly however, the prevalence was highest in samples collected before the introduction of ACTs in Kenya, and decreased steadily over time. In the efficacy study, 24% of these SNPs were present. There was no increase in PfMDR copy

number observed. This data indicate unlike what was observed in SEA, these markers are not associated with ACT CRs, and are more likely associated with pressure from chloroquine which was removed from circulation at the time when reduction in the prevalence of the SNPs is observed.

307

PFCRT HAPLOTYPE DIVERSITY IN CAMEROON AND EMERGENCE OF THE S(AGT)VMNT TYPE

Huguette Gaelle Ngassa Mbenda

National Institute of Malaria Research, New Delhi, India

Evolution and spread of chloroquine-resistant-malaria parasite *Plasmodium falciparum* have posed great challenge in malaria control efforts all over the malaria endemic countries of the globe. Although chloroquine (CQ) has been banned from national malaria control programs of many endemic countries; due to high efficacy, easy avaibility and affordability, CQ still continues to be non-officially used in many countries including Africa. The main objective of this study is to unravel distribution of different Pfcrt genotypes in central, littoral, eastern, southern and in international bordering part of Cameroon. This is because, (i) chloroquine (CQ)-resistant (CQR) malaria parasite *Plasmodium falciparum* is of wide occurrence in Cameroon, (ii) mutations in the 72nd - 76th amino acid positions of the Pfcrt gene are known to confer resistance to CQ and (iii) only a single CQR haplotype (C72V73I74E75T76) has so far been reported in Cameroon. We have followed molecular approach with DNA sequencing of the 2nd exon of the Pfcrt gene to identify Single Nucleotide Polymorphisms (SNPs) in 180 *P. falciparum* field isolates sampled in five different locations in Cameroon. The CQR-Pfcrt CVIET haplotype was mostly abundant, followed by the wild CVMNK haplotype. Five hitherto-unreported CQR-Pfcrt haplotypes were detected for the first time in Cameroonian *P. falciparum*, including the surprise appearance of the S(agt) VMNT haplotype. Observations on the high haplotype diversity of the CQR-Pfcrt haplotypes coupled with appearance of the S(agt)VMNT type is daunting and can pose a greater challenge to the malaria control program of Cameroon than before.

308

PARASITOLOGIC AND MOLECULAR ASSESSMENT OF EFFICACY OF SOME ANTIMALARIAL DRUGS USED DURING PREGNANCY IN LAGOS, NIGERIA

Chimere O. Agomo[1], Wellington A. Oyibo[2], Mary Oguike[3], Rachel Hallet[3], Colin Sutherland[3]

[1]Nigerian Institute of Medical Research, Lagos, Nigeria, [2]University of Lagos, Lagos, Nigeria, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom

The prevention of malaria in pregnancy (MIP) reduces the adverse effect of malaria on both mother and foetus. Resistance to sulphadoxine-pyrimethamine (SP) has led to discontinuation of the use of SP in treatment of malaria, but used only in intermittent preventive treatment of malaria during pregnancy (IPTp). It is therefore essential to continuously monitor the efficacy of SP during pregnancy. Other non-recommended drugs but commonly used for antimalarial chemoprophylaxis during pregnancy (e,g, chloroquine and pyrimethamine) were assessed using molecular markers of resistance. Protective efficacy of SP in IPTp and the equivalence of monthly IPTp-SP to two-dose IPTp-SP was assessed in a longitudinal study from the second trimester to delivery in Lagos. A total of 1084 pregnant women were screened, but 259 were recruited (122 and 137 in two-dose and monthly-dose arms respectively) into the longitudinal study. The protective efficacy of SP was similar in both study arms (P>0.05): 98.3%, 99.1%, 100% and 100% at months 1,2,3 and 4 respectively in the two-dose arm versus 98.5%, 100%, 100% and 100% at months 1,2,3 and 4 respectively in the monthly-dose arm. The outcome of pregnancy (low birth weight and live births) was similar in the two study arms (P>0.05) irrespective of gravidity and age of the women. The frequency of Pfcrt haplotypes were 24.1, 53.7% and 22.2% for CVMNK, CVIET and CVIET+CVMNK respectively. The dhfr haplotypes were 26.7%, 6.7% and 66.7% for ACNCSVI, ACICNVI and ACIRNVI respectively. The pfmdr1 haplotypes were 53.6%, 17.9%, 21.4% and 7.1% for NYSND, YYSND, NFSND and YFSND respectively. SP was effective in IPTp and 2-dose was equivalent to monthly-dose SP regimen in Lagos. The high levels of resistance markers for pyrimethamine and chloroquine indicates that antimalarial treatment/chemoprophylaxis in pregnancy with either pyrimethamine or chloroquine may not be effective.

309

THERAPEUTIC EFFICACY AND SAFETY OF ARTEMETHER/LUMEFANTRINE FOR TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA AND PARASITE GENETIC FACTORS ASSOCIATED WITH PARASITE CLEARANCE OR TREATMENT FAILURE

Alex Shayo

The Nelson Mandela African Institution of Science and Technology, Arusha, United Republic of Tanzania

Following deployment of artemisinin based combination therapy (ACTs), regular testing for efficacy is recommended by the World health Organisation (WHO). This study assessed the efficacy and safety of Artemether/Lumefantrine (AL) and parasite genetic factors associated with parasite clearance or treatment failure in an area where the transmission has significantly declined in recent years. This was an open-label, single-arm trial, involving 88 children (6 months to 10 years) with uncomplicated falciparum malaria attended at Mkuzi Health Centre in Muheza, Tanga. . Follow-up was for 28 days and the primary end point was parasitological cure on day 28. Secondary end points included: haemoglobin improvement at day 28 and occurrence and severity of adverse events. Parasite sequencing is being performed at Sanger Institute, UK. Of the 163 patients screened, 88 were enrolled in the study. Geometric mean parasite density was 18,551 asexual parasites/µl (range: 256-200,000) and mean haemoglobin level was 10.4±1.8g/dL. Nine patients (10.2%) were lost during follow up and there was no early treatment failure. Before PCR correction; 78.4% (n=40) under-fives and 75.0% (n=21) children aged ≥5years hadadequate clinical and parasitological response (ACPR) . Late clinical failure (LCF) was seen in 5.6% of under-fives (n=51) and 3.6% children aged ≥5years (n=28). Furthermore, 15.7% and 21.6% of the patients had late parasitological failure (LPF) among under-fives and children aged ≥5years respectively. After PCR correction, ACPR was 100% in both groups. Reported adverse events included abdominal pain (11.9%), cough (59.7%), diarrhoea (1.5%), fever (23.8%), headache (1.5%), and skin rashes (1.5%). The association between treatment outcome and parasite genetic factors will be performed later. AL was safe and efficacious for the treatment of uncomplicated malaria. Since Mkuzi/Muheza has been a hotspot of drug resistance in Tanzania, surveillance needs to be continued to detect future changes in parasite sensitivity to ACTs.

310

INDEPENDENT EMERGENCE OF THE SUPER RESISTANCE-CONFERRING MUTATION AT *DHPS* CODON 581 IN EAST AFRICAN *PLASMODIUM FALCIPARUM* POPULATIONS

Sidsel Nag[1], Michael Alifrangis[1], Mette Lett Schousboe[1], Deus Ishengoma[2], John Lusingu[2], Hirva Pota[3], Reginald Kavishe[4], Richard Pearce[3], Rosalynn Ord[3], Caroline Lynch[3], Seyoum Dejene[5], Jonathan Cox[3], John Rwakimari[6], Daniel Minja[2], Martha Lemnge[2], Cally Roper[3]

[1]University of Copenhagen, Copenhagen, Denmark, [2]National Institute of Medical Research, Tanga, United Republic of Tanzania, [3]London School of Hygiene and Tropical Medicine, London, United Kingdom, [4]Kilimanjaro Clinical Research Institute, Moshi, United Republic of Tanzania, [5]Médécins Sans Fronitères, Humera, Ethiopia, [6]Ministry of Health, Kampala, Uganda

Sulphadoxine-Pyrimethamine (SP) is no longer applied as treatment against malaria, due to a variety of mutations in *Plasmodium falciparum* populations conferring resistance to the drug. However, SP has been shown to still provide protection as preventive treatment against malaria. WHO now recommends SP in preventive treatment in pregnant women and in infants, as well as in combination with Amodiaquine for seasonal malaria chemoprevention. Unfortunately, the parasites still harbor the resistance-conferring mutations, which in East Africa consist of a triple mutation in the *Pfdhfr* gene, and a double mutation in the *Pfdhps* gene. Now it is being shown in East Africa that a third mutation has appeared in the *Pfdhps* gene, terminating the efficacy of SP as preventive treatment. Emergence of this "super resistance"-conferring mutation threatens the hitherto successful control measure of intermittent preventive treatment in pregnancy. In this study, we have analysed the origin of the "super resistant" triple mutant parasites in Ethiopia, Uganda and Tanzania, with regard to the previously established double mutants. We have applied microsatellite analysis of the genetic region flanking the *Pfdhps* gene, to analyse the lineage of the double and triple mutants. In Ethiopia both double and triple mutants were derived from a single lineage which was distinct from those in Uganda and Tanzania. This correlates well with previous studies showing distinct parasite populations in Northeast and Southeast Africa. In Uganda and Tanzania, we also found triple mutants which were derived from the previously characterized Southeast African lineage. However, a novel microsatellite allele incorporated into the Tanzanian triple mutant lineage since 2004, exceeding in numbers the previously described Southeast lineage, illustrates the local expansion of a new triple mutant lineages. We conclude that the A581G mutation has occurred independently and multiple times on local *Pfdhps* double mutant backgrounds.

311

QUANTITATIVE ANALYSIS OF THE ANTIMALARIAL DRUG PYRONARIDINE USING DRIED BLOOD SPOTS

Daniel Blessborn, Karnrawee Kaewkhao, Nicholas J. White, Joel Tarning

Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand

The fixed-dose antimalarial drug combination of artesunate and pyronaridine (PYRAMAX®) has recently been developed and introduced in the treatment of falciparum and vivax malaria. Pyronaridine have a long terminal elimination half-life and it is possible to detect and quantify the drug for several weeks after the last dose. To be able to measure pyronaridine from a small quantity of blood, a sensitive quantification method was developed for therapeutic drug monitoring and pharmacokinetic studies. Pyronaridine have a high blood-to-plasma ratio and this also makes whole blood the most suitable sample matrix. Therefore, a method to measure pyronaridine in dried blood spots was developed and validated. This method use 50 µl of whole blood applied as blood spots on a filter paper to be dried at ambient temperature. The extraction process followed by separation and detection by electrospray LC-MS/MS will be presented as well as the stability tests of pyronaridine in dried blood spots. The developed method was validated according to the US FDA bioanalytical method validation guideline. Dried blood spots, as a sample collection technique, have become an increasingly useful tool in rural areas where transportation is problematic and where laboratory facilities are minimal. The accurate and sensitive method presented here requires a very small sample volume, which can be collected by finger or heal pricks. The work presented here may be particularly advantageous in studies involving small children, in whom it is ethically and practically impossible to collect large volumes of blood.

312

THE EFFECT OF ANTIMALARIAL DRUGS ON INTRACELLULAR PH IN *PLASMODIUM FALCIPARUM*

Juliane Wunderlich, Petra Rohrbach

McGill University, Sainte-Anne-de-Bellevue, QC, Canada

The acidic digestive vacuole (DV) of *Plasmodium falciparum* is the site of action of several antimalarial drugs and its membrane contains transporters that are implicated in the regulation of drug resistance. The low pH of the DV lumen is necessary for hemoglobin degradation and the detoxification of the toxic byproduct heme and its maintenance is thus crucial to the survival of the parasite. The quinoline drugs chloroquine (CQ), mefloquine (MQ) and quinine (QN) have previously been shown to raise DV pH using spectrofluorimetry, however, no accurate quantitative measurements have been performed. In this study, CQ-resistant Dd2 and CQ-sensitive 3D7 parasites were transfected with a DNA construct encoding the ratiometric pH-sensitive green fluorescent protein pHluorin alone or pHluorin with a targeting peptide that mediates trafficking of the fluorescent protein to the DV. Quantitative pH determinations were carried out using confocal fluorescence microscopy of single live parasite-infected erythrocytes under physiological conditions. MQ had the strongest effect on DV pH at a concentration of 500 nM and caused the organellar pH to rise by 0.7 pH units, whereas CQ and QN addition resulted in a pH increase by 0.3 - 0.5 pH units. To test whether the acidification of the cytosol in response to CQ, MQ and QN may be due to H^+-coupled drug export from the DV into the cytosol mediated by *P. falciparum* chloroquine resistance transporter (PfCRT), the pH effects of PfCRT inhibitor verapamil (VP) were measured in combination with a quinoline drug. VP prevented the cytosolic acidification caused by CQ and QN in Dd2 parasites, supporting the hypothesis that these drugs are expelled from the DV in symport with protons in drug-resistant strains. In contrast, VP did not alter the pH effects of MQ, which is probably not transported by PfCRT. The specific V-ATPase inhibitor concanamycin A at a concentration of 100 nM abolished the transvacuolar pH gradient between DV lumen and cytosol within 20 min, indicating that the V-ATPase might play a central role in maintaining this gradient.

313

PREVALENCE OF *DHFR* AND *DHPS* MOLECULAR MARKERS IN *PLASMODIUM FALCIPARUM* PARASITES IN PREGNANT WOMEN OF NCHELENGE DISTRICT, NORTHERN ZAMBIA

Mwiche P. Siame[1], James Chipeta[2], Phil Thuma[3], Charles Michelo[2], Sungano Mharakurwa[4]

[1]Macha Research Trust/The University of Zambia, Choma, Zambia, [2]The University of Zambia, School of Medicine, Lusaka, Zambia, [3]Macha Research Trust, Choma, Zambia, [4]Johns Hopkins School of Public Health/ Macha Research Trust, Choma, Zambia

Sulphadoxine pyrimethamine (SP) is the recommended drug for Intermittent Preventive Treatment in pregnancy (IPTp) in most African countries including Zambia. However, malaria is still one of the leading causes of morbidity and mortality in pregnant women despite reports of greater than 50% of women taking at least two doses of SP in IPTp. Studies have shown that resistance to SP is associated with mutations in the *dhfr* and *dhps* gene of the *Plasmodium falciparum* parasite. Few studies have been done to determine the prevalence of these mutations

in parasites found in pregnant women in Zambia. This cross-sectional study was conducted in Nchelenge, Northern Zambia in February-April 2013. Nchelenge is a hyper-endemic area with estimated perennial malaria prevalence of 50%. Three Rural Health Centers were randomly selected and a census survey carried out at each health center. A questionnaire was administered and malaria testing done using RDT and microscopy, with collection of a dried blood spots. Parasite DNA was extracted from dried blood spots using the chelex method followed by nested PCR. Positive samples then underwent mutation specific enzyme restriction digestion. The overall results (n=385), showed a mean age of 25. The prevalence of malaria was 22%. Multivariate analysis showed that there was an association between malaria and anaemia (AOR: 2.94) and women aged >25 years old (AOR: 0.40) were less likely to have malaria. The prevalence of *dhfr* codon S108N was 94%; codon C59R was 93.4%; and codon A16N was 5.54% *dhps* codon 436 was present at 97% and no mutation was found on *dhps* codon 540. This study showed a high number of mutations in the *dhfr* and *dhps* genes than previously reported. The high malaria endemicity in the general population of this area may have contributed to the high prevalence of resistant parasites in pregnant women. Other studies have shown that in highly endemic areas resistant parasites tend to spread quickly. These findings call for close and more detailed monitoring and evaluation of IPTp in Zambia to ascertain its effectiveness.

314

THE EFFICACY OF ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN THE GAMBIA: 2013 FOLLOW UP STUDY

Abdullahi Ahmad[1], Joseph Okebe[1], Balla Gibba[2], Serign J. Ceesay[1], Julia Mwesigwa[1], Haddy Nyang[1], Alfred Ngwa[1], Umberto D'Alessandro[1]

[1]Medical Research Council Unit, Banjul, Gambia, [2]National Malaria Control Programme, Banjul, Gambia

Artemisinin-based combination therapy (ACT) remains the main stay of case management of uncomplicated *Plasmodium falciparum* malaria. Because of increasing threat of artemisinin resistance, routine efficacy monitoring is recommended and World Health Organization guideline recommends a change in the choice of a first-line ACT when its treatment failure rate exceeds 10% in a 28 or 42-day *in vivo* therapeutic efficacy study. In 2008, The Gambia introduced artemether-lumefantrine (AL) for treatment of uncomplicated *P. falciparum* malaria and here, we report on the therapeutic efficacy monitoring of AL in the Western region of the country in the 2013 malaria transmission season. In a one-arm prospective study, 129 children aged >1 to 12 years with uncomplicated *P. falciparum* mono infection of between 1,000 to 100,000 asexual parasites/µl were treated with a weight-based oral AL and followed up for 28 days for clinical and parasitological response. Parasite density was determined using Giemsa stained thick blood films on day 0 and on days 1, 2, 3, 7, 14, 21, 28 or on any other day a child had symptoms of malaria. Filter paper spots were obtained during each of the follow up visits for polymerase chain reaction (PCR) analysis to determine if a recurring parasitemia was a re-infection or a recrudescence. Blood samples were obtained on day 0 and on whichever day a parasitemia >1,000 parasites/µl was detected for *ex vivo* parasite susceptibility drug testing. In the per protocol study population (n=100), PCR uncorrected results showed that proportion of children with adequate clinical and parasitological response by day 28 was 93% (93/100). Of these, 56% and 100% had complete parasite clearance by days 3 and 7 respectively. All the cases of parasitemia detected by day 3 were low grade (<1,000 parasites/µl). All the treatment failures (n=7) encountered were late parasitological failures between days 14 and 28. These results suggest that AL remains efficacious for the treatment of uncomplicated *P. falciparum* malaria in the Western region of The Gambia. A high proportion of low grade parasitemia by day 3 was however observed.

315

MALARIA AND MIGRANTS IN THAILAND: TREATMENT-SEEKING (AND OTHER) BEHAVIORS

Jintana Chaiwan[1], Prayuth Sudathip[2], Nardlada Khantikul[3], Piyarat Butraporn[4], Arantxa Roca Feltrer[5], Glaister Leslie[6]

[1]Malaria Consortium, Chiang Mai, Thailand, [2]Bureau of Vector-Borne Diseases, Thailand Ministry of Public Health, Bangkok, Thailand, [3]Office of Disease Prevention and Control #10, Thailand Ministry of Public Health, Chiang Mai, Thailand, [4]Mahidol University, Bangkok, Thailand, [5]Malaria Consortium, Phnom Penh, Cambodia, [6]Malaria Consortium, Bangkok, Thailand

Artemisinin-resistant malaria has emerged as an urgent concern in South East Asia. One strategy to address the problem is to improve the access of mobile and migrant populations (i.e. those considered at highest risk of malaria) to health services. This study sought to understand the knowledge, attitudes and practices (KAP) of different migrant groups in Thailand, in order to design strategies to increase migrants' health access. In September 2013, 386 migrants who visited selected health facilities along the Thai-Myanmar border participated in a KAP retrospective study. The results showed that net usage among migrants who crossed borders daily was lower than migrants who stayed in Thailand for longer periods. Less than half (48.3%) of fever cases in the past 3 months sought treatment at public health facilities, even though 36.4% of those who were tested for malaria were positive. Lower net usage, as well as delayed treatment seeking for fever, was also reported among rubber plantation workers. New intervention packages (such as insecticide-treated net delivery and behaviour change communication delivered at official and unofficial border points) must be devised to reach migrants as well as those who work in rubber plantations, as both groups are more likely to sleep without mosquito nets, and delay seeking treatment.

316

THERAPEUTIC EFFICACY OF CHLOROQUINE FOR THE TREATMENT OF *PLASMODIUM VIVAX* IN SOUTH ETHIOPIA

Seleshi K. Mekonnen, Girmay Medhin, Nega Berhe, Abraham Aseffa

Armauer Hansen Research Institute and Addis Ababa University, Addis Ababa, Ethiopia

The development and spread of chloroquine resistance *Plasmodium vivax* threatens the health of millions of people and poses a major challenge in the control of malaria .Monitoring of drug efficacy in two years interval is an important tool in establishing rational antimalarial drug policies. The current study assessed therapeutic efficacy of chloroquine for the treatment of *Plasmodium vivax* in South Ethiopia. A one-arm prospective evaluation of clinical and parasitological responses for uncomplicated microscopically confirmed vivax malaria was conducted to monitor the therapeutic efficacy of chloroquine from September to December 2012 in South Ethiopia. A total of 90 patients above 6 months and greater than 5kg with symptomatic, microscopically confirmed, uncomplicated P.vivax and fulfill the inclusion criteria were selected and included. All eligible patients were treated with CQ and followed for 28 days. PCR was conducted to differentiate re-infection from recrudescence. Pvmdr1 gene, the orthologue of pfmdr1, were sequenced and analyzed for the presence of mutations at positions 976 and 1076. Result: Of the 2500 febrile patients, 92 *P. vivax* positive cases were enrolled and treated with chloroquine. Out of 92 vivax cases 90 completed 28 days follow up period. Parasites reappeared on 12.2 % (11/90) patients. Among 11 patients, 9 were late treatment failures and 2 were early treatment failures. On the day of recurrent parasitaemia, the level of chloroquine/desethylchloroquine (CQ-DCQ) was above the minimum effective concentration (>100 ng/ml) in 9, but lower in 2 patients. We found that 8.9 % (8/90) of the isolates carried the F976 single nucleotide polymorphism. There was no mutation at codon 1076 in all *P. vivax* isolates in our study area. In conclusion, reappearance of the parasite within the 28 days follow-up period might

be due to parasite resistance to chloroquine. This finding alarms the need to launching a monitoring program of chloroquine for the treatment of *P. vivax*.

317

STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE SUGAR PHOSPHATASE PFHAD1, A REGULATOR OF THE MEP PATHWAY FOR ISOPRENOID BIOSYNTHESIS IN *PLASMODIUM FALCIPARUM*

Jooyoung Park, Ann M. Guggisberg, Rachel L. Edwards, Megan L. Kelly, Dana M. Hodge, Audrey R. Odom, Niraj H. Tolia

Washington University School of Medicine, St. Louis, MO, United States

The emergence of drug resistance to currently available antimalarial drugs is a major challenge in malaria control efforts, and thus, understanding the mechanisms that underlie drug resistance is of great importance. The methylerythritol phosphate (MEP) pathway for isoprenoid precursor biosynthesis is an attractive target for novel antimalarial drug development, and the small molecule compound fosmidomycin has been validated as a competitive inhibitor of the MEP pathway enzyme deoxyxylulose 5-phosphate (DXR). Fosmidomycin was employed in a forward genetics approach to gain understanding into regulation of the MEP pathway. Fosmidomycin-resistant *Plamodium falciparum* strains were selected *in vitro*, and genetic analysis revealed that mutations in PfHAD1 are associated with fosmidomycin resistance. PfHAD1 is a previously uncharacterized enzyme with homology to haloacid dehalogenase-like sugar phosphatases. The crystal structure of PfHAD1 was solved in order to define the structural basis for loss-of-function mutations in PfHAD1. The identified point mutations map to tightly packed hydrophobic inner core regions or catalytic regions of PfHAD1, resulting in either protein misfolding or interference with substrate binding. Biochemical investigations show that PfHAD1 dephosphorylates a variety of sugar phosphate compounds, including intermediates of glycolysis, which feed into the MEP pathway. Several of these sugar phosphates have been co-crystallized with PfHAD1 in order to investigate the structural basis for its diverse substrate specificity. Finally, metabolic profiling reveals that fosmidomycin-resistant parasite strains lacking PfHAD1 have substantial increases in MEP pathway metabolites. Together, these results demonstrate that PfHAD1 regulates substrate availability to the MEP pathway and that loss of PfHAD1 function confers fosmidomycin resistance in *P. falciparum*.

318

DIVERSITY OF *PLASMODIUM* MALARIA AND MOLECULAR MARKERS OF SULPHADOXINE RESISTANCE IN TANZANIA

Vito Baraka[1], Alex Shayo[2], Deogratias Ngatunga[2], Erasmus Kamugisha[3], Celine Mandara[1], Rashid Madebe[1], Joram Buza[2], Martha M. Lemnge[1], Jean Pierre van Geertruyden[4], Deus S. Ishengoma[1]

[1]National Institute for Medical Research, Tanga, United Republic of Tanzania, [2]Nelson Mandela Institute of Science and Technology, Arusha, United Republic of Tanzania, [3]Catholic University of Health and Allied Sciences - Bugando, Mwanza, United Republic of Tanzania, [4]International Health, University of Antwerp, Antwerp, Belgium

Intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) is widely deployed in the control of pregnancy-associated malaria. However widespread SP resistance threatens its effectiveness. This study aimed at assessing diversity of *Plasmodium* species as well as identifying molecular markers of sulphadoxine resistance in the current situation of changing malaria epidemiology. A cross-sectional study was conducted in three districts of Tanzania with different transmission intensity; Muheza and Muleba (hypoendemic), and Nachingwea (hyper-endemic/ holoendemic). Patients with a history of fever or fever at presentation (≥37.50) were screened using malaria rapid diagnostic tests (mRDTs) and confirmed by microscopy. Molecular markers of sulphadoxine resistance were detected by nested-PCR followed by sequence specific oligonucleotide probes-enzyme- linked immunosorbent assay (SSOP-ELISA). A total of 466 (56.9%) patients were tested positive by mRDTs and 397 (48.5%) microscopy. Nachingwea had higher positivity rate compared to other sites, at 29.0% and 27.7% by mRDT and microscopy respectively. *Plasmodium falciparum* species was the predominant specie at all sites while P.ovale was only detected at Muleba and Nachingwea. Prevalence of P.falciparum gametocytes was higher in Muleba (13.7%) followed by Nachingwea (7.2%) and the lowest was observed in Muheza (0%). Additionally, fever was a strong predictor of malaria parasite rates at Nachingwea ($p<0.001$). Prevalence of sulphadoxine resistance involving Pfdhps codon 540E (96.6%) and 581G (53.4%) were higher in Muheza compared to the other sites.This study showed that Nachingwea had high malaria positivity rate compared to other sites. High Sulphadoxine resistance observed at Muheza could be attributed to local use of SP and it may spread to other areas. Further studies to monitor the spread of sulphadoxine resistance in other regions are warranted

319

INVESTIGATION OF STRUCTURE-ACTIVITY RELATIONSHIPS OF ANTIMALARIAL DRUGS THROUGH NOVEL *PLASMODIUM FALCIPARUM* CHLOROQUINE RESISTANCE TRANSPORTER (PFCRT) HAPLOTYPES

Stephanie Rasmussen[1], Andres Gutierrez[1], Hernandez Jessica[1], Nicole Flordeliza[1], Melissa Forbush[1], Nyachare Gatkouth[1], Samuel Lemus[1], Ian Nickel[1], Jillian Nunes[1], Bianca Sanchez Mora[1], Alexis Valdovinos[1], Stephanie Huezo[1], Jianbing Mu[2], Thomas E. Wellems[2], Roland A. Cooper[1]

[1]Dominican University of California San Rafael, CA, United States, [2]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Mutation in the *Plasmodium falciparum* resistance chloroquine transporter (PfCRT) protein causes chloroquine resistance and alters susceptibility to various antimalarial drugs. Mutant PfCRT transports chloroquine out of the parasite digestive vacuole; however, the molecular details of drug-receptor interaction are poorly understood. The antiviral drug amantadine has moderate potency against *P. falciparum* and acts to reduce the level of chloroquine resistance determined by the *pfcrt* haplotype. In accordance, chloroquine-resistant parasites show enhanced sensitivity to amantadine. We used continuous, step-wise amantadine selection on three chloroquine-resistant lines of the *P. falciparum* 106/1 strain. Each of these lines contains a unique *pfcrt* allele differing only by a non-synonymous SNP in codon 76, resulting in PfCRT K76I, K76N or K76T, but otherwise contains an identical genetic background. Stepwise selection with amantadine resulted in parasite lines with six novel *pfcrt* alleles. Parasites showed resistance to amantadine, increased chloroquine sensitivity, and changes in susceptibility to other digestive vacuole-targeting drugs. The results shed light on new topographical regions of the PfCRT protein and amino acid residues involved in drug interactions and digestive vacuole morphology. Two additional amantadine-selected parasite clones, derived from 106/1 K76N and K76I, showed amantadine resistance and altered quinoline susceptibility without accompanying mutations in *pfcrt*. These parasite lines are currently under investigation. Our results increase the understanding of how digestive vacuole-targeting drugs interact with the PfCRT protein and explain patterns of cross-resistance in the malaria parasite.

320

INVESTIGATION OF THE THAI MULTIDRUG RESISTANT *PLASMODIUM FALCIPARUM* C2A STRAIN IN THE *AOTUS* MONKEY MODEL

Nicanor III Obaldia[1], Bryan L. Smith[2], Geoffrey S. Dow[2], Lucia Gerena[2], Dennis Kyle[3], William Otero[4], Pierre-Yves Mantel[1], Nicholas Baro[1], Rachel Daniels[1], Manoj Duraisingh[1], Sarah Volkman[1], Dyann Wirth[1], Matthias Marti[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Department of Global Health, University of South Florida, Tampa, FL, United States, [4]Antimalarial Drugs and Vaccines Evaluation Center, Tropical Medicine Research, Panama City, Panama

Antimalarial drug resistance is the biggest threat to the current malaria eradication agenda (MaLERA) based on oral artemisinin-based combination therapies (ACTs). ACTs are the standard of care for uncomplicated malaria, but reports of clinical treatment failures (defined as a reduced parasite clearance rate or persistence of parasites on the third day of treatment) of artesunate (AS) / mefloquine (MQ) combinations are rapidly emerging from South-East Asia. The Thai *Plasmodium falciparum* C2A clone is a Multiple Drug Resistant (MDR) isolate obtained from a patient in Thailand in 1992 before deployment of this ACT, first introduced in 1994 and adopted in 2005, though, artemisinins had been in use in western Cambodia since the late 70's (Witkowski et al, 2013). In a previous *in vivo* adaptation study in Aotus monkeys, we observed that even in the absence of MQ pressure, C2A appeared to gradually loose its susceptibility to MQ as passage levels increased. In order to further characterize this observation we assessed the efficacy of MQ and AS alone or in combination in infected splenectomized Aotus monkeys. Our results confirmed the previous findings of resistance to oral MQ at 40 mg / Kg given once or to oral AS at 33 mg / Kg x 3 days alone or in combination with MQ at 40 mg / kg once. Strikingly, in the AS alone group, clearance occur in 1 / 2 animals on day 7 post-treatment (PT) but recrudesce on day 8 PT (slow clearance phenotype) and persisted in the other one until day 10 PT when it was finally rescue treated. As a comparison, AS at 20 mg / Kg orally x 3 days against infections of the Vietnam CQ resistant FVO strain, clears infection within 1-2 days PT in spleen intact Aotus. Only when AS was administered IV at 20 mg / Kg x 3 days in combination with oral MQ at 40 mg / Kg, infections were cured in 4 / 5, but cleared and recrudesce in 1 / 5 rescue treated animals. Additional *in vitro* assays of C2A demonstrated reduced susceptibility to MQ, CQ, artemisinin, dihydro-arteminsinin, and atovaquone-proguanil, arteether and AS compared to the sensitive D6 strain, the *in vitro* adapted TM90-C2A line or passage III Aotus adapted C2A when compared to the later monkey passage X. Analysis of drug resistance loci and copy number variation is underway to determine the genetic basis for the observed drug resistance profile. We anticipate that these studies will shed light on the early spread and evolution of multi drug resistance and reduced artemisinin susceptibility in South-East Asia.

321

EFFECT OF DRUG PRESSURE ON THE PREVALENCE OF SULFADOXINE-PYRIMETHAMINE RESISTANT HAPLOTYPES AND SELECTIVE SWEEP CHARACTERISTICS IN MALAWI

Elena M. Artimovich[1], Miriam K. Laufer[1], Kristan Schneider[2], Terrie E. Taylor[3], James G. Kublin[4], Ananias A. Escalante[5], Christopher V. Plowe[1], Shannon Takala-Harrison[1]

[1]University of Maryland Baltimore, Baltimore, MD, United States, [2]Department of MPI, University of Applied Sciences Mittweida, Mittweida, Germany, [3]Blantyre Malaria Project, University of Malawi College of Medicine, Blantyre, Malawi, [4]Fred Hutchinson Cancer Research Center, Seattle, WA, United States, [5]School of Life Sciences, Arizona State University, Tempe, AZ, United States

The reemergence of chloroquine sensitive parasites in Malawi following the switch to sulfadoxine-pyrimethamine (SP), raised hope that abandoned drugs might later find clinical utility. Malawi replaced SP with artemisinin-based combination therapy (ACT) in 2007 in response to failing efficacy. Here we examine the effect of SP pressure on prevalence of SP-resistant haplotypes and the characteristics of associated selective sweeps. Pyrosequencing and microsatellite genotyping were performed on samples from three time periods in the history of SP use: high SP-use, when SP was the first-line treatment; the transition period from SP to ACTs; and low SP-use, five years after the switch to ACTs. An increase in the prevalence of dhfr triple and dhps double mutants occurred between the high SP use and transition periods ($p<0.0001$), but there was no significant change in haplotype prevalence 5 years after reduction of SP pressure. The prevalence of the dhps triple mutant 437G/540E/581G increased despite reduced SP pressure ($MHp<0.0001$). A reduction in the prevalence of less resistant dhfr and dhps was also observed. Microsatellite analysis identified sweeps flanking dhfr triple and dhps double mutant haplotypes. Changes in sweep characteristics were seen at both distal and proximal markers. At most dhps flanking loci there was a decline in expected heterozygosity after the high SP use period, however changes at proximal markers suggest that change in SP pressure may not affect all markers equally. Changes flanking dhfr were not as pronounced as dhps, suggesting that drug pressure affects sweeps at different rates. Our data suggest that SP-resistance may be longer lived than chloroquine resistance. Maintenance of SP resistant genotypes and sweep characteristics could be due to continued pressure from residual SP use for intermittent preventive treatment and trimethoprim-sulfamethoxazole prophylaxis in persons living with HIV individuals, and/or to a lack of fitness cost of resistance.

322

ADDRESSING ARTEMISININ-RESISTANT MALARIA BY IDENTIFYING MALARIA HOTSPOTS IN CAMBODIA

Soy Ty Kheang

University Research Co. LLC, Phnom Penh, Cambodia

During the past decade Cambodia has made significant strides toward malaria reduction and now, elimination. The last remaining cases will be the hardest to reach, as they are located in remote border areas. Efforts are also threatened by the emergence of artemisinin-resistant malaria (ARM). The PMI/USAID Control and Prevention of Malaria (CAP-Malaria) Project developed a model to effectively identify malaria hotspots and treat remaining malaria cases in remote villages through household active case detection of ARM and a comprehensive response. Key strategies include individual index malaria case detection serving as the focal point for subsequent comprehensive response to surrounding areas. Village Malaria Workers (VMWs) are trained to diagnose malaria in their communities and take a blood smear. Following patient treatment through directly observed therapy (DOT), the VMW takes a second blood smear after 72 hours of the treatment initiation which is analyzed at a health center laboratory. Cases that are still positive on day-3, and therefore an early warning sign for ARM, receive further investigation and case management, including follow-up to day 28. In addition, 40-50 people surrounding the index case are screened for malaria regardless of symptoms and treated if infected, and any gaps in community coverage of long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and malaria knowledge are addressed by a team including the VMW, health center staff, and district malaria officials. Between September 2010 and December 2013, 1,733 malaria patients were diagnosed with malaria by VMWs from 11 targeted health facilities in catchment areas where evidence of ARM has been found. Among the residents from four catchment areas, 1,585 *Plasmodium falciparum* and mixed infection malaria cases, 109 (7.7%) were positive on day-3. During the three year time period, however, the number of day-3 positive cases detected decreased, even while overall malaria enrolled cases increased. Potential reasons for the drop in day-3 positive cases could include the systematic DOT provided by the VMWs, ensuring treatment adherence and improved quality of the drug supply.

323

REAL-TIME PCR AND HIGH RESOLUTION MELTING (HRM) ANALYSIS OF POLYCLONAL *PLASMODIUM FALCIPARUM* INFECTIONS

Khalid B. Beshir[1], Nouhoum Diallo[2], Aminatou Kone[2], Antoine Dara[2], Oumou Maiga-Ascofare[2], Abdoulaye Djimde[2], Colin Sutherland[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Malaria Research and Training Centre, Department of Epidemiology of Parasitic Diseases, Faculty of Pharmacy, Bamako, Mali

Recurrent *Plasmodium falciparum* parasitaemia in treated malaria patients results from either recrudescence of parasite clones which survived drug action, or acquisition of new clones that emerge from the liver during follow up after antimalarial treatment. One molecular method used to distinguish between the two is PCR-correction using size polymorphisms of various genes and microsatellites . The recurrent infection is commonly categorized by comparing the size of polymorphisms in genetic markers of the genes on enrolment samples (day-0) and on follow-up day on which parasite are detected by microscopy (day of parasitological failure). We have previously reported that by increasing the parasite genotyping to multiple time-points (day-0, day-1 and day-2), recrudescent clones are identified more often than by the conventional approach. However, most currently used gel-electrophoresis methods, particularly when multiple time-points are incorporated, are time-consuming, laborious and the interpretation of results can be ambiguous. The development of an alternative, rapid, simple and accurate method based on Real-time PCR and High Resolution Melting (HRM) analysis will be reported. Its usefulness for *ex vivo* drug sensitivity and complexity of infection studies will be discussed.

324

K13-PROPELLER, MAL10 AND MAL13 AND POLYMORPHISMS IN *IN VIVO* ARTEMISININ SUSCEPTIBLE *PLASMODIUM FALCIPARUM* PARASITES FROM BOUGOULA-HAMEAU, MALI

Amed Ouattara[1], Aminatou Kone[2], Matthew Adams[3], Bakary Fofana[4], Amelia Walling-Maiga[5], Jose P. Gil[6], Anders Bjorkman[7], Ogobara K. Doumbo[4], Christopher V. Plowe[3], Abdoulaye Djimde[4]

[1]University of Maryland School of Medicine and University of Science, Techniques and Technologies of Bamako, Mali, Baltimore, MD, United States, [2]University of Science, Techniques and Technologies of Bamako, Mali and Karolinska Institutet, Stockholm, Sweden, [3]University of Maryland School of Medicine, Baltimore, MD, United States, [4]University of Science, Techniques and Technologies of Bamako, Bamako, Mali, [5]Duke University School of Medicine, Durham, NC, United States, [6]Karolinska University Hospital/Karolinska Institutet, Stockholm, Sweden, [7]Karolinska Institutet, Stockholm, Sweden

Artemisinin resistance has been documented in South-East Asia and may already be spreading in that region**.** Molecular markers are important tools in monitoring the spread of antimalaria drug resistance. Recently, two SNPs on chromosomes 10 and 13 (MAL10-688956 and MAL13-1718319) were shown to be useful markers of delayed parasite clearance in surveillance for artemisinin resistance in South-East Asia. In addition, mutations in the PF3D7_1343700 kelch propeller domain ('K13-propeller') were associated with artemisinin resistance *in vitro* and *in vivo*. The prevalence of these molecular markers of artemisinin resistance is unknown in sub-Saharan Africa. We therefore extracted DNA from dried blood spots of pre-treatment falciparum malaria infections in Bougoula-Hameau, Mali where we recently showed that artemisinin monotherapy was highly efficacious with a median parasite elimination slope half-life of 2 hours. Polymorphisms of MAL10-688956 and MAL13-1718319 were genotyped by Nested PCR followed by restriction enzyme digestion with *NsiI* and *MslI*, respectively. Mutations in K13-propeller were determined by direct sequencing of Nested PCR amplicons. One hundred children aged 1-10 years were included in this study. Sample processing and statistical analyses are underway and results will be presented at the meeting. This study will provide information on the prevalence of molecular markers of artemisinin resistance in a population of sub-Saharan African malaria parasites fully susceptible to artemisinin.

325

IDENTIFICATION OF MALARIA-INFECTIOUS INDIVIDUALS BY FIELD-BASED DIAGNOSTIC METHODS: LOOP-MEDIATED ISOTHERMAL AMPLIFICATION AND LIGHT MICROSCOPY

Bronner P. Gonçalves[1], Spencer Polley[1], André Lin L. Ouédraogo[2], Lynn Grignard[1], Sophie Jones[1], Moussa W. Guelbeogo[2], Issa Nebie[2], Colin Sutherland[1], Iveth J. González[3], Teun Bousema[1], Chris Drakeley[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [3]Foundation for Innovative New Diagnostics, Geneva, Switzerland

Malaria elimination strategies will require sensitive diagnostic tools capable of detecting most human falciparum infections in endemic communities. Malaria loop-mediated isothermal amplification (LAMP) is a field-friendly assay that has greater diagnostic accuracy compared to microscopy and consequently might have a key role in mass screening campaigns. Here, we assessed the infectiousness of 130 randomly-selected individuals living in a malaria endemic area of Burkina Faso by membrane feeding assays (N=307 assays) and related infectivity results to malaria infection status determined by two field-based methods (LAMP and light microscopy), and by quantitative-nucleic acid sequence-based amplification (QT-NASBA). In 72.0% of samples, *Plasmodium falciparum* infection was detected by LAMP. Parasites were identified by microscopy (asexual or sexual parasites) and 18s NASBA in 45.0% and 94.8% of samples, respectively. In 75.5% of feeding assays where at least one mosquito was infected, parasites were detected by LAMP, similar to light microscopy (74.4%). Our results confirm previous findings that LAMP is more sensitive than microscopy for detecting falciparum infection. A similar proportion of infectious individuals had parasites detected by LAMP and microscopy, suggesting that high parasite density, higher than microscopy detection threshold, is an important predictor of infectiousness. Further research is warranted to verify whether this pattern also occurs at low transmission intensities, where most infectious individuals have low-level infections.

326

UNEARTHING THE HIDDEN INTRICACIES BETWEEN FISHING AND MALARIA: A CASE STUDY OF RUSINGA ISLAND, WESTERN KENYA

Jacqueline E. Oduke, Wolfgang R. Mukabana, Collins K. Mweresa

International Centre of Insect Physiology and Ecology, Nairobi, Kenya

Establishment of underlying factors of malaria exposure remains key for identification of ideal preventive measures.It is apparent that malaria risk behaviours are rooted in wider aspects of local livelihoods, and socio-cultural beliefs and practices. This research work draws on empirical data derived from in-depth, one-to-one semi-structured interviews, focus groups and questionnaire administration to participants in villages in Rusinga Island to explore the nature of these practices and their potential impact on malaria exposure risk. Participants were fish crew, both men and women. By eliciting local understandings of malaria-related behaviours, we explore how malaria risks are played out in people's everyday lives. Our findings reveal the problem of non-usage of bednets by community members resulting from their livelihood and socio-cultural practices and events. These practices contravene the consistent and sustained use of the bednet which are called for by public health policies. In particular, we try to explain how in reality livelihood activities and lifestyle play a role in exposure to malaria by Rusinga fish crew around dawn and dusk.

327

A COMMUNITY-BASED SURVEY ON KNOWLEDGE, ATTITUDE AND PRACTICE OF DIAGNOSIS AND TREATMENT FOR VIVAX MALARIA IN SOUTH BENGKULU AND MINAHASA DISTRICTS, INDONESIA

Lenny L. Ekawati

Eijkman-Oxford Clinical Research Unit, Jakarta, Indonesia

Plasmodium vivax malaria globally threatened an estimated 2.49 billion people in 2010. In Indonesia, *P. vivax* is transmitted among over six million pregnancies and 130 million individuals, with overall prevalence of 3.84%. On the contrary to a conventional philosophy, growing evidence suggests that *P. vivax* demonstrate as debilitating illness and life-threatening for adults and children in eastern archipelago. Management of *P. vivax* malaria is challenging due to the presence of relapsing liver stage (hypnozoites), which can be killed by the only available drug, Primaquine (PQ) and administration with blood schizontocides acts as radical cure of vivax malaria. According to current recommendations, suspected fever cases should be confirmed by microscopy or rapid diagnostic tests (RDTs) prior to treatment. However, a vast majority of fever cases in Indonesia are clinically diagnosed and Artemisinin Combination Therapy (ACT) is not properly administered. PQ failure has been attributed to inadequate treatment regimen and dosage, and patients' lack of compliance. Further, PQ can induce hemolytic toxicity in individuals who are deficient in the enzyme glucose-6-phosphate dehydrogenase (G6PD). Treatment and diagnostic practices essential for optimal PQ efficacy are inadequate in Indonesia, as indicated by high levels of drug resistance. To date research into malaria-related knowledge and behaviours has been widely conducted with household surveys. These measurements reveal information on determinants of malaria diagnosis and treatment processes. Cross sectional surveys were conducted between April and August, 2013. Three questionnaires were implemented in the household and primary health centers (PHCs). Total number of respondents was 562. Clinicians and laboratory technicians were queried in ten selected PHCs. Data was analyzed by STATA ver. 9. Overall, the processes involved in vivax malaria diagnosis and treatment must be evaluated in order to provide control programs with a "pulse of community. Most people stated an understanding about malaria, symptoms, severity level and consequence of untreated malaria. However, despite a call for malaria elimination program, community awareness related to early diagnosis and effective treatment should be strengthened. Health providers require multi-faceted interventions to support better laboratory confirmation and treatment towards vivax malaria infections.

328

SINGLE DOSE OF PRIMAQUINE FOR MALARIA ELIMINATION IN A POPULATION WITH HIGH PREVALENCE OF G6PD DEFICIENCY

Germana Bancone[1], Raweewan Somsakchaicharoen[1], Nongnud Chowwiwat[1], Ladda Kajeechiwa[1], Suradet Thinraow[1], Slight Naw Nyo[1], Clare Ling[1], Jacher Wiladphaingern[1], Naw Lily Kirichareon[1], Nicholas J. White[2], Francois Nosten[1]

[1]Shoklo Malaria Research Unit, Mae Sot, Thailand, [2]Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand

Malaria caused by multi-drug resistant *Plasmodium falciparum* (including artemisinin resistance) is common in the population living along the Thai-Myanmar border. In the context of malaria elimination to combat the spread of artemisinin resistance, the Shoklo Malaria Research Unit has evaluated the safety of single dose of 0.25 mg base/kg of primaquine with an ACT in a population of over 2000 subjects. Blood collection for hemoglobin levels and sensitive detection of malaria parasite were performed every 3 months during one year in 4 different villages. G6PD was characterized by Fluorescent Spot Test in all study participants and genotyping performed for the most common local mutations in subjects with abnormal phenotype. Hemoglobin levels of subjects treated with PMQ before and after primaquine administration were analyzed. Results will be presented showing the effect of the single low dose primaquine in G6PD normal and deficient participants.

329

PROTEIN MICROARRAY ANALYSIS OF HUMAN ANTIBODY RESPONSES IN *PLASMODIUM VIVAX* RELAPSE VS. REINFECTION IN THE PERUVIAN AMAZON

Raul Chuquiyauri[1], Douglas Molina[2], Huw Davies[3], Eli Moss[4], Ruobing Wang[5], Malcolm J. Gardner[5], Kimberly C. Brouwer[6], Sonia M. Torres[7], Robert Gilman[8], Hugo Rodriguez[9], Alejandro Llanos-Cuentas[7], Daniel E. Neafsey[4], Philip Felgner[3], Joseph M. Vinetz[1]

[1]Division of Infectious Diseases, University of California San Diego, San Diego, CA, United States, [2]Antigen Discovery Inc., Irvine, CA, United States, [3]University of California Irvine, Irvine, CA, United States, [4]Broad Institute of MIT and Harvard University, Cambridge, MA, United States, [5]Seattle BioMed, Seattle, WA, United States, [6]University of California San Diego, San Diego, CA, United States, [7]Instituto de Medicina Tropical 'Alexander von Humboldt', Universidad Peruana Cayetano Heredia, Lima, Peru, [8]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [9]Directorate of Health, Iquitos, Peru

Accurate estimations of parasite prevalence, malaria disease incidence, and hence prioritization of control efforts require new, cost-effective, and field-deployable methods. Because determining the presence of malaria parasitemia is time-intensive, often insensitive, and expensive, interest has grown for using serological tools to monitor infection status and transmission dynamics. Because *Plasmodium vivax* is only available in relatively low parasitemia from humans and in limited quantities from non-human primates, microarray seroepidemiology using *P. vivax* asexual stage parasite lysates has only infrequently been done. The field activities of this study were carried out from 2005-2008 in the Santo Tomas, San Jose de Lupuna, and Padrecocha villages of the Peruvian Amazon. Genotyping of *P. vivax* isolates was performed using PCR-restriction fragment length polymorphism (PCR-RFLP) analysis of the *P. vivax* merozoite surface protein-3alpha (PvMSP3α). Relapse was defined as identical RFLP patterns of the primary and subsequent infection parasites. Reinfection was defined as different RFLP patterns of the primary and subsequent infection parasites. Of 111 subjects, 96 had 2 episodes, 10 had 3 episodes, and 5 had 4 episodes over a 24-month follow-up period. Using PvMSP3α PCR-RFLP genotyping of the recurrent infections, 38 were classified as relapse; 88 were classified as reinfection; and 5 showed no RFLP band and thus were not classified. Using microarray analysis, the antigens differentially recognized by *P. vivax*-infected subjects were more likely to have significantly higher signal for subjects with PvMSP3α genotypes different than the previous infection than for subjects with PvMSP3α genotypes similar to the previous infection. Proteins recognized by Human IgG Antibodies are more likely to have amino acid changes than proteins coded by the genome as a whole. Proteins recognized by sera are more likely to have SNPs than the genome as a whole, suggesting selection arising from host-pathogen interactions, immune or otherwise. The protein microarray analysis results will provide tools for applying sero-epidemiology to surveillance, control and elimination; establishing tools for decay kinetics.

330

EFFICACY OF INDOOR RESIDUAL SPRAYING (IRS) WITH ACTELLIC® 300CS IN SENEGAL

Ousmane Sy[1], Badara Cissé[1], Abdoulaye Diallo[1], Fassiath Tairou[1], Elhadji Ba[2], Jean Louis Abdourahim Ndiaye[1], Lassana Konaté[1], Oumar Gaye[1], Paul Milligan[3], Ousmane Faye[1]

[1]Universite Cheikh Anta Diop, Dakar, Senegal, [2]IRD, Dakar, Senegal, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom

In areas of low malaria transmission, such as western Senegal, where scaling up of control measures has been effective in reducing malaria incidence, additional measures are required to eliminate the disease. Targetting additional control measures to communities with persisting high transmission of malaria may be more effective than blanket control measures. As part of a cluster randomized trial of a targetted control strategy, efficacy of Actellic 300CS (pirimiphos methyl) for IRS delivered by district spray teams was evaluated. Hotspot villages were identified on the basis of the number of cases of malaria the previous year. In August 2013, IRS with Actellis300CS was delivered to all households in these villages by community health workers, trained and supervised by staff of the department of Public Health (Service National d'Hygiène). Efficacy was assessed in 4 villages over 4 months. In each village, 5 treated rooms were selected, and untreated rooms selected as controls, and bioassays were done on each of three walls per room to measure knock-down and 24-hour mortality of lab reared *Anopheles gambiae* and of wild mosquitoes locally caught. A household survey was conducted to assess completeness of coverage and acceptability. Results and conclusion: After two, three and four months the 24-hour mortality, adjusted for control mortality using Abbott's formula, was 92% (95%CI 85%,96%), 72% (63%,80%), and 37% (29%,45%) respectively. Sensitivity tests to different insecticides using local *Anopheles* showed 100% sensitivity to pirimiphos methyl in all four sites, and to bendiocarb and permethrin in three sites with lower sensitivity in one district, with resistance to DDT in all sites. These results show that IRS with Actellic 300CS was highly effective but the duration of efficacy was shorter than expected.

331

APPLICATION OF MOLECULAR BARCODE AND PRE-AMPLIFICATION TECHNIQUES IN TRACKING *PLASMODIUM FALCIPARUM* POPULATION STRUCTURE CHANGES DURING SCALE-UP MALARIA INTERVENTIONS IN SOUTHERN AFRICA

Sungano Mharakurwa[1], Rachel Daniels[2], Phil Thuma[3], Alan Scott[4], William Moss[4], Sarah Volkman[2], Dyann Wirth[2]

[1]National Institute of Health Research, Harare, Zimbabwe, [2]Harvard School of Public Health, Boston, MA, United States, [3]Macha Research Trust, Choma, Zambia, [4]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Malaria is receding from many endemic countries in the wake of scaled-up interventions for possible elimination of the disease. However, the decline is heterogeneous within and between countries and the resilient scourge persists as low-grade submicroscopic infections among semi-immune members of the population. This transitioning epidemiology not only poses risk of possible resurgences typical for malaria, but also presents challenges for detection and tracking of intervention impact on the surviving parasite reservoir. Here we demonstrate application of molecular barcode and pre-amplification technology for the successful detection and documentation of *Plasmodium falciparum* population structure changes in relation to control interventions in areas of southern Africa. Dry blood spot (DBS) samples were collected through prospective cross-sectional surveys, between 2003 and 2013, in three areas of southern Africa exhibiting contrasting malaria epidemiology under control, namely Choma district in southern Zambia (successfully declining malaria), Mutasa district in eastern Zimbabwe (rebounding malaria) and Nchelenge district in northern Zambia bordering the Democratic Republic of Congo (persistently holoendemic malaria). Following DBS screening by realtime quantitative PCR (with preamplification for low-grade infections), *P. falciparum* infections were genotyped using a 24 SNP molecular barcode assay. Analysis of population structure across the 24 barcode markers demonstrated significant loss of genetic diversity and population structure decline for Choma (M-W U = 178.0, p = 0.023) but not for Mutasa (MW U = 10, p = 0.487) or Nchelenge, where the genetic diversity was even higher than Mutasa (MW U = 35.0, p = 0.029). Barcoding and pre-amplification techniques afford an instrumental tool for parasite identification, tracking and documenting the impact of interventions on parasite populations during malaria control and elimination programmes when parasitaemia is expected to decline to submicroscopic levels.

332

MALARIA AND HEALTH NEEDS ASSESSMENT AMONG MIGRANT FARM WORKERS IN AMHARA REGION, ETHIOPIA: A VENUE-BASED APPROACH

Rebekah Stewart Schicker[1], Neway Hiruy[2], Berhanu Melak[3], Woyneshet Gelaye[4], Belay Bezabih[5], Rob Stephenson[6], Amy E. Patterson[7], Zerihun Tadesse[2], Paul M. Emerson[8], Frank O. Richards, Jr.[8], Gregory S. Noland[8]

[1]Emory University, Department of Global Health, Rollins School of Public Health, Nell Hodgson Woodruff School of Nursing, Atlanta, GA, United States, [2]The Carter Center, Addis Ababa, Ethiopia, [3]The Carter Center, Bahir Dar, Ethiopia, [4]Amhara Regional Health Research Laboratory Center, Bahir Dar, Ethiopia, [5]Amhara National Regional State Health Bureau, Bahir Dar, Ethiopia, [6]Emory University, Department of Global Health, Rollins School of Public Health, Atlanta, GA, United States, [7]The Carter Center, Agnes Scott College, Atlanta, GA, United States, [8]The Carter Center, Atlanta, GA, United States

Mobile populations present unique challenges to malaria control and elimination. Each year, around 350,000 people travel to areas of North Gondar Zone, Ethiopia to work on large-scale farms, doubling the population of areas with the heaviest malaria burden in Amhara Region. The farming season overlaps the major malaria season (September to December). Little is known about these migrant workers, including migration patterns, access to, and use of malaria prevention measures, housing accommodations, and health care seeking behavior. In order to assess these factors and prevalence of malaria and anemia, a venue-based survey of migrants who traveled for farm labor in two districts of North Gondar was conducted in July 2013 at the beginning of the farming season. A total of 605 migrants ≥18 years of age were recruited from three venue types: farms (58% of survey population), roads between farms (16%) and towns (26%). Most (73.1%) had started farm work at the time of survey and 99% were male. Mean age was 22.8 years, with 74.7% less than 25 years. Nearly all (95.8%) came from Amhara, with half (51.8%) of those coming from other districts in North Gondar. Most (77.4%) migrants arrived in June or July, and 46.3% intended to leave in September. Around half (52.1%) lived in temporary shelters, while 20.8% had no sleeping accommodations. Malaria was the leading identified health concern. LLINs were available to 12.0% of participants. Reported net use last night was 74.6% among those with access to a net and 8.8% overall. *Plasmodium* prevalence by RDT was 12.0%, with 9.6% *Plasmodium falciparum*, 1.7% *P. vivax*, and 0.7% mixed infections. Anemia (Hb <13 mg/dl) was detected in 28.3% of migrants. Around one-third (30.3%) reported having fever within the past two weeks, of whom 31.3% sought treatment. Distance to a healthcare facility and cost were important reported barriers to seeking treatment. These results are being utilized to develop interventions tailored to this population.

333

COMMUNITY PERCEPTIONS AND PRACTICES TOWARDS MALARIA CONTROL MEASURES IN RWANDA: A DESCRIPTIVE QUALITATIVE STUDY

Chantal M. Ingabire[1], Alexis Rulisa[2], Jane Alaii[3], Emmanuel Hakizimana[1], Fredrick Kateera[1], Claude Muvunyi[1], Leon Mutesa[4], Bart Van Den Borne[5]

[1]Rwanda Biomedical Center, Kigali, Rwanda, [2]Rwanda Social Security Board, Kigali, Rwanda, [3]Family Health International, Nairobi, Kenya, [4]National University of Rwanda, Kigali, Rwanda, [5]Maastricht University, Maastricht, Netherlands

Long lasting insecticidal nets (LLINs), indoor residual spraying (IRS) and malaria case treatment with artemisinin based combination therapy have been proved to reduce malaria significantly. Elimination requires consideration of other factors yet underexplored. Our objective was to explore behavioral and environmental predictors associated with malaria transmission, to draw operational recommendations. In December 2013, we conducted nine focus group discussions, in Ruhuha, Southern East of Rwanda with 81 participants. Various cooperatives members, community health workers, health care professionals, lay community, schools teachers, youth representatives, as well as local and religious leaders were included. Discussions were recorded, transcribed in English and coded using Nvivo 10. Study protocol was approved by Rwanda National Ethics Committee. Malaria ranked first among the top five diseases in the area. High knowledge on transmission and symptoms was observed. The notion of malaria elimination by the community was acknowledged but challenges noted. A recent outbreak of bedbugs was found hindering the use of LLINs in addition to the hot weather characterizing the dry season. IRS was generally perceived as no longer killing insects including mosquitos unlike spreads them. Seeking care at the health center as first choice was related with having a community based health insurance (CBHI) while buying medicines in pharmacies was commonly seen among those without a CBHI or who claim spending much time at the health center. A recent wealth categorization has had an impact on the amount to be contributed to get a CBHI. A large number of participants argued that the categorization was unfairly done and leading some families unable to afford the CBHI. In conclusion, joint efforts are needed to tackle issue of bedbugs for proper use of LLINs at household level. Further studies are needed to test the efficacy of IRS and look for appropriate chemicals if needed. Lastly, to uphold high coverage of CBHI, government is suggested to revise the wealth categorization.

334

EVALUATION OF THE LOOP MEDIATED ISOTHERMAL DNA AMPLIFICATION (LAMP) FOR MALARIA DIAGNOSE IN A *PLASMODIUM VIVAX* ENDEMIC SETTING

Andres F. Vallejo[1], Iveth González[2], Myriam Arévalo-Herrera[1], Socrates Herrera[1]

[1]Caucaseco Scientific Research Center and Malaria Vaccine and Drug Development Center, Cali, Colombia, [2]Foundation for Innovative New Diagnostics, Geneva, Switzerland

Current malaria diagnostic tests, including microscopy and antigen-detecting rapid tests, cannot reliably detect low-density infections. Molecular methods such as polymerase chain reaction (PCR) are highly sensitive but remain too complex for field deployment. We assessed the field applicability of a new molecular assay for malaria diagnosis based on loop-mediated isothermal amplification (mLAMP) in *Plasmodium vivax* endemic setting of Colombia. A prospective cohort of patients was used to assess the diagnostic performance of the mLAMP. After signing the informed consent, volunteers were diagnosed using microscopy and LAMP at point of care (POC). To assess the field performance of mLAMP for detection of submicroscopic infections, a cross-sectional survey was conducted in 3 regions of Colombia with different epidemiological profiles. The mLAMP results were interpreted by visual reading under ultra violet light. Real time PCR and nested real time PCR in discordant samples was used as reference. LAMP sensibility and specificity was comparable to real time PCR for both *P. vivax* and *P. falciparum* detection at point of care diagnosis. 278 febrile patients were enrolled at Tierralta, Cordoba, the mLAMP sensitivity of for both *P. falciparum* and *P. vivax* was 100% and 90% respectively compared with PCR. All false-negative LAMP results involved samples of *P. vivax* with parasitaemia levels detectable by 3-well nested real time PCR but very low or undetectable by qPCR. In the cross-sectional, survey 980 volunteers in 10 sentinel sites at 3 different malaria endemic regions of Colombia were enrolled. LAMP detected 14.8 times more cases than microscopy with a prevalence across all sentinel sites of 6.02% (n=59) and 0.4% (n=4) respectively. *Plasmodium falciparum* infections accounted for 23.9% and *P. vivax* infections for 76.1%. No cases of mixed infections were identified by PCR. The proportion of asymptomatic infections among mLAMP confirmed cases was 98.0%. Malaria LAMP in a *P. vivax* low endemic setting achieved sensitivity similar to that of single-well nested PCR in a reference laboratory. LAMP showed detection limits comparable to PCR under minimum infrastructure condition under field conditions and dramatically increase the detection of asymptomatic malaria providing a new tool for diagnosis, surveillance, and screening in elimination strategies.

335

A NOVEL CROWD-SOURCING TECHNIQUE FOR PREDICTING DENSITIES AND DISTRIBUTION OF DISEASE-TRANSMITTING MOSQUITOES IN RURAL TANZANIA

Stephen P. Mwangungulu

Ifakara Health Institute, Morogoro, United Republic of Tanzania

Lack of reliable techniques that can be used for large-scale programmatic monitoring of distribution and densities of disease-transmitting mosquitoes is a major challenge to public health authorities, especially in rural and remote communities where high-tech GIS and remote sensing facilities are not readily applicable for regular use. We developed and evaluated a new community-based participatory mapping approach that relies simply on the knowledge and experiences of residents to rapidly identify areas where disease-transmitting mosquitoes are most abundant. The method is proposed for use in spatial targeting of mosquito control interventions. Such simplified methodologies for mapping vector densities will be particularly necessary for optimal placement of new interventions to complement existing ones such as long-lasting insecticide treated nets (LLINs) for malaria control. This new crowd-sourcing technique consisted of 5 steps. We initially mapped three test villages comprehensively identify major land marks (step 1). We then selected 60 community members monthly, taught them basic map-reading and offered them gridded maps of their own villages so they could identify locations where they think mosquitoes are most abundant, by simply ranking the grids on scale of 1-5 (step 3). The data generated was interpolated in ArcGIS using inverse distance weighting method and classified to show places where people thought there were high, medium and low mosquito densities (step 4). Finally, mosquito sampling was done using an effective odor-baited sampling tool, to verify outdoor mosquito densities in locations pre-identified by community members as having high, medium and low mosquito densities, and to validate this crowd-based prediction method. Maps were derived from community knowledge and opinions on the mosquito density distributions. For twelve months in three villages, entomological surveys depicted the same vector densities and distribution pattern as the crowd-sourcing technique. This study thus provides evidence that we can rely on community knowledge and experience to identify suitable areas where mosquitoes are most abundant and where to locate outdoor complementary interventions. Such a method will be cheaper, quicker and easier even for planning and implementing large-scale vector control operations.

336

EVALUATION OF THE EFFECTIVENESS OF MALARIA CONTROL ACTIVITIES BY SIMULTANEOUS NATIONWIDE CROSS-SECTIONAL AND CASE-CONTROL SURVEYS IN MADAGASCAR

Thomas Kesteman, Milijaona Randrianarivelojosia, Patrice Piola, Christophe Rogier

Institut Pasteur de Madagascar, Antananarivo, Madagascar

The Malaria Control Program of Madagascar has set since 2007 an objective to pre-eliminate malaria. In order to help decision making to reach pre-elimination, we aimed at evaluating the actual effectiveness of malaria control activities. We conducted a nationwide survey in 2012-2013 in 62 sites representing all transmission patterns. This survey included (1) a cross-sectional study to measure the effectiveness of each control intervention on reducing the transmission, and (2) a concurrent case-control study to measure the effectiveness on reducing the morbidity. We present here the results related to organized vector control activities, i.e. Long Lasting Insecticidal Nets (LLIN) distribution and Indoor Residual Spraying (IRS) campaigns. The cross-sectional survey included 15,734 individuals among which 3.7% had a positive Rapid Diagnostic Test (RDT). LLIN daily use was 52.3% in areas covered by universal distribution and IRS coverage was 64.8% in targeted areas. 818 uncomplicated clinical malaria cases were compared to 7,767 controls living in the same villages. Multilevel analysis of factors associated with a positive RDT or with the occurrence of an episode of non-complicated malaria revealed that LLIN daily use had a 45% protective effectiveness (PE) against infection (aOR 0.55 [95%CI 0.42, 0.72]) and a 48% PE against morbidity (aOR 0.52 [0.28, 0.96]). The PE of IRS was evaluated to be 23% against infection (aOR 0.77 [0.53, 1.13]) and 49% against morbidity (aOR 0.51 [0.39, 0.66]). In areas where both activities are implemented, coverage of LLIN was 21.3 percentage points lower than in areas where LLIN only were deployed. Combining IRS with LLIN provided almost no gain in preventing infection, but the PE of LLIN use against morbidity increased from 51% (aOR 0.49 [0.20, 1.20]) to 66% (aOR 0.34 [0.16, 0.74]) when IRS was added, although non significantly. Our results show that, taken separately, LLIN and IRS perform satisfactorily but that their concurrent use might have a limited benefit as compared with efforts to improve the coverage of a single intervention.

337

IDENTIFICATION OF ASYMPTOMATIC MALARIA INFECTION IN BORDER CROSSING POPULATIONS IN CAMBODIA

Chandary Rang[1], Hannanh Edwards[1], Sophal Uth[1], Sara Canavati[1], Hai Van[1], Sovannaroth Siv[2], Ly Po[2], John Hustedt[1], Arantxa Roca-Feltrer[1]

[1]Malaria Consortium, Phnom Penh, Cambodia, [2]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia

Cross-border population movement can hinder elimination of malaria by importing new infection, maintaining hot-spots of high transmission, and contributing to the development and spread of drug resistance. Cross-border movement is common in Cambodia yet there is limited surveillance at border points. To investigate malaria infection in border-crossing populations, three border crossing points were chosen as study sites, one from each of Cambodia's borders with Thailand, Vietnam and Lao. From August 2013 to February 2014, 3,206 border crossers were tested for malaria (by RDT and PCR) and fever, and were interviewed regarding *a priori* risk factors for infection. Univariate and multivariate logistic regression were performed to investigate potential exposure variables against the outcome of malaria infection. Based on RDT analysis there were 103 cases of malaria (67 Pf, 34 Pv and 2 mixed) giving an overall prevalence of 3.21% (PCR prevalence and drug resistance analysis in progress and available April 2014). Prevalence differed between boarder sites (0.09% Thailand, 0.99% Vietnam and 8.04% Laos). Sixty-nine (67%) cases were asymptomatic. Main risk factors for infection determined by multivariate logistic regression were previous episode of malaria (OR 5.52, p<0.0001), Forest-goers (OR 5.26, p<0.0001), <15 years old (OR 4.94, p=0.003), presence of fever (OR 4.03, p<0.0001), and working as security/armed forces (OR 3.11, p=0.02). Knowledge of two or more prevention methods gave a protective effect from infection (OR 0.45, p=0.008). The Lao border point had greater prevalence of forest-goers, high-risk occupations, previous malaria episodes, and low knowledge of prevention methods; leading to higher prevalence of malaria infection. Cross-border malaria will hinder elimination where border-crossing populations have high prevalence of risk factors for infection. Lessons learned from this project have set the basis for a comprehensive cross-border surveillance platform in the Greater Mekong Subregion. Similar border points should continue to be identified and targeted surveillance put in place for countries across the GMS where population movement is common.

338

CHANGING TRENDS IN MALARIA AMONG MOZAMBICAN PREGNANT WOMEN: EFFECT ON ANTIMALARIAL IMMUNITY AND CLINICAL IMPACT OF *PLASMODIUM FALCIPARUM* INFECTIONS

Alfredo Mayor[1], Azucena Bardají[1], Eusebio Macete[2], Tacilta Nhampossa[2], Anna Maria Fonseca[1], Raquel González[1], Sonia Maculuve[2], Pau Cisteró[1], Maria Rupérez[1], Joe Campo[1], Carlota Dobaño[1], Anifa Vala[2], Betuel Sigaúque[2], Alfons Jiménez[1], Sonia Machevo[2], Laura de la Fuente[1], Abel Nhama[2], Leopoldina Luis[2], John Aponte[1], Sozinho Acácio[2], Arsenio Nhacolo[2], Chetan Chitnis[3], Esperanza Sevene[2], Pedro Luis Alonso[1], Clara Menéndez[1]

[1]Barcelona Centre for International Health Research, Barcelona, Spain, [2]Manhiça Health Research Center, Manhiça, Mozambique, [3]International Centre for Genetic Engineering and Biotechnology, New Delhi, India

Understanding the determinants and consequences of malaria declines and resurgences as well as the timescales over which antimalarial immunity is gained and lost, has become a priority in the context of current goals of malaria elimination and eradication. Epidemiological changes provide a unique opportunity to investigate how anti-malarial immunity is lost following reductions in transmission and how this might impact disease burdens in potential resurgences. Here we present the results from a study that combines epidemiological and immunological description of malaria among pregnant women delivering between 2003 and 2012 at antenatal clinics in the Manhiça District hospital in Southern Mozambique. *Plasmodium falciparum* qPCR-positivity decreased from 33% in 2003 to 2% in 2010 (p<0.001) and increased to 6% in 2012 (p=0.026), with antimalarial IgGs mirroring these malaria trends. Parasite densities in peripheral blood and in the placenta were higher in 2010-12 than in 2003-5, whereas prevalence of peripheral infections that were submicroscopic followed an opposite trend. An attenuation of the parity effect on parasite densities and on IgGs against placental-type parasites was observed during 2010-12 compared to 2003-5. Malaria infection was associated with a larger reduction in maternal haemoglobin levels and in the birthweight during 2010-12 compared to 2003-5. These results suggest that a sustained reduction in the exposure to malaria parasites leads to a weakening of immune regulation of parasite densities that can increase the potential for occurrence of high-density infections and malaria-related harmful effects. These findings reinforce the importance of sustaining efforts when moving from control to elimination to avoid rebounds of malaria associated with the reduction in naturally acquired immunity.

339

PROGRESS TOWARD DEVELOPMENT OF A LOW DENSITY INFECTION-DETECTION TEST TO SUPPORT ACTIVE DETECT-AND-TREAT INTERVENTIONS AIMED AT REGIONAL MALARIA ELIMINATION

Paul LaBarre, Kathleen Tietje, Kenneth Hawkins, Christine Clerk, Kelly Ebels, Chris Crudder, Robert Burton

PATH, Seattle, WA, United States

As successful malaria control programs dramatically reduce malaria prevalence, strategic and programmatic changes are required to eliminate malaria transmission altogether. Development of new, game-changing, and possibly disruptive malaria infection-detection technologies can enable more efficient elimination interventions in the most challenging malaria-endemic environments. For active detect-and-treat interventions, the essential product characteristics required are: low limit-of-detection, rapid turn-around times, ease of use, high sensitivity, portability, and appropriate pricing. PATH's Diagnostics for Malaria Elimination Toward Eradication (DIAMETER) project is focused on bridging the gap between the rapidly evolving set of detect-and-treat tactics and the existing diagnostic capabilities. We aim to ensure that elimination interventions are not hampered by test performance. The goal of the DIAMETER project is to catalyze rapid access to the most cost-effective and temporally-effective elimination infection-detection tools in the product development pipeline. Our phase I activities have produced an elimination market analysis, a six-country field investigation of user requirements, and a landscape of technologies in the product development pipeline. Our phase II activities focus on development of infection-detection tests (IDTs) for qualitative detection of low-density *Plasmodium falciparum* infections. We envision that IDTs will be used by malaria elimination programs to identify and treat subclinical, low-parasite-density populations that serve as reservoirs of parasite biomass. We will present our phase I findings, the process used to develop the IDT target product profile, and the landscape of promising platform technologies that can be leveraged to achieve the required performance characteristics.

340

G6PD DEFICIENCY IN LATIN AMERICA: PREVALENCE, VARIANTS AND IMPLICATIONS FOR MALARIA ELIMINATION

Fernando F. A. Val[1], Wuelton M. Monteiro[1], André Siqueira[1], Gabriel P. Franca[1], Vanderson Sampaio[1], Gisely Melo[1], Anne Almeida[1], Marcelo A. M. Brito[1], Henry M. Peixoto[2], Douglas Fuller[3], Quique Bassat[4], Gustavo A.S. Romero[5], Maria Regina F Oliveira[5], Marcus V. G. Lacerda[1]

[1]Fundação de Medicina Tropical Doutor Heitor Vieira Dourado, Manaus, AM, Brazil, [2]Faculdade de Medicina, Universidade de Brasília, Brasília, DF, Brazil, [3]Department of Geography and Regional Studies, University of Miami, Coral Gables, FL, United States, [4]Barcelona Centre for International Health Research (CRESIB, Hospital Clínic-Universitat de Barcelona), Barcelona, Spain, [5]National Institute for Science and Technology for Health Technology Assessment, Porto Alegre, RS, Brazil

Glucose-6-phosphate dehydrogenase deficiency (G6PDd) is the most prevalent human genetic disease worldwide. Predominance of *Plasmodium vivax* malaria in Latin America (LA) is especially relevant, since *P. vivax* radical cure requires the use of primaquine (PQ). It is important to consider that PQ may cause severe drug-induced hemolysis and may lead to acute intravascular haemolysis in G6PDd individuals. This could further hamper malaria control efforts. Despite clinical and epidemiological significance of G6PDd and malaria interaction, G6PDd prevalence has not been well measured in LA populations. A systematic review using existing bibliographic and biomedical databases was made to better understand the distribution of G6PDd in Latin America and the Caribbean. The review resulted in low prevalence rates in Argentina, Bolivia, Mexico, Peru and Uruguay. Studies performed in Curaçao, Ecuador, Jamaica, Saint Lucia, Suriname and Trinidad, as well as some surveys carried out in some areas of Brazil, Colombia and Cuba showed high prevalence (>10%) of G6PDd. G6PD encoded by the *G6PD A-^{202A}* mutation was the most broadly distributed across LA, being identified in 81.1% of deficient individuals surveyed. It was observed a virtual absence of G6PDd among Amerindians, suggesting that PQ use is safe in this population. In order to avoid unnecessary exposure to hemolysis inducing drugs, as PQ, it is crucial to develop a rapid and accurate G6PDd diagnostic test to be used in LA field conditions. Issues related to the radical cure of *P. vivax* infection and an eventual elimination of malaria in LA, such as massive drug treatment, must be linked to information about the genetic background of the population. Although considering the inexistence of multicenter studies with similar methodologies, it is possible to estimate roughly that G6PD deficiency in LA is a recognized phenomenon, however with the evidence that some populations are probably not affected, such as the indigenous.

341

QUALITY OF ARTEMISININ COMBINATION THERAPIES IN SUB SAHARAN AFRICA AND CAMBODIA, ASSESSED USING LABORATORY ANALYTICAL TECHNIQUES

Harparkash Kaur

London School of Hygiene & Tropical Medicine, London, United Kingdom

Artemisinin combination therapies (ACTs) are recommended as first line treatment for malaria by the World Health Organisation (WHO) and officially implemented in 79 of 88 countries. Reports of ad hoc surveys from SE Asia showed that up to 50% of the artesunate monotherpy sold was falsified. Alarmingly the first case of falsified artesunate (that almost resulted in a fatality) and ACTs were also reported from Africa where the predominant parasite species is the potentially fatal *Plasmodium falciparum*. Resource poor countries do not have the technical (medicines control laboratories), financial, or human resources required to inspect and police the drug supply. The lack of reliable estimates of poor quality drug prevalence and its causes makes it difficult for national regulatory authorities to determine the need and scale of interventions to assure drug quality. We purchased and carried out qualitative and quantitative content analyses on over 10,500 ACTs in total from Cambodia, Ghana (Kintampo), Tanzania, Nigeria (Enugu and Ilorin) and Equatorial Guinea (Bioko Island) following varying sample collections methods to provide effective surveillance of ACTs available in a given geographical region. The percentage active pharmaceutical ingredient (% API), determined by laboratory chromatographic analysis, was used to classify the medicines as good quality (85% - 115%), as recommended by the United States Pharmacopeia for the analysis of single tablet samples. Poor quality samples included substandard samples (115% of both APIs), degraded samples (improper storage and/or transport) and falsified samples (absence of stated APIs). Most of the samples contained the stated APIs, with the exception of samples from Enugu (1% out of 2865), Ilorin (0.8% out of 1449) and Bioko (7.3% out of 683) which did not contained the stated APIs. Of concern are the monotherapy tablets on sale in countries with falsified ACTs. The results were disseminated to the country-specific ministry of health, as well as relevant manufacturers and logged on the WHO Rapid Alert System for the surveillance and monitoring of Substandard/ Spurious/Falsely Labelled/ Falsified Counterfeit, SSFFC, medicinal products.

342

SURVEILLANCE SYSTEMS TO FACILITATE MALARIA ELIMINATION

Colin Ohrt[1], Kathryn W. Roberts[1], Hugh J. Sturrock[1], Jennifer A. Wegbreit[1], Bruce Lee[2], Roly Gosling[1]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Johns Hopkins University, Baltimore, MD, United States

Robust and responsive surveillance systems are critical for the success of malaria control and elimination. In elimination settings surveillance must be an intervention, where immediate action is taken in response to case

identification. An ideal surveillance system for malaria elimination should include: rapid and complete case reporting, incorporation of data from other relevant systems, central data storage and management, automated and expert data analysis, customized outputs and feedback, and timely and targeted response. Spatial information enhances a surveillance system, ensuring that cases are tracked to the household level and are mapped over time. Data-sharing and coordination across borders to address imported malaria are vital and the incorporation of new technologies can improve the speed, accuracy or quality of data. Ideally, a malaria elimination surveillance system needs to take information from many sources, in multiple formats, conduct some degree of automated analysis and disseminate tailored reports to multiple levels to guide action. While many countries' surveillance systems include one or several of these ideal elements, no single system contains all components. Understanding countries' successes and challenges designing and implementing surveillance systems facilitates the refinement or creation of systems in the future. Many parts of the ideal system for malaria elimination surveillance have already been developed, but have yet to be linked together in a coherent system. To facilitate this process, malaria elimination programs should support the implementation and refinement of existing systems and ensure political and financial commitment for this process. Malaria elimination programs should also strive to: develop systems to improve the access and utility of surveillance databases, establish and pilot cross border databases, and support the creation of standard indicators for malaria surveillance. Ultimately, investment in a timely and targeted surveillance system is necessary to achieve elimination goals.

343

THE DIMINISHING RETURNS OF ATOVAQUONE-PROGUANIL FOR ELIMINATION OF *PLASMODIUM FALCIPARUM* MALARIA: MODELLING MASS DRUG ADMINISTRATION AND TREATMENT

Richard J. Maude[1], Chea Nguon[2], Arjen M. Dondorp[1], Lisa J. White[1], Nicholas J. White[1]

[1]Wellcome Trust Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, [2]National Centre for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia

Artemisinin resistance is a major threat to current efforts to eliminate *Plasmodium falciparum* malaria which rely heavily on the continuing efficacy of artemisinin combination therapies (ACT). It has been suggested ACTs should not be used in mass drug administration (MDA) in areas where artemisinin resistant *P. falciparum* is prevalent and that atovaquone-proguanil (A-P) might be a preferable alternative. However, a single point mutation in the cytochrome b gene confers high level resistance to atovaquone, which arises frequently during treatment and this would affect the potential efficacy of atovaquone-proguanil as a tool for elimination. A deterministic population level mathematical model was developed based on data from Cambodia to explore the possible effects of large-scale use of A-P compared to ACT for mass drug administration and/or treatment of *P. falciparum* malaria, with and without adjunctive primaquine (PQ), with the aim of local elimination. The model showed the initial efficacy of ACT and A-P for MDA to be similar. However, each round of A-P MDA resulted in rapid acquisition and spread of atovaquone resistance. Even a single round of MDA could compromise its efficacy sufficient to preclude its use for treatment or prophylaxis. A switch to A-P for treatment of symptomatic episodes resulted in a complete loss of efficacy in the population within 4-5 years of its' introduction. For malaria elimination, A-P for MDA or treatment of symptomatic cases should be avoided. A combined strategy of ACT+PQ MDA, long-lasting insecticide treated bed nets and high coverage with ACT for treatment of symptomatic episodes would be preferable.

344

USING ROUTINE AGGREGATED MALARIA CASE DATA FROM SWAZILAND TO PRODUCE FINE SCALE RISK MAPS

Hugh J. Sturrock[1], Justin Cohen[2], Andy Tatem[3], Arnaud Le Menach[2], Nyasatu Ntshalintshali[2], Simon Kunene[4], Michelle Hsiang[1], Roly Gosling[1]

[1]Global Health Group, San Francisco, CA, United States, [2]Clinton Health Access Initiative, Boston, MA, United States, [3]University of Southampton, Southampton, United Kingdom, [4]National Malaria Control Program, Mbabane, Swaziland

Mapping malaria risk is an integral component of effective and efficient resource allocation. As transmission declines and infections become increasingly rare, countries have to move from using infection prevalence data to case data for stratification, mapping and prediction of transmission risk. While some programs collect information on the household locations of individual cases, facilitating fine scale risk mapping, doing so is resource and time intensive. Routine health facility data is more convenient to collect, but its utility for extrapolating risk across surrounding regions is presently unclear. Using routinely collected case data from health facilities in Swaziland between 2011-2013, here we explore the use of a method termed point sampling, in conjunction with random forest models, to produce fine scale risk maps (~300m resolution). Using known household locations of cases to validate models, results show that all three variations of the approaches tested here have very good predictive ability (AUC values ≥0.83) with the optimal approach incorporating the spatial distribution of the population. Predictions from the best performing model also showed good correspondence with a reference risk map generated using the household locations of cases (mean error = 0.01, absolute error = 0.02). Cross-scale models utilizing routine health facility level data can provide fine scale prediction of malaria risk in low transmission settings and hold promise for other cross-scale disease prediction problems. Bayesian approaches to cross-scale prediction will also be discussed.

345

RETREATED BUT NOT DEFEATED: THE ROLE OF HEALTH SYSTEMS IN IMPROVING THE EFFECTIVENESS OF ARTEMISININ COMBINATION THERAPIES (ACTS) FOR MALARIA CONTROL AND ELIMINATION

V. Bhargavi Rao[1], Jamie Griffin[1], IMPACT 2 Study Team[2], Lucy Okell[1], David Schellenberg[3], Azra Ghani[1]

[1]Imperial College London, London, United Kingdom, [2]Ifakara Health Institute (Tanzania)/Centers for Disease Control and Prevention (USA)/ London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom

Artemisinin Combination therapies (ACTs) are highly efficacious first-line antimalarials. However their use in sub-Saharan Africa, both as treatment and control measure, is hampered by weak health systems and a poorly controlled diversity of antimalarial sources. We extended an existing mathematical model of malaria transmission to include health systems factors: dimensions of access to sources of ACTs in the private, public and tertiary sectors, and quality of care for malaria and non-malarial febrile illness (NMFI). Data from the IMPACT 2 study in Tanzania was used to parameterise the model. Our aim was to estimate the impact of overcoming health systems barriers in 3 settings, differing by epidemiology and prevailing health systems. Outcomes included malaria mortality and parasite prevalence (transmission risk). Primary level interventions (private and public sectors) had most impact on transmission. In low-prevalence scenarios, modelling single interventions, e.g. ensuring 100% treatment seeking for fever, eliminated parasites. Improving quality of care, e.g. introduction of diagnostic-led therapy with adequate stocks of ACTs was as effective in all settings as a policy of presumptive treatment; reducing parasite prevalence in under-fives (U5s) in moderate transmission settings by up to 86% depending on the sector of implementation. In contrast, a policy of presumptive treatment for U5s only was not

effective in any region, likely due to reservoirs of infection in older age-groups. Strengthening public facilities was ineffectual in contexts with strong private sector preference. The model outcomes demonstrate optimal packages of interventions at a national level are not always ideal interventions at a regional level. Improving the effectiveness of ACT delivery is highly dependent on local epidemiology, existing health systems provision and population preferences for either primary health facilities or private drug shops. Context-specific planning and consideration of local priorities e.g. reducing ACT wastage on NMFI versus reducing transmission, may improve progress towards international targets to decrease clinical disease and mortality, and in low transmission settings, to approach elimination.

346

CONSEQUENCES OF THE TIMING OF GESTATIONAL ANEMIA ON NEWBORN'S HEMOGLOBIN CONCENTRATION IN A MALARIA ENDEMIC AREA

Smaila Ouedraogo

Public Health Department, University of Ouagadougou, Ouagadougou, Burkina Faso

Because of the high prevalence of gestational anaemia (GA) in developing countries, anaemia-related adverse effects on birth outcomes are expected to have an important public health impact. We studied the relationship between GA and newborn's anaemia, taking into account the timing of GA within the MiPPAD trial of intermittent preventive treatment in pregnancy (IPTp) (http://clinicaltrials.gov/ct2/show/NCT00811421). Study design Prospective cohort of pregnant women followed up from early pregnancy until the time of delivery. Study site, population and procedures The study was conducted in the district of Allada (southern Benin) where malaria is perennial. The study population was composed of HIV negative women less than 28 weeks of gestational age and their newborns. Socio-demographic data were collected on inclusion (before first IPTP administration, IPTp1), clinical and biological data at each visit (IPTp1, second IPTp administration -IPTp2- and delivery). Children's clinical and biological data were collected at birth. Statistical analysis Logistic and linear regressions were performed to evaluate at each time point the relation between maternal and newborns' Hb levels. Direct and indirect associations between Hb levels in pregnancy and at birth, mediated by Hb levels at different times of pregnancy, were then tested using a path analysis. Newborn anaemia was common (63.5%) in the 862 analyzed mother-newborn pairs. Linear regression showed a significant association between maternal and newborns' Hb levels at delivery only (P = 0.004). Path analyses confirmed the strong direct association between maternal Hb at delivery and newborns' Hb, and also found an indirect effect of maternal Hb at IPT1 and IPT2 (P = 0.006 and P = 0.005). In conclusion, the strong association between mothers' and newborns' Hb status at delivery shows that late GA has important consequences on the baby. The indirect effect of Hb levels at earlier stages of pregnancy suggests that preventive measures such as IPTp and micronutrient supplementations also play a role to lower the consequences of GA and should be reinforced.

347

SPACE-TIME MAPPING OF MALARIA AND ITS CO-DISTRIBUTION WITH STUNTING AMONG CHILDREN BELOW FIVE YEARS IN SOMALIA USING BAYESIAN GEO-STATISTICAL APPROACH

Damaris K. Kinyoki[1], James A. Berkley[1], Grainne M. Moloney[2], Abdisalan M. Noor[1]

[1]Kenya Medical Research Institute - Wellcome Trust Research Programme, Nairobi, Kenya, [2]Kenya United Nations Children's Fund, Nairobi, Kenya

Several studies in developing countries have shown that malaria is associated with greater malnutrition morbidity and mortality and that there is a transient weight loss in young African children following a malaria attack. The majority of studies that have investigated the association of malaria and malnutrition were done in clinical settings and there are limited population-wide investigation of the co-epidemiology of malaria and malnutrition. In 2011, the rate of malnutrition in Somalia was cited to be the highest worldwide. *Plasmodium falciparum* prevalence in Somalia also varies from very low to moderate transmission. This study sought to map the distribution of malaria and stunting among children under the age of five years at similar spatial and temporal resolutions and determine their co-distribution using a large household cross-sectional survey data undertaken bi-annually from 2007 to 2010 in Somalia. We developed a Bayesian hierarchical space-time model through stochastic partial differential equation (SPDE) approach using R-INLA library to produce risk maps of malaria and stunting at 1 x 1 km spatial resolution and predict to each year of study from 2007 to 2010. To determine co-distribution of the two health issues, we developed a shared component model over space and time in a geo-statistical framework. We observed that the prevalence of malaria was high in the Central South zone with a prevalence of 30% followed by the Puntland, 15% and in Somaliland with less than 5%. This same pattern was observed in the rate of stunting where Central South zone had a mean rate of 30% followed by Puntland, 20% and lowest in Somaliland with mean rate of 18%. Results showed that the shared component distribution (representing social economic and environmental determinants) had a larger effect on malaria and malnutrition in the southern and central part of Somalia. Multivariate mapping models provide a better understanding of co-morbidity between health outcomes. In particular, the analyst of multiple disease outcomes can assess the underlying common and divergent spatial distributions of the diseases to optimally integrate disease management required to address the multiple burden of diseases.

348

HOT SPOT OR NOT: A COMPARISON OF SPATIAL STATISTICAL METHODS TO PREDICT PROSPECTIVE MALARIA INFECTIONS

Jacklin F. Mosha[1], Hugh J. Sturrock[2], Brian Greenwood[3], Colin J. Sutherland[3], Nahla B. Gadalla[3], Sharan Atwal[3], Simon Hemelaar[3], Joelle M. Brown[2], Chris Drakeley[3], Gibson Kibiki[4], Teun Bousema[3], Daniel Chandramohan[3], Roland D. Gosling[2]

[1]National Institute for Medical Research Tanzania, Mwanza, United Republic of Tanzania, [2]The Global Health Group, University of California San Francisco, CA, United States, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom, [4]Kilimanjaro Clinical Research Institute and Kilimanjaro Christian Medical College, Kilimanjaro, United Republic of Tanzania

Within affected communities, *Plasmodium falciparum* infections may be skewed in distribution such that single or small clusters of households consistently harbour a disproportionate number of infected individuals throughout the year. Identifying these hotspots of malaria transmission would permit targeting of interventions and a more rapid reduction in malaria burden across the whole community. This study set out to compare different statistical methods of hotspot detection using different indicators for prediction of infection the following year. Two full surveys of four villages in Tanzania were completed over consecutive years, 2010-2011. In both surveys, infection was assessed using nested polymerase chain reaction. In addition in 2010, serologic markers of exposure were assessed. Baseline clustering of infection and serological markers were assessed using three geospatial methods: spatial scan statistics, kernel analysis and weighted local prevalence analysis. Methods were compared in their ability to predict infection in the second year of the study using random effects logistic regression models, and comparisons of the area under the receiver operating curve (AUC) for each model. Sensitivity analysis was conducted to explore the effect of varying radius size for the kernel and weighted local prevalence methods and maximum population size for the spatial scan statistic. Guided by AUC values, the kernel method and spatial scan statistics appeared to be more predictive of infection in the following year. Hotspots of PCR-detected infection and seropositivity to AMA-1 were predictive of subsequent infection. For the kernel method, a 1km window was optimal. Similarly, allowing hotspots to contain up to 50% of the

population was a better predictor of infection in the second year using spatial scan statistics than smaller maximum population sizes. Clusters of AMA-1 seroprevalence or parasite prevalence that are predictive of infection a year later can be identified using geospatial models. Kernel smoothing using a 1km window and spatial scan statistics both provided accurate prediction of future infection.

349

FINE SCALE PARTICIPATORY MAPPING OF MALARIA INFECTION CLUSTERS BY RELATING ROUTINE DIAGNOSTIC RESULTS OF PATIENTS ATTENDING HEALTH FACILITIES TO THE NAMES OF THEIR LOCAL LEADERS IN URBAN DAR ES SALAAM, UNITED REPUBLIC OF TANZANIA

Yeromin P. Mlacha

Ifakara Health Instituite, Dar es Salaam, United Republic of Tanzania

Standard methods for assessing malaria infection burden such as cross-sectional and incidence cohort surveys are limited by sample size in terms of the sensitivity and the spatial resolution they can achieve, especially as transmission declines. This study investigated whether diagnostic data collected routinely at health facilities can provide an affordable alternative for identifying malaria transmission hot spots in urban Dar es Salaam, Tanzania. The study was performed in two adjacent wards of Buguruni and Vingunguti whose total population is 178,000. Anonymised, routinely collected clinical, and test result data for patients tested for malaria were collated from June 2012 to January 2013 from a laboratory registry book at Buguruni Health Centre, located close to the boundary between the two wards. Additionally, patients were asked to provide the name of their local *Ten Cell Unit Leader,* the elected local representative responsible for residential housing clusters. Geographic coordinates of these local leaders' houses were mapped with GPS receivers so that patients' residential locations could be traced and mapped in the absence of further data or a residential address system. Geographic information systems and spatial scan statistics were deployed to detect clustering of malaria cases. Among 2,407 patients diagnosed for malaria, 1,941 (80.6%) provided the name of their respective local leader, 1,121 (57.7%) could successfully be traced to their residential location. Only 240 patients (21.4%) tested positive for malaria. Six geographic clusters of high malaria infections (*hot spots*) and four clusters of low malaria risk (*cold spots*) ($p<0.05$) were identified. Cluster radii for hot spots and cold spots varied from 0 to 276 meters, and 160 to 477 meters respectively. Participatory mapping by recording simple points of reference that community member can readily relate to (e.g. names of local leaders) during routine health facility visits can be used to map hot and cold spots of malaria infections on fine geographic scales as an affordable alternative.

350

MALARIA CASE-FINDING AND TREATMENT STRATEGIES IN AN INTERNALLY DISPLACED PERSONS (IDP) CAMP IN THE DEMOCRATIC REPUBLIC OF CONGO

Claude Masumbuko Kasereka[1], Jean Paul Makelele Katsuva[2], Michael Hawkes[3]

[1]University of Nairobi, Nairobi, Kenya, [2]Medecins d'Afrique, Goma, Democratic Republic of the Congo, [3]University of Alberta, Edmonton, AB, Canada

Social upheaval and chronic human insecurity related to an internecine civil and international war in the Democratic Republic of Congo (DRC) have led to a 70% increase in mortality relative to pre-war levels, and large-scale population displacement. The principal causes of mortality are preventable and treatable infectious diseases such as malaria, and internally displaced persons (IDPs) in temporary shelters may be at elevated risk. Accurate case finding and treatment of malaria is a cornerstone of malaria control efforts; therefore, we explored several possible strategies for active case-finding and treatment in an IDP camp at Mugunga, in Eastern DRC. Beginning with a consecutive sample of 100 febrile patients under 5 years of age from the IDP camp presenting to a nearby clinic for management of a fever episode, we detected 19 cases of uncomplicated malaria diagnosed using HRP2-based rapid diagnostic test, who were then treated with artemisinin combination therapy (ACT). We engaged community health workers in the IDP camp to screen the household members of all 100 children for malaria. The median household size was 5 (range 3 to 9) and there was a median of 1 (range 1 to 2) children under 5 years of age per household, representing a total of 377 screened individuals. We detected 29 cases of malaria through this active case-finding procedure who had not presented for medical treatment, who were then treated with ACT. Fourteen cases were asymptomatic, while 1 participant reported fever, 6 headache, and 8 myalgia. Based on this data, we examined several hypothetical strategies for anti-malarial drug administration in this cohort including: [1] mass drug administration without screening; [2] screen all IDPs with RDT and treat positive cases; [3] screen selectively based on symptoms, then treat positive cases; [4] screen selectively based on household contacts of a child with uncomplicated malaria, then treat positive cases. Under these 4 strategies, the number of missed cases, unnecessary treatments, and cost per case detected and treated were: [1] 0, 348 (92%), $46; [2] 0, 0, $23; [3] 14 (49%), 0, $7; [4] 24 (83%), 0, $25. Although imperfect because nearly half of cases remain undetected, population-level symptom-based screening and treatment appears to be a low-cost strategy to detect and treat cases that might not otherwise present to local health clinics.

351

MALARIA REPORTING THROUGH ELECTRONIC INTEGRATED DISEASES SURVEILLANCE AND RESPONSE (EIDSR) IN KAGERA REGION OF TANZANIA

Michael Oresto[1], Anold Mutafungwa[1], Ritha Willilo[1], Renatha Mandike[2], Elibariki Mwakapeje[3], Ismail Kaloleni[4], Mwenesi Mwendwa[5], Winfred Mwafongo[2], Frank Chacky[2], Mahdi Ramsan[1], Naomi Kaspar[6], Lynn A. Paxton[7], Jeremiah Ngondi[1]

[1]RTI International, Dar es Salaam, United Republic of Tanzania, [2]Ministry of Health, National Malaria Control Program, Dar es salaam, United Republic of Tanzania, [3]Ministry of Health, Epidemiology Section, Dar es salaam, United Republic of Tanzania, [4]University of Dar es salaam, Dar es salaam, United Republic of Tanzania, [5]Ministry of Health, Planning Section, Dar es salaam, United Republic of Tanzania, [6]President's Malaria Initiative/United States Agency for International Development, Research Triangle Park, NC, United States, [7]Malaria Branch and President's Malaria Initiative, Centers for Disease Control and Prevention, Dar es Salaam, United Republic of Tanzania

Malaria surveillance is an important component of malaria control program. Currently, malaria and other disease data are collected through a paper based system that faces challenges of delayed reporting and poor data quality. . We describe the results of weekly reporting of health facility based malaria surveillance data through electronic Integrated Disease Surveillance and Response (IDSR) The Tanzania Ministry of Health and Social Welfare (MoHSW) and the Department of Computer Science - University of Dar es Salaam (UDSM) designed a system of data transfer through the use of mobile phone technology. A five day training of health facilities was undertaken in March 2013 on tallying of data from outpatient registers, recording of data on weekly reporting booklets, and electronic transmission of data. The key malaria indicators collected were: number of people tested for malaria; number tested positive; and number treated presumptively for malaria (clinical malaria cases). Malaria data were transmitted to the District Health Information System (DHIS2) on a weekly basis which enabled establishment of malaria early epidemic detection system (MEEDS), preparedness and response to unusual increase in malaria cases. A total of 289 health facilities in 8 districts of Kagera region participated in the training. In the first 10 weeks of implementation (week 12 to 20 of 2014),87.6% of all expected reports were transmitted on time (by Monday 3pm). Over the same period, a total of 201,431 suspected malaria cases were reported of whom 184,191 (91.4%) were tested for malaria while 17,240 (8.6%) were clinical malaria cases. Malaria

positivity among those tested was 46.4%. Even at the very early stages of the system implementation, electronic disease surveillance has proven advantageous over the former paper based system. This data show an improvement in completeness and timeliness of reporting malaria illnesses, allowing the National Malaria Control Programme (NMCP) to better tailor its programmatic activities and potentially detect and respond to outbreaks in a more timely manner.

352

GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* FROM WESTERN KENYA HIGHLAND AREAS PRONE MALARIA EPIDEMICS

Beth M. Adamowicz

University of Minnesota, Minneapolis, MN, United States

Transmission of *Plasmodium falciparum* malaria in the east African highlands is unstable and frequently epidemic, and recent analyses of globally sampled *P. falciparum* showed high levels of genetic diversity positively associated with variation in transmission intensity. In highland areas of unstable malaria transmission, parasite genotyping can provide valuable information on genotype-specific immunity, drug efficacy in the population, and potential etiologies of increased incidence. *P. falciparum* genotyping was conducted on filter paper blood samples from individuals with clinical malaria in two adjoining highland areas in western Kenya. Genotyping was performed at the antigen locus merozoite surface protein-2 (MSP-2), and on a panel of 12 microsatellite loci. At both sites, more than 70% of individuals had infection with >1 parasite genotype. Multiplicity of infection (MOI) was detected at similar frequencies by MSP-2 and microsatellite genotyping. However, microsatellite genotyping detected significantly greater parasite diversity than MSP-2 genotyping, indicating that microsatellite genotypes are more useful for interrogating parasite population diversity and structure. Neither MOI nor genetic diversity differed by age. All individuals with repeated symptomatic infections had infection with a new genotype rather than recrudescence, implying that treatments given to these individuals in an earlier episode of infection were effective and/or there was active immunity against earlier infections. There was also no difference in these metrics between the two areas, indicating a common *P. falciparum* life history and potentially the effectiveness of similar control measures.

353

MOLECULAR MARKER TRENDS IN *PLASMODIUM FALCIPARUM* DIHYDROFOLATE REDUCTASE AND *PLASMODIUM FALCIPARUM* DIHYDROPTEROATE SYNTHASE GENES IN KENYAN ISOLATES BETWEEN THE YEARS 2008 TO 2012

Dennis W. Juma

United States Army Medical Research Unit-Kenya, Kisumu, Kenya

Sulfadoxine-pyrimethamine (SP), an antifolate was replaced by artemether-lumefantrine as the first-line malaria drug treatment in Kenya in 2004 due of the wide spread of resistance. However, SP still remains the recommended drug for intermittent preventive treatment in pregnant women and infants (IPTP/I) owing to its safety profile. This study assessed the prevalence of mutations in dihydrofolate reductase (Pfdhfr) and dihydropteroate synthase (Pfdhps) genes associated with SP resistance in samples collected in Kenya between 2008 and 2012. Field isolates collected from Kisumu, Kisii, Kericho and Malindi district hospitals were assessed for genetic polymorphism at various loci within the two genes by sequencing. Among the Pfdhfr mutations, codons N51I, C59R, S108N showed highest prevalence in all the sites combined at 95.5%, 84.1% and 98.6% respectively. Pfdhfr S108N prevalence was highest in Kisii at 100%. A temporal trend analysis showed steady prevalence of mutations over time except for codon Pfdhps 581 which showed an increase in mixed genotypes. Triple Pfdhfr N51I/C59R/S108N and double Pfdhps A437G/K540E had high prevalence rates of 86.56% and 87.86% respectively. The Pfdhfr/Pfdhps quintuple, N51I/C59R/S108N/A437G/K540E mutant has been shown to be the most clinically relevant marker for SP resistance was observed in 75.71% of the samples.

354

ASSOCIATION BETWEEN LENGTH OF NIGHT TIME EXPOSURE AND *PLASMODIUM FALCIPARUM* MALARIA INFECTION RISK IN DAR ES SALAAM, TANZANIA

Daniel F. Msellemu

Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania

Little is known about the role of time as a risk factor in areas that have various forms of malaria interventions. Socioeconomic demands, human behaviours and extended periods of outdoor activities are major contributing factors for malaria transmission in areas where other interventions like long lasting insecticidal nets (LLINs) are widely used. We investigated the associations between sleeping time and *Plasmodium falciparum* parasite prevalence (PfP) among residents in Dar es Salaam, Tanzania. From March 2009 to May 2012 a cross-sectional study was conducted among individuals aged 3-months to 98 years. A total of 4000 people were tested for malaria paracitemia by using rapid diagnostic test (RDT). Individual level information on length of time a person was potentially exposed to mosquitoes after 6:00pm (i.e. time before going inside and under a bed net), demography, house quality and bed-net use were also collected. Hierarchical model was used to assess malaria infection and identify time, social economic status (SES) and net use as risk factors. A total of 411 people (10%) surveyed tested positive for malaria parasitemia. People with outdoor exposure of 5 hours had the highest parasitemia prevalence of (PfP=14.4%) followed by children of 0-5 years (PfP=13%). People with the longest periods of time spent indoors (>5 hours) had the lowest prevalence (PfP=6%). At least 76% of people surveyed slept under bed-net. Multivariate analysis showed a strong evidence that people spending 5 hours outdoors had higher infection prevalence than people spending less time outdoors (OR [95%CI]=2.32 [1.62-3.33] P<0.001). Interestingly, this study also determined that people who owned bed-nets were more likely to have malaria, compared to those without (OR=2.60 [1.76 - 3.84], P=0.04). In conclusion, the findings indicate an increased risk of malaria transmission for the part of population that spends most of the evening time outdoors in Dar Es Salaam because of either behaviours or socioeconomic activities. It is therefore important to plan interventions that can offer protection to this part of the population.

355

INTERNALLY DISPLACED PERSONS (IDPS) ARE AT INCREASED RISK OF MALARIA RELATIVE TO NEIGHBORING VILLAGE CONTROLS

Jean Paul Makelele Katsuva[1], Claude Masumbuko Kasereka[2], Tsongo Kibendelwa[3], Michael Hawkes[4]

[1]Medecins d'Afrique, Goma, Democratic Republic of the Congo, [2]University of Nairobi, Nairobi, Kenya, [3]Universite de Goma, Goma, Democratic Republic of the Congo, [4]University of Alberta, Edmonton, AB, Canada

Globally, 26 million internally displaced persons (IDPs) have been forced to flee their homes as a result of armed conflict (yet remain within their country of nationality). Civil and international war in the Democratic Republic of Congo (DRC) has generated tens of thousands of IDPs residing within temporary housing camps, at high risk of poor mental and physical health. Malaria is the chief cause of death among children in the area, but the burden of malaria has not been well described among children within IDP camps in the DRC. We conducted 2 surveys in Walikale district in Eastern DRC to compare the point-prevalence of *P. falciparum* antigenemia among (1) children residing in an IDP camp versus children from the surrounding village, and (2) febrile children presenting for medical care at a health clinic serving both the IDP camp and surrounding village. For the first community-based survey, a random sample of 200 temporary shelters

within the Bilobilo IDP camp was visited and a child under 5 from each household was tested for malaria using HRP2-based rapid diagnostic test. As a control group, we partnered with a public vaccination drive in the surrounding village of Mubi to survey 200 households. Participants from the IDP camp reported that they had fled their homes a median of 13 (10-17) months earlier, and had suffered varying degrees of community violence including theft (50%), physical assault (9%), rape (6%), gunshot and knife injury (1 participant each). Children tested in the IDP camp and surrounding village (controls), were of a median (range) age of 2.6 (0.1-5) years (IDPs) vs 2.4 (0.7-5) years (controls). Household bednet ownership was 34% (IDPs) vs 68% (controls) [p<0.001]. Bednet use by the index child the previous night was 56% (IDP) vs 25% (controls) [p<0.001]. The point-prevalence of malaria was 19% (IDPs) vs 9.5% (controls) [relative risk 2.3 (95%CI 1.3-4.1), p=0.0095). We next examined malaria prevalence among children with acute febrile illness. We tested 100 children presenting to the clinic from Bilobilo IDP camp and 100 from Mubi village. Malaria RDT was positive in 78% (IDPs) vs 39% (controls) [relative risk 2.0 (95%CI 1.5-2.6), p<0.001]. Taken together, these data suggest that displacement is a risk factor for malaria infection, both community carriage and acute febrile illness. IDPs represent a high risk group for malaria and targeted control measures may reduce the burden of malaria in this vulnerable group.

356

TEMPORAL ASSOCIATION BETWEEN FISHING ACTIVITIES AND MOSQUITO ACTIVITY PATTERNS IN RUSINGA ISLAND, WESTERN KENYA

Everline A. Olanga[1], Lucy Irungu[2], Wolfgang R. Mukabana[1]

[1]International Centre of Insect Physiology and Ecology, Nairobi, Kenya, [2]University of Nairobi, Nairobi, Kenya

Fishing is a major source of livelihood for residents in Rusinga Island on the shores of Lake Victoria, western Kenya. This activity is conducted outdoors during the day but predominantly at night. Malaria is endemic in western Kenya and areas around the lake shore are associated with mosquito breeding habitats. The main objective of this study was to determine the association between fishing activities and mosquito activity patterns in a fishing beach in western Kenya. A longitudinal entomological survey on a fishing beach was conducted for a period of 5 months. Kolunga fishing beach was selected for this study as fishermen registered at this beach conduct fishing activities at night. Mosquitoes were captured using battery-powered MMX traps baited with a potent mosquito attractant. The traps were positioned approximately 10 metres inland from the shoreline. Hourly mosquito collections were conducted from 6pm till 7am the next day. The number of people at the beach who conducted fishing activities was recorded at hourly intervals. A total of 1279 mosquitoes were collected, of which 93 were malaria vectors. Of the 93 malaria vectors collected, 28 (33.7%) were *Anopheles gambiae s.l.* while 65 (66.3%) were *An. funestus*. The peak mosquito biting activity for *An. gambiae s.l.* was 9 - 10pm and 3 - 4am while *An. funestus* was recorded as 9 - 10pm and 4 - 5am. The peaks in mosquito biting times coincided with fishermen's activities at the fishing beach. In conclusion, individuals conducting fishing-related activities at the beach during the peak biting times are at high risk of receiving infectious mosquito bites.

357

MALARIA PREVENTION IN VULNERABLE GROUPS NIGERIA, 2011-2012

Hannatu J. Dimas[1], Olufemi Ajomobi[2], Kabir Sabitu[3], Patrick Nguku[1]

[1]Nigeria Field Epidemiology and Laboratory Training Programme, Abuja, Nigeria, [2]National Malaria Control Programme, Abuja, Nigeria, [3]Ahmadu Bello University, Zaria, Nigeria

Malaria prevention is vital to its control. At least 80% of children less than 5 years (U5) and pregnant women (PW) sleep under long lasting insecticidal nets (LLIN) while all PW attending ante-natal clinics should receive at least two doses of Sulphadoxine-pyrimethamine for Intermittent Preventive Treatment (IPT). Even though there is gradual attempt to scale-up access to these interventions, little information is available about their currents. We analysed access to LLINs and IPT in Nigeria using secondary data.We collected national summary data on malaria preventive services from January 2011 to December 2012. Data on uptake of LLINs in U5 and PW, and uptake of IPT in PW from all reporting health facilities (HF) were extracted. Analysis was done by state using Microsoft excel. In 2011, of the 2,741,485 PW who attended ANC in 96,418 HFs, 622, 317 (22.7%) and 649, 732 (23.7%) received LLIN and IPT respectively. In 2012, of the 3, 392, 363 PW who attended ANC in the 114, 667 HFs, (9%) and (16.6%) received LLIN and IPT respectively. In 2011 and 2012 199289(3.2%) of the 6,220,672 and 301896(3.2%) of the 9,521,932 U5 seen received LLINs respectively. Despite increase in ANC attendance and reporting HF, access to LLIN and IPT remains low. In Nigeria, universal access to malaria preventive commodities and services has not yet been achieved in high risk groups. High antenatal clinic (ANC) attendance alone is not sufficient to ensure high IPT and LLIN coverage as the Roll Back Malaria target of 80% was not achieved.SP for IPT should always be made available at all HF's and health workers should be trained on the need to increase IPT coverage.

358

MALARIA PREVALENCE, SPATIAL CLUSTERING AND RISK FACTORS IN A LOW ENDEMIC AREA OF EASTERN RWANDA: A CROSS SECTIONAL STUDY

Stephen Rulisa[1], Fredrick Kateera[2], Jean Pierre Bizimana[3], Steven Agaba[4], Janvier Dukuzumuremyi[4], Lisette Baas Baas[4], Jean de Dieu Harelimana[4], Petra F. Mens[5], Kimberly R. Boer[5], Peter J. De Vries[5]

[1]University of Rwanda, Kigali University Teaching Hospital, Kigali, Rwanda, [2]Rwanda Biomedicalcentre, Kigali, Rwanda, [3]Geography Department, Faculty of Science, National University of Rwanda, Butare, Kigali, Rwanda, [4]Amsterdam Institute for Global Health and Development, INTERACT Project, Kigali, Rwanda, [5]Academic Medical Center, Division of Infectious Diseases, Tropical Medicine and AIDS, Amsterdam, Netherlands

Rwanda reported significant reductions in malaria burden following scale up of control intervention from 2005 to 2010. This study sought to; measure malaria prevalence, describe spatial malaria clustering and investigate for malaria risk factors among health-centre-presumed malaria cases and their household members in Eastern Rwanda. A two-stage health centre and household-based survey was conducted in Ruhuha sector, Eastern Rwanda from April to October 2011. At the health centre, data, including malaria diagnosis and individual level malaria risk factors, was collected. At households of these Index cases, a follow-up survey, including malaria screening for all household members and collecting household level malaria risk factor data, was conducted. Malaria prevalence among health centre attendees was 22.8%. At the household level, 90 households (out of 520) had at least one malaria-infected member and the overall malaria prevalence for the 2634 household members screened was 5.1%. Among health centre attendees, the age group 5–15 years was significantly associated with an increased malaria risk and a reported ownership of ≥4 bednets was significantly associated with a reduced malaria risk. At the household level, age groups 5–15 and >15 years and being associated with a malaria positive index case were associated with an increased malaria risk, while an observed ownership of ≥4 bednets was associated with a malaria risk-protective effect. Significant spatial malaria clustering among household cases with clusters located close to water- based agro-ecosystems was observed. In conclusion, malaria prevalence was significantly higher among health centre attendees and their household members in an area with significant household spatial malaria clustering. Circle surveillance involving passive case finding at health centers and proactive case detection in households can be a powerful tool for identifying household level malaria burden, risk factors and clustering.

359

CASE-CONTROL STUDY OF EFFECTIVENESS OF INSECTICIDE-TREATED BEDNETS IN CENTRAL SENEGAL

Fassiatou Tairou[1], Badara Cisse[1], C. Flach[2], A. Diallo[1], M. Ndiaye[1], Ousmane Sy[1], El-h. Ba[3], O. Gaye[1], Paul Milligan[2]

[1]Université Cheikh Anta Diop de Dakar, Dakar, Senegal, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Institut de Recherche pour le Développement, Dakar, Senegal

The effectiveness of bednet (LLIN) programs in Senegal is threatened by resistance to insecticides. The aim of this study was to determine the effectiveness of LLIN use by the individual and in the local community in reducing the incidence of malaria, in central Senegal. A matched case control study was conducted in rural parts of the district of Fatick, central Senegal, in an area covered by a demographic surveillance system (DSS). All confirmed cases of uncomplicated malaria presenting at health posts in the district between October 2011 and February 2012 were included in the study. For each case, one or two controls of the same age group, selected by random sampling from the DSS listing for the district, were recruited concurrently if a malaria rapid diagnostic test was negative. For cases and controls, and members of their households and of surrounding households, bednet use and approximate age was recorded after inspecting sleeping places. Information about bednet use was also obtained for all households in the district when visited in demographic surveillance rounds from January to December 2011. Conditional logistic regression was used to estimate the rate ratio associated with use of LLIN, and with the level of local coverage of LLINs in the community grouped by quintiles, adjusted for potential confounding factors including distance to health facility, socio-economic status, and distance to mosquito breeding sites. 540 malaria case-control pairs were recruited. Sleeping under an LLIN, and local coverage of LLINs, were independently associated with reduced malaria incidence. Malaria incidence for individuals in communities with coverage in the upper quintile (>81% of persons sleep under an LLIN) was 64% (95%CI 18%, 84%) lower than in communities in the lowest quintile (<50% LLIN coverage). In conclusion, LLINs remain effective in central Senegal but high coverage is required to maximize impact. LLIN distribution programs should target communities with low coverage.

360

DEFINING THE RELATIONSHIP BETWEEN *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX* PARASITE RATE AND CLINICAL DISEASE

Katherine E. Battle[1], Markku S. Karhunen[1], Samir Bhatt[1], Carlos A. Guerra[1], Nick Golding[1], Kirsten A. Duda[1], Rosalind E. Howes[1], Iqbal R. Elyazar[2], J. Kevin Baird[2], Peter W. Gething[1], Simon I. Hay[1]

[1]Spatial Ecology and Epidemiology Group, University of Oxford, Oxford, United Kingdom, [2]Eijkman-Oxford Clinical Research Unit, Jakarta, Indonesia

Quantifying of the number of malaria cases that occur annually in endemic countries is essential to national control program development and evaluation. Clinical incidence of malaria has proven to be difficult to enumerate. Strategies that adjust routine case reports or model incidence values using prevalence surveys have been applied to estimates of malaria as a whole and *Plasmodium falciparum*-specific incidence, but have yet to be developed for *Plasmodium vivax*. Infection prevalence, or parasite rate, is one of the most widely available metrics of malaria endemicity and is collected using simple methodologies. To help inform the number of clinical infections of *P. falciparum* and *P. vivax* per year, it is therefore useful to quantify the relationship between prevalence and clinical incidence for each parasite. Here we present updated methods to estimate *P. falciparum* incidence and a new equivalent model for *P. vivax*, developed to accommodate the unique epidemiological features of the parasite. A systematic search for active case detection surveys was performed. Recorded incidence values were then matched to measures parasite rates. Two separate hierarchical Bayesian models, using a flexible Gaussian process prior, were fitted to the matched falciparum and vivax data. Temporal variation was modelled by using a Poisson-Gamma mixture. The fitted relationships, along with appropriate uncertainty metrics, allows for estimates of clinical incidence of known confidence to be made from wherever *P. falciparum* or *P. vivax* prevalence data are available. This will allow for updated estimates of *P. falciparum* burden as well as novel measures of the global burden of *P. vivax*.

361

AN IMPROVED MATHEMATICAL MODEL OF MALARIA INCIDENCE IN COLOMBIA

Sebastian Bernal Garcia, Luisa Díez, Santiago Arango, Juan Suaza, Sandra Uribe, Liliana Jaramillo, Germán Poveda

Universidad Nacional de Colombia, Medellín, Colombia

Malaria is an endemic disease in the lowlands of the tropical Americas. In Colombia, it constitutes a serious public health problem with 105,000 cases per year during 2005-2011, but showing: (1) an increasing trend during the last 50 years, and epidemic outbreaks during the occurrence of the warm phase of El Niño/Southern Oscillation (El Niño) over the tropical Pacific. Here we develop a mathematical model to represent the temporal dynamics of malaria incidence in endemic regions of the Pacific coast of Colombia, involving the complex interactions between environmental and climatic factors, the diverse developmental stages and the feeding habits of the vector (*Anopheles* albimanus), and their interaction with humans and with the parasite (*Plasmodium falciparum*). Our model overcomes diverse limitations and shortcomings of the model introduced by Ruiz et al. (2006), and provide a much-improved tool towards the development of an early warning system for malaria prevention and control in Colombia.

362

MALARIOMETRIC SURVEY OF IBESHE COMMUNITY IN IKORODU, LAGOS STATE, NIGERIA: DRY SEASON

Oluwagbemiga O. Aina, Chimere O. Agomo, Yetunde A. Olukosi, Bamidele A. Iwalokun, Hilary I. Okoh

Nigerian Institute of Medical Research, Lagos, Nigeria

Malariometric surveys generate data on malaria epidemiology and dynamics of transmission necessary for planning and monitoring of control activities. This study determined the prevalence of malaria and the knowledge, attitude and practice (KAP) towards malaria infection in Ibeshe, a coastal community. The study took place during the dry season in 10 villages of Ibeshe. All the participants were screened for malaria. A semi-structured questionnaire was used to capture socio-demographic data and KAP towards malaria. A total of 1489 participants with a mean age of 26.7 ±20.0 years took part in the study. Malaria prevalence was 14.7% (95% CI 13.0-16.6%) with geometric mean density of 285 parasites/µl. Over 97 % of participants were asymptomatic. Only 40(2.7%) of the participants were febrile while 227(18.1%) were anemic. Almost all the participants (95.8%) identified mosquito bite as a cause of malaria, although multiple agents were associated with the cause of malaria. The commonest symptoms associated with malaria were hot body (89.9%) and headache (84.9%). Window nets (77.0%) were preferred to LLIN (29.6%). Malaria is mesoendemic in Ibeshe during the dry season. The participants had good knowledge of symptoms of malaria, however there were a lot of misconceptions on the cause of malaria.

363

THE IMPACT OF URBANIZATION CLASSIFICATION AND POPULATION DENSITY ON CHILDHOOD *PLASMODIUM FALCIPARUM* PARASITE PREVALENCE RATES IN AFRICA

Caroline W. Kabaria[1], Abdisalan M. Noor[1], Robert W. Snow[1], Catherine Linard[2]

[1]KEMRI Wellcome Trust Research Programme, Nairobi, Kenya, [2]Biological Control and Spatial Ecology, Université Libre de Bruxelles, Brussels, Belgium

Residents of urban areas have been shown to have lower rates of malaria infection compared to rural neighbors. There are, however, many challenges in defining urbanization. The transition from a rural settlement to one best described as urban does not follow a definite boundary. Defining the gradient between rural and urban settlements is an important factor in defining malaria prevalence patterns. In this study, we use boosted regression tree models to determine using whether (i) urbanization had a significant effect on malaria transmission and if this effect varied with urbanization definition used (ii) if population density had significant effect on malaria parasite prevalence and if so, could population density replace urban classifications in modeling malaria transmission patterns. The study focuses on 21 household surveys from the Demographic & Health Surveys (DHS) and Malaria Indicator Survey (MIS) conducted between 2006 and 2013 in 14 malaria endemic countries across Africa. Although analysis is expected to be complete by June 2014, preliminary results show that the BRT model with population density was found to perform significantly better (p-values <0.001) in predicting malaria risk when we compared the model's predictive performance to the other models with urbanization classification. However, adding urbanization to the BRT model with population density produced a better performing model implying there are aspects of the influence of urbanization on malaria prevalence that cannot be sorely explained by population density or population distribution patterns.

364

THE EFFECTS OF MALARIA IN THE FIRST TRIMESTER MEASURED BY REPEATED FETAL CROWN RUMP LENGTH: AN OBSERVATIONAL STUDY

Aung M. Min[1], Rose McGready[2], Francois Nosten[3], Marcus J. Rijken[1], Aris T. Papageorghiou[4], Stephen H. Kennedy[4], Suporn Kiricharoen[1], Supan Thiptharakun[1]

[1]Shoklo Malaria Research Unit, Mae Sod, Thailand, [2]Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand, Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom, Mae Sod, Thailand, [3]Shoklo Malaria Research Unit, Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom, Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand, Mae Sod, Thailand, [4]Nuffield Department of Obstetrics and Gynaecology and Oxford Maternal and Perinatal Health Institute – Green Templeton College, University of Oxford, Oxford, United Kingdom

Malaria in pregnancy causes low birth weight although the mechanism of this adverse effect is not characterized. At Shoklo Malaria Research Unit on the Thai-Myanmar border we have observed reduced fetal head diameter when women had malaria from < 14 to < 24 weeks of pregnancy. This investigation aims to determine if there is a reduction in growth with malaria infection in the first trimester (7+5 to 14 weeks) by using ultrasound confirmed fetal crown rump length measurement. Shoklo Malaria Research Unit on the Thai-Myanmar border has offered fetal biometry to all pregnant women since 2001 at their first antenatal visit to determine viability and gestation and the scan is repeated if necessary for ongoing care. The training manual and protocol for trans-abdominal CRL were from the British Medical Ultrasound society recommendations. Observed versus the expected increase in CRL measurements will be compared for women with malaria, women with fever from other causes and for women without malaria. Data collection is still in process but repeated CRL measurements are currently available for 1300 women of whom more than 100 have had malaria in the window of two CRL measurements. The final results will be available in May 2014

365

UTILIZATION OF MALARIA PREVENTIVE SERVICES IN PREGNANT WOMEN ATTENDING PUBLIC HEALTH FACILITIES IN OYO STATE, SOUTHWEST NIGERIA, 2013

Olukemi T. Olugbade[1], Maureen O. Anyanwu[2], Sholakunmi Olusanya[3], Taiwo O. Ladipo[2], Olufemi Ajumobi[4], Adebola Olayinka[1], Ikeoluwa Ajayi[5]

[1]Nigeria Field Epidemiology Laboratory Training Programme, Abuja, FCT, Nigeria, [2]Malaria Control Programme, Ministry of Health, Ibadan, Oyo State, Nigeria, [3]Roll Back Malaria Programme Association for Reproductive and Family Health, Ministry of Health, Ibadan, Oyo State, Nigeria, [4]National Malaria Elimination Programme, Abuja, FCT, Nigeria, [5]Nigeria Field Epidemiology Laboratory Training Programme/Faculty of Public Health, Department of Public Health, University of Ibadan, Abuja, FCT/ Ibadan, Oyo State, Nigeria

Malaria is a major cause of maternal and newborn mortality globally. In areas with high malaria endemicity an estimated 50 million women become pregnant annually; and half of these women reside in Africa. Malaria in Pregnancy (MIP) is an obstetric, social, economic and medical emergency. It has been documented to be responsible for 11% of maternal deaths, 2-5% of maternal anemia, 8-15% of low birth weight infants, and 3-8% of infant deaths, in developing countries. In 2004 use of Sulphadoxine- Pyrimethamine (SP) for Intermittent Preventive treatment (IPTp), was adopted in Nigeria within the focused antenatal care package, in addition to health education, personal protection by sleeping inside Long Lasting Insecticidal Nets (LLINs), and effective case management. We determined the utilization of these preventive services and their uptake in pregnant women attending public health facilities in Oyo state. We reviewed malaria specific summary data abstracted from the Oyo state National Health Management Information Systems health facility registers of 462 facilities from January to December 2013. Descriptive analysis of MIP, and uptake of preventive strategies was done, using Microsoft excel 2007. Results: Of the 929,843 outpatient clinic attendees, 39218 (4%) were Pregnant women (PW) with fever. Of PW with fever, MIP accounted for 22,052(56%) of fever cases; 5887(27%) were clinically diagnosed and 16,165(73%) had laboratory confirmation. Of the 322,514 PW who attended ANC, 70,216(22%) and 5105(2%) received at least 2 doses of SP for IPTp and LLINs respectively. In conclusion, despite good ANC coverage, there was low utilization of laboratory diagnosis, low uptake of SP for IPTp, and poor ownership of LLINs, all less than the State's 80% target for each indicator. We recommend consistent health education to PW for prevention of MIP and adherence to guidelines for effective management of MIP by health workers, with the aim of reducing malaria burden in PW, thereby improving pregnancy outcomes and making pregnancy safer.

366

AN ELITE INFECTION-CONTROL PHENOTYPE WITH IMMUNOLOGICAL CORRELATES IN A TANZANIAN BIRTH-COHORT EXPOSED TO INTENSE MALARIA TRANSMISSION

Scott D. Nash[1], Rebecca Prevots[1], Edward Kabyemela[2], Kun-Lin Lee[1], Michal Fried[1], Patrick E. Duffy[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Seattle Biomedical Research Institute, Seattle, WA, United States

Malaria incidence is highly heterogeneous even in hyperendemic areas, although no one has shown that innate or naturally acquired resistance can completely prevent infection. We examined immunoparasitological evidence for an elite infection-control phenotype from a birth-cohort followed 2002-2006 in Muheza, Tanzania, an area of intense transmission. Children (n=688) provided blood smears every 2 weeks during infancy and

monthly thereafter. We documented maternal and childhood demographic and clinical characteristics, cord-blood cytokine levels, and antibody responses to preerythrocytic (LISP1, SLARP, among others) and common blood stage (MSP1, AMA1) malarial antigens every 6 months. Antibody seroprevalance comparisons were assessed through generalized estimating equations, and associations of cord-blood cytokines with elite infection-controller status were estimated through logistic regression. Sixty (8.7%) children had no blood-smear positive slides over an average of two years (range: 1 to 3.5 years, ~121 person-years) and were identified as elite infection-controllers. Elite controllers were similar to non-controllers with respect to completeness of follow-up and most maternal and childhood behavioral and biomedical risk factors. Antibody seroprevalence was similar between elite and non-controllers for five of six preerythrocytic antigens, and increased with age in both groups. Elite controllers had a lower seroprevalence to MSP1 (5.6% vs. 29.3%; P<0.0001) and AMA1 (25.9% vs. 60.3%; P<0.0001) compared with non-controllers. In addition, elite controllers were over twice as likely as non-controllers to have cord levels of IL1-B(odds ratio(OR): 2.5 (1.1,5.8)), TNF-α (OR: 2.6 (1.2, 5.8)), or TNF-R1(OR: 2.2 (1.0, 4.8)) in the top third of the distribution. These data suggest that a subset of naturally exposed children is able to control malaria infection before patent parasitemia, and this control is associated with a unique immunologic profile at birth. Research on these children may identify mechanisms for highly effective naturally acquired immunity to malaria.

367

MAPPING OF 'HOT SPOTS' AND SIGNIFICANCE OF REACTIVE CASE DETECTION IN DETERMINING THE MALARIA BURDEN IN AN URBAN SETTING

Alex Eapen

National Institute of Malaria Research, Chennai, India

Malaria is a serious public health problem causing high morbidity and mortality in India. Chennai, a metropolitan city is endemic for malaria and the transmission is perennial with a peak between July and October. *Plasmodium vivax* is the predominant parasite species accounting for 93.6 to 99.8% of the prevalence in the city. The focus of national programme is to identify and treat the individuals presenting to the health care facility. In fact, human malaria parasite reservoir consists of both symptomatic and asymptomatic infections among people in a given area. It is well known that addressing the individuals who do not seek treatment and capturing asymptomatic subjects by active surveillance is essential to move forward for malaria elimination. As a part of the study of complex malaria in an urban setting, the objective was to estimate the burden of malaria (both symptomatic and asymptomatic) adopting reactive case detection strategy and finding spatial clustering of infections. Reactive case detection activity is initiated immediately in response to positive case reported at malaria clinic involving screening of all the household members of the index case. Individuals living in close proximity (<50m radius) to passively detected case and additionally households within 100m radius are surveyed for presence of fever, also randomly selected control households without any history of fever in previous 2 weeks are screened for presence of malaria parasites by microscopy, RDT and PCR. Coordinates of all the index case and enrolled households are captured by global positioning system (GPS) to provide information on spatial distribution of infections. Preliminary data from the ongoing study indicates reactive surveillance would help to identify malaria infection which otherwise would be missed by routine passive case detection. This strategy will help to identify the malaria reservoir which includes the asymptomatic individuals harbouring malarial parasites in the community and serve as vital information on the actual magnitude of the problem for the programme to tackle and undertake appropriate intervention measures to interrupt transmission.

368

ACTIVE CASE DETECTION IN MALARIA ELIMINATION SETTINGS: TIMELINESS AND COMPLETENESS OF MALARIA CASE NOTIFICATION IN ZANZIBAR

Jeremiah M. Ngondi[1], Abdul-wahid Al-mafazy[2], Issa Garimo[1], Michael McKay[3], Naomi Kaspar[4], Mahdi Ramsan[1], Abdulla S. Ali[1]

[1]RTI International, Dar Es Salaam, United Republic of Tanzania, [2]Zanzibar Malaria Elimination Programme, Zanzibar, United Republic of Tanzania, [3]RTI International, Washington, DC, United States, [4]United States Agency for International Development, Dar Es Salaam, United Republic of Tanzania

Zanzibar Malaria Elimination Programme (ZAMEP) introduced malaria early epidemic detection system (MEEDS) in 2008 and malaria case notification (MCN), an active malaria surveillance system, in August 2012. MCN is an active surveillance system whereby individual malaria cases are transmitted in real time through the use of mobile phones and android tablets to a central database. We evaluated the timeliness of case notification after diagnosis and timeliness of case follow-up after notification. For MCN reporting, a massage for each case is sent from Health Facility to the server and the server generates an alert to the District Malaria Surveillance Officer's (DMSO) mobile phone and tablet. DMSO follows up notified cases to their households. All case-household members are tested for malaria using rapid diagnostic tests (mRDT). People with positive malaria test results are treated with artemisinine-based combination therapy (ACT). A total of 2,603 index malaria cases were notified in 2013 of which 75.8% were followed-up. Of the 1,974 cases followed-up notification after diagnosis was done within 24 hours in 50.6%, between 24 to72 hours in 25.8%, while 23.8% of cases were notified after 72 hours. The median time from diagnosis to case notification was 1.2 days (interquartile range [IQR]=0.5 to 2.6). Household follow-up was undertaken within 48 hours in 54.2%, between 48 hours and 1 week in in 21.5%, while 23.4% of cases were followed after more than 1 week. The median time to household follow-up was 3 days (IQR=1 to 8). A total of 1,812 (70% of those notified) had the household follow-up completed. Active case detection and treatment alongside other preventative interventions is likely to reduce malaria transmission and enhance malaria elimination efforts in Zanzibar. Following successful implementation of malaria case notification, ZAMEP has set performance standards for malaria case notification and case follow-up with a target of 90% of cases to be notified with 24 hours of diagnosis, 100% follow-up of all notified cases, and 90% of cases to be followed up within 48 hours of notification. Therefore more resources will be required to enable Zanzibar achieve these standards in order to make MCN more effective.

369

COMPARISON OF PFHRP2-BASED RDTS AND PCR IN AN AREA OF DECLINING MALARIA TRANSMISSION IN SOUTHERN ZAMBIA

Natasha M. Laban[1], Tamaki Kobayashi[2], David Sullivan[2], Clive J. Shiff[2], William J. Moss[2]

[1]Macha Research Trust, Choma, Zambia, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Histidine-rich protein-2 (HRP-2)-based malaria rapid diagnostic tests (RDTs) are valuable in detecting *Plasmodium falciparum* infection; however, various factors can affect test validity. The test misses low level parasitaemia and will not detect non-*P. falciparum* species. More sensitive and species-specific tools such as PCR will become necessary to identify parasite reservoirs in elimination settings. To address this, HRP2-based RDTs, pan *Plasmodium* species nested PCR and species-specific q-PCR were compared over a five-year period in a region of declining malaria transmission in southern Zambia. Cross-sectional surveys were conducted in Choma District, Zambia from 2008 to 2012. A total of 3,292 individuals were enrolled and tested with RDT and samples collected for microscopy and dried blood spots (DBS) with latter used for pan *Plasmodium* nested PCR. Species-specific q-PCR was performed on samples that tested positive

either by microscopy, RDT or nested PCR. There were 12 individuals positive by microscopy (0.3%), 42 positive by RDT (1.3%) and 57 positive by nested PCR (1.7%). Thirty percent of 57 samples positive by nested PCR were also RDT positive. Eighty seven samples positive by either microscopy, RDT or nested PCR were also tested for the presence of Pfhrp gene. Of the 87, sixty one were positive by q-PCR for *P. falciparum* (53% of which were also RDT positive) and 7 were positive by q-PCR for P. malariae. Of the 7 positive for P. malariae, five represented co-infection with *P. falciparum*, two of which were also RDT positive. Mean copy number of Pfhrp for RDT-positive and negative samples was 1152 and 160, respectively and the difference was not statistically significant [p=0.126] indicating that the RDT-negative, q-PCR-positive samples results were likely due to low parasite density and not Pfhrp gene deletions. Findings illustrate that individuals with low-level parasitaemia and non-falciparum malaria will not be identified by HRP-2 RDTs and the importance of more sensitive and specific diagnostics to achieve malaria elimination.

370

MALARIA VECTOR ABUNDANCE AND MALARIA PREVALENCE AFTER IMPLEMENTATION OF INSECTICIDE TREATED NETS IN A RURAL MALARIA ENDEMIC DISTRICT IN WESTERN KENYA

Andrew A. Obala[1], Judith Mangeni[1], Kristin Corey[2], Lucy Abel[1], Daniel Aswa[1], Wendy O'Meara[2]

[1]Moi University, Eldoret, Kenya, [2]Duke University, Durham, NC, United States

Data on morbidity due to malaria in Webuye Health and Demographic Surveillance System (HDSS) area, Bungoma East district suggests control efforts have not had expected impact in spite of insecticide treated nets (ITN) ownership exceeding 70% and availability of Affordable Medicine Facility-malaria(AMFm).The current study was designed to determine malaria transmission and malaria prevalence after implementation of ITNs and AMFm. Malaria vectors were captured in sentinel villages and households in Webuye HDSS using pyrethrum spray catch (PSC), CDC light traps, exit traps (ET), and light traps with rotating cups. Captured mosquitoes were primarily identified, gonotrophic stages of each malaria vector recorded and the mosquitoes preserved for further analyses. Parasitological surveys are done quarterly using malaria rapid diagnostics test (RDT) kits on all household members in the same households where mosquitoes are captured by PSC and ET and their immediate neighbors. Our preliminary data show a three-fold majority (70.9%; 567/799) of unfed malaria vectors were captured resting or leaving huts compared to other gonotrophic stages (23.5%; 188/799). There were more gravid malaria vector components (57.1%; 8/14) caught in exit traps than other gonotrophic stages. PCR analysis on a small proportion (no=81) of *An gambiae* group showed *An. gambiae* ss comprised 64% while *An arabiensis* consisted of 20.3%, but 15.6% were unidentified. The sporozoite rate was low (1.4%; 1/69). 370 participants were tested for malaria; 21.6% were positive with the highest prevalence of 41% reported among children 5-10 years. From these preliminary data, we show that high number of gravid malaria vectors were captured in exit traps and the large number of the vectors caught resting indoors by other techniques confirm endophily among these vectors in spite of increased ITN coverage. However, malaria prevalence declined compared to high prevalence previously reported. Data on molecular analysis of *An gambiae* group and malaria transmission efficiency, presence of KDR mutation, and malaria prevalence; *An gambiae* characteristics will be compared to malaria prevalence and incidence at the household and neighborhoods after one year of data collection.

371

MALARIA OUTBREAK IN THE INTERNALLY DISPLACED PERSONS ALONG THE CHINA-MYANMAR BORDER

Guofa Zhou[1], Guiyun Yan[1], Liwang Cui[2], Zhaoqing Yang[3], Ying Wang[4]

[1]University of California Irvine, Irvine, CA, United States, [2]Pennsylvania State University, University Park, PA, United States, [3]Kunming Medical University, Kunming, China, [4]Third Military Medical University, Chongqing, China

Internally displaced persons (IDPs) represent special populations with special public health concerns, particularly infectious diseases such as malaria. In such settings, infectious disease incidence rate is usually high, but innovative strategies for accessing vulnerable populations and delivering basic public health interventions may ultimately reduce transmission of infectious diseases. Contrary to common belief and results of previous studies, we report lower malaria incidence rates in IDPs compared to local residents. Malaria passive case and active case surveillances were conducted in two IDP camps and two local villages along the China-Myanmar border in the Laiza area of Myanmar from April 2011 to December 2013. Malaria vector populations were monitored using CDC light traps. The use of malaria preventive measures was investigated. Information on aid agencies and their activities during the study period was obtained through questionnaire surveys. Malaria was confirmed in 1,321 patients. Of these cases, 83.4% were *Plasmodium vivax* malaria. Seasonal malaria outbreaks were observed both in the IDP camps and in the local villages in 2013; malaria incidence in local villages peaked 4 weeks before the peaks in IDP camps. The malaria annual incidence rates were 56.5 and 164.7 cases/1,000 population in IDP camps and local villages, respectively. Older children of 5-14 yrs had the highest incidence rate in IDP camps regardless of gender and study community, while male adults had significantly higher incidence rates than females in local villages. The parasite prevalence rate was in general <5%. Over 99% of households in both communities owned bed nets, and bed net usage rates were 76.4% and 66.3% in local residents and IDPs, respectively. *Anopheles* vector density was nine-fold higher in local villages than in IDP camps. There were more active aid agencies in the IDP camps than in local villages. Temperatures in 2012 and early 2013 were 1-3°C above normal in the study area. Refugee/IDP camps are usually located in underdeveloped areas. If management practices are effective and facilities are relatively adequate, infectious diseases can be significantly reduced in IDP camps; however, under certain environmental conditions, disease outbreaks may be unavoidable. The long-term impact of malaria control strategies must be closely monitored.

372

DO THE DYNAMICS OF CLASSICAL MALARIA EPIDEMICS DEMONSTRATE *PLASMODIUM FALCIPARUM'S* SURVIVAL STRATEGY?

G. Dennis Shanks

Australian Army Malaria Institute, Enoggera, Australia

Areas of marginal transmission can generate enormous lethal malaria epidemics when factors favouring the parasite shift only slightly. Although usually described in terms of vectorial capacity, medical scientists working in India in the early 20th century came to the conclusion that "an epidemic of relapses" was the key triggering event of malaria epidemics. This explanation has been largely discarded as the biology of *Plasmodium falciparum* recrudescence was differentiated from *P vivax* relapse. Using detailed data from the 1908 Punjab malaria epidemic investigated by Rickard Christophers, the genesis of epidemics has been re-examined in order to inform current control efforts. Several aspects of the 1908 epidemic were well documented. It was highly focused geographically depending on recent rainfall and canal overflow. The epidemic arose very suddenly and simultaneously in several places. Malaria spleen surveys indicated very little recent malaria transmission and blood smears showed

very few gametocytes just prior to the epidemic. Population stress as indicated by high grain prices due to a poor harvest caused by drought the previous year was a key risk factor for malaria epidemics. Although increased female anopheline survival due to increased humidity played an important part in the magnification of the epidemic, it does not explain its genesis. Current studies using large blood volume molecular methods in SE Asia indicate that a large number of asymptomatic individuals exist with very low falciparum parasitemia. Human population stress triggering a shift towards gametocytogenesis is hypothesized as the key initiation factor for malaria epidemics. Its evolutionary significance may be that it allows the parasite to match the tropical agricultural cycle. Public health programs will have to eliminate all parasites in asymptomatic persons probably by mass drug administration in order to eliminate the risk of malaria epidemics during war and natural disaster.

373

SCHOOL-BASED COUNTRYWIDE SEROPREVALENCE SURVEY INDICATES SPATIAL HETEROGENEITY IN MALARIA TRANSMISSION IN THE GAMBIA

Joseph Okebe, Muna Affara, Omar Janha, David Jeffries, Serign Ceesay, Davis Nwakanma, Umberto D'Alessandro

Medical Research Council Unit, Banjul, Gambia

As the geographical distribution of malaria transmission becomes progressively clustered, identifying such residual pockets of transmission is important for research and for targeting interventions. Malarial antibody-based surveillance is increasingly recognised as a valuable complement to classic methods for the detection of infection foci. The study presents serological evidence of transmission heterogeneity among school children in The Gambia, measured during the dry season. Healthy primary school children were randomly selected from 30 schools across the country and screened for malaria infection (microscopy) and antimalarial antibodies ($MSP1_{19}$). Antibody distribution was modelled using finite mixture models with the Akaike's Information Criterion (AIC) used to determine best model fit. Factors associated with a positive serological status were identified in a univariate model and then combined in a multilevel mixed-effects logistic regression model, simultaneously adjusting for variations between individuals and school. A total of 4140 children, 1897 (46%) boys, were enrolled, with mean age 10.2 years (SD 2.6, range 4- 20 years). Microscopy results available for 3640 (87.9%) children showed that 1.9% (69) were positive for *Plasmodium falciparum* infections, most of them (97.1%, 67/69) asymptomatic. The overall sero-prevalence was 17.4% (720/4140) with values for the schools ranging from 0.6% to 57.5%. Age (OR 1.54, 95% CI 1.26- 1.89, P <0.001) and malaria infection (OR 4.95, 95% CI 2.76- 8.87, P <0.001) were strongly associated with seropositivity. Serological tests could identify individuals who were or had been infected, and clusters of residual transmission. Field-adapted antibody tests could guide mass screening and treatment campaigns.

374

MOLECULAR EPIDEMIOLOGY OF *PLASMODIUM FALCIPARUM* IN AREAS OF DIFFERENT MALARIA ENDEMICITY - TANZANIA

Felista W. Mwingira[1], Seif Shekalaghe[2], Salim Abdulla[2], Marcel Tanner[1], Ingrid Felger[1]

[1]University of Basel, Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Ifakara Health Institute - Bagamoyo Research and Training Centre, Bagamoyo, United Republic of Tanzania

Malaria endemicity in sub Saharan Africa continues to decline in recent years as a result of disease intervention strategies. While symptomatic malaria is recognized and treated, asymptomatic infections become increasingly important for interrupting transmission as they contribute substantially the infective reservoir to mosquitoes. To determine the asymptomatic carriage in urban and rural settings in Tanzania, crossectional community surveys were conducted using highly sensitive molecular tools. A total of 440 finger prick blood samples were collected in Dar es Salaam (DSM, urban malaria with low transmission), and 506 samples from coastal Rufiji, (high malaria transmission). *Plasmodium falciparum* (Pf) was detected by mRDT (Pan/Pf), light microscopy (LM) and quantitative PCR (qPCR) or qRT-PCR targeting the S-type 18S rRNA genes or transcripts of A-type 18s rRNA respectively. Pf gametocytes were detected by light microscopy and qRT-PCR targeting transcripts of gametocyte specific marker pfs25. Pf Prevalence was 73%, 49.4%, 47.43% and 25.3% by qRT-PCR, qPCR, RDT and LM in the coastal area (high transmission); while in DSM (low transmission) prevalence was 10.4% 5.23%, 1.59 and 3.41%, respectively. Comparing the two endemic areas, we observed up to 10-fold differences in prevalence rates by the three detection methods. Gametocyte prevalence was 0.23% by LM and 0.9% by qRT-PCR in DSM. In high transmission gametocyte prevalence was 10 fold higher than in low transmission (2.57% by LM and 43.7 % by qRT-PCR). The huge difference in prevalence observed in rural Tanzania argues for reliable molecular tools in malaria surveillance, since 50% of infections would remain undetected by microscopy diagnostics. Such precise molecular data on asymptomatic carriage seems instrumental for planning of malaria interventions. Gametocyte prevalence was about 50% of asexual prevalence at high endemic settings and mirrored asexual prevalence rates. Thus, the difficult and costly molecular detection of gametocytes seems dispensable from surveillance.

375

A MULTI-PATHOGEN SPATIO-TEMPORAL MODEL TO PREDICT INCIDENCE OF RARER DISEASES

Denis Valle

University of Florida, Gainesville, FL, United States

The quantification of spatial and temporal patterns of disease risk is often hampered by substantial noise associated with small population sizes, particularly for rarer diseases. Here, we propose a novel Hierarchical Bayesian model that relies on spatial and temporal correlation, as well as between-pathogen correlation, to improve the characterization of disease risk. We illustrate this model with malaria incidence data from the Brazilian Amazon collected from 2004 to 2008 by the government surveillance system, totaling an area of 3.6 million km2 and 2.4 million malaria cases. In this region, *Plasmodium falciparum* is much rarer than *P. vivax*, making it hard to estimate *P. falciparum* disease risk. However, there is greater public health concern regarding *P. falciparum* because it tends to result in more adverse health outcomes. Preliminary findings reveal striking geographical differences in disease risk for both pathogens, which seem to be strongly influenced by forest cover. Our model also reveals a strong correlation between *P. vivax* and *P. falciparum* malaria incidence, which suggests that *P. vivax* data can help in inferring *P. falciparum* risk in a way that is above and beyond the information contained in our covariates. We anticipate conducting a 10-fold cross-validation exercise by August 2014 to better assess the relative importance of temporal, spatial, and between-pathogen correlation in improving predictions of *P. falciparum* disease risk. The modeling framework proposed here can be valuable for inferring disease risk of a rarer (but more severe) disease based on a more common disease. Finally, although geospatial models have been extensively used to create interpolated surfaces of disease risk, our preliminary malaria results suggest that the uncertainty associated with these surfaces is large and that relatively few areas have significantly high disease risk (hotspots). This finding is important from a public health perspective because it reveals the critical role of uncertainty in determining priority areas for malaria interventions.

376

A COUNTRYWIDE CROSS-SECTIONAL SURVEY OF THE PREVALENCE OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTION IN THE GAMBIA

Julia Mwesigwa[1], Joseph Okebe[1], Muna Affara[1], Gian Luca Di Tanna[2], Davis Nwakanma[1], Omar Janha[1], Alfred Amambua Ngwa[1], Umberto D'Alessandro[1]

[1]Medical Research Council Unit, The Gambia, Banjul, Gambia, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

Malaria indicators have decreased substantially in The Gambia with pre-elimination status targeted for 2015. Achieving this goal requires in-depth understanding of the burden of asymptomatic *Plasmodium falciparum* parasitaemia (APFP) as asymptomatic carriers can maintain or re-initiate transmission. A cross-sectional survey was conducted to determine the prevalence of APFP in the Gambia, describe its heterogeneity and its associated risk factors. A cross-sectional survey was conducted in 36 villages in six geographical locations in November 2012 during the peak of malaria transmission. 350 persons across all age groups were randomly selected in each village (whole populations for villages <350). Finger-prick blood sample were collected for microscopy, species-specific PCR and haemoglobin estimation. Information on bed net use and previous antimalarial treatment were also collected. Data were analysed for the prevalence of APFP by village; summary statistics and factors associated with APFP are presented. Of the 9094 participants enrolled; 59.6% females and 27.2 % were aged <5 years. The median (IQR) age and mean (±SD) haemoglobin were 12 (5, 28) years and 11.7 (±2.3) g/dl, respectively. The prevalence of APFP was 16.01% with marked heterogeneity between villages ranging from 1.6% to 49.1%. The mean parasite density was 4329.7/ul. 95% of participants owned bed nets, 78.8% reported indoor residual spraying (IRS). The odds of APFP were higher in children aged 5-15 years (OR1.93; 95% CI: 1.63 -2.30) and adults (OR1.62; 95% CI: 1.35 -1.95) relative to those aged < 5 years. Participants with severe anemia had twice the odds of APFP (OR 2.76; 95% CI: 2.01 - 3.79). Other predictors associated with increased odds of APFP were; axillary temperature, sleeping on verandas at night and IRS. APFP is substantially high in the villages in the eastern region of the country despite high coverage of bed nets and use of IRS. Any intervention aiming at interrupting transmission should target asymptomatic carriers as they represent a substantial reservoir of infection.

377

GENETIC INVESTIGATION OF VAR2CSA VARIANTS ASSOCIATED WITH LOW BIRTH WEIGHT IN WOMEN WITH PLACENTAL MALARIA IN MALAWI

Jaymin C. Patel[1], Steve M. Taylor[2], Christian M. Parobek[1], Mwayi Madanitsa[3], Victor Mwapasa[3], Feiko O. ter Kuile[4], Jonathan J. Juliano[1], Steven R. Meshnick[1]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]Duke University Medical Center, Durham, NC, United States, [3]University of Malawi, Blantyre, Malawi, [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Each year 125 million pregnant women worldwide and 32 million pregnant women in sub-Saharan Africa are at risk of falciparum malaria infection during the course of their pregnancy. Malaria in pregnancy contributes to approximately one million cases of infant low birth weight (LBW) and of maternal anemia, with an estimated 10,000 maternal and 70,000 to 200,000 infant deaths worldwide. The effect of *Plasmodium falciparum* on infants is largely due to placental malaria, in which infected erythrocytes sequester in the placenta owing to the interaction between VAR2CSA proteins on the erythrocyte surface and placental chondroitin sulfate A (CSA). The association between specific VAR2CSA protein domains and adverse birth outcomes, such as LBW, has not been well characterized due the size and diversity of the protein. Recent studies have implicated the ID1-DBL2X-ID2 region of var2csa (~2.7 kb) as critical to placental pathogenesis because it binds to the CSA with same avidity as the whole protein. Using placental samples from 135 *P. falciparum*-infected pregnant women enrolled in a Malaria In Pregnancy Consortium (MIPC) prevention trial in Malawi, a molecular epidemiology study is being conducted to characterize the diversity of the var2csa ID1-DBL2X-ID2 region using next generation deep sequencing methods and to evaluate associations of specific ID1-DBL2X-ID2 haplotypes with LBW. A pooled case-control study design will be employed wherein parasite DNA samples will be pooled from women with and without LBW babies and deep-sequenced across the targeted domain using PacBio Circular Consensus Sequencing (CCS) to determine the frequency of each domain variant in the pool. After haplotype reconstruction from these pooled sequencing steps, associations with LBW will be examined by comparing frequencies of var2csa variants between the two groups. The results from this study will allow us to characterize the diversity of var2csa for the first time using high resolution data. It will help identify pathogenic variants of var2csa and inform efforts currently underway to develop a vaccine for pregnant women living in falciparum malaria endemic areas.

378

HOUSEHOLD HEALTH CARE-SEEKING COSTS: EXPERIENCES FROM A RANDOMIZED, CONTROLLED TRIAL OF COMMUNITY-BASED MALARIA AND PNEUMONIA TREATMENT AMONG UNDER-FIVES IN EASTERN UGANDA

Fred Matovu[1], Aisha Nanyiti[2]

[1]Makerere University, Kampala, Uganda, [2]Development Economics Group, Wageningen University and Research Centre, Holland, Kampala, Uganda

Home and community-based combined treatment of malaria and pneumonia has been promoted in Uganda since mid 2011. The combined treatment is justified given the considerable overlap between the symptoms of malaria and pneumonia among infants. There is limited evidence about the extent to which community-based care reduces health care-seeking costs at the household level in rural and urban settings. This paper assesses the rural-urban differences in direct and indirect costs of seeking care from formal health facilities compared to community medicine distributors (CMDs). Exit interviews were conducted for 282 (159 rural and 123 urban) caregivers of children below five years who had received treatment for fever-related illnesses at selected health centers in Iganga and Mayuge districts. Data on the direct and indirect costs incurred while seeking care at the health center visited were obtained. Using another tool, household level direct and indirect costs of seeking care from CMDs were collected from a total of 470 caregivers (304 rural and 166 urban). Costs incurred at health facilities were then compared with costs of seeking care from CMDs. Household direct costs of seeking care from health facilities were significantly higher for urban-based caregivers than the rural (median cost = US$0.42 for urban and zero for rural; p<0.0001). The same is true for seeking care from CMDs (p=0.0038). Overall, caregivers travelled for an average of 75 min to reach health centers and spent an average of 80 min at the health center while receiving treatment. However, households in rural areas travelled for a significantly longer time (, p<0.001 to reach health care facilities than the urban-based caregivers. Besides travelling longer distances, rural caregivers spent 150 min seeking care from health facilities compared to 30 min from CMDs. In conclusion**,** time and monetary savings for seeking care from CMDs are significantly larger for rural than urban households. Thus, home and community-based treatment of child febrile illnesses is much more cost-saving for rural poor communities, who would spend more time travelling to health facilities - which time could be re-directed to productive and income-generating activities

379

HIGH GENETIC DIVERSITY IN *PLASMODIUM* POPULATIONS IN AREAS OF LOW TRANSMISSION: A COMPARATIVE STUDY OF COLOMBIAN POPULATIONS

M. Andreina Pacheco[1], Andres Vallejo[2], Nora Martinez[2], John Garcia[2], Socrates Herrera[2], Myriam Arevalo-Herrera[2], Ananias A. Escalante[1]

[1]Arizona State University, Tempe, AZ, United States, [2]Caucaseco Scientific Research Center and Centro Internacional de Vacunas, Cali, Colombia

The success of malaria programs is measured by the spatial scales at which disease burden is reduced or eliminated. Consequently, spatial patterns of transmission are of particular interest. In this study, we describe changes in the population structure of *Plasmodium vivax* (Pv) and *P. falciparum* (Pf) among endemic areas of Colombia with different transmission intensities. In 2012, some representative areas like Buenaventura (Valle) reported 979 cases in 2012, whereas Tumaco (Nariño) and Tierralta (Cordoba) reported 1,475 and 7,482 respectively. These areas display differences in the relative importance of Pf and Pv (e.g. Valle Pv 90%, Tierralta Pv 92%, and Tumaco Pv 6.9%). We analyzed a total of 550 samples, including both Pv (358) and Pf (192) from these areas using a set of unlinked microsatellite loci. We found different pattern of population structure in each parasite. Using Structure v2.3.3, two clusters were identified for Pf and 9 for Pv. We estimated a total of 37 haplotypes for Pf (Tierralta: 6, Tumaco: 33), sharing only 2 between populations, indicating limited gene flow between these areas. The heterozygosity for Pf was higher in Tumaco (He: 0.871) than in Tierralta (He: 0.453). In contrast, the genetic diversity was very similar and high in the three Pv populations (Buenaventura He: 0.976, Tierralta He: 0.985 and Tumaco He: 0.979). A total of 195 haplotypes were found in Pv and all were private haplotypes (Buenaventura: 20; Tierralta: 131 and Tumaco: 44) indicating strong population structure. Overall these set of microsatellites allowed us the identification of locally related, but geographically distinctive genotypes that potentially can be tracked in time and space. These characteristics makes possible their use in molecular surveillance (e.g. separating local cases from migrants) in the context of control and elimination.

380

PLASMODIUM VIVAX MOLECULAR PREVALENCE IN THE SAHEL REGION OF MALI

Amadou Niangaly[1], Youssouf Tolo[1], Jennifer Junghyon Rentz[2], Mark A. Travassos[2], Amed Ouattara[2], Matthew Adams[2], Matthew B. Laurens[2], Bourema Kouriba[1], Issa Diarra[1], Modibo Daou[1], Antoine Dara[2], Drissa Coulibaly[1], Karim Traore[1], Christopher V. Plowe[2], Mahamadou A. Thera[1], Ogobara K. Doumbo[1]

[1]Malaria Research and Training Center/DEAP/FMOS, Bamako, Mali, [2]Center for Vaccine Development, Baltimore, MD, United States

The prevalence of *Plasmodium vivax* infection is traditionally reported as low in West and Central Africa because of the high prevalence of the Duffy-negative antigen [Fy(a- b-)]. Recent studies have found cases of *P. vivax* infection in Duffy negative people, and sporozoites of *P. vivax* have also been found in the salivary glands of *Anopheles* mosquitoes in endemic areas inhabited by a predominantly Duffy-negative population. In these regions, a Duffy-positive minority may serve as a reservoir of *P. vivax*. Infection of Duffy-negative individuals may occur, with selection of parasites capable of invading erythrocytes lacking the Duffy antigen. The potential presence of *P. vivax* infection in a Duffy-negative population in Africa may be a significant public health problem in the era of malaria elimination/eradication, where strategies are mainly directed against falciparum malaria. We hypothesized that there is endemic circulation of *P. vivax* in our study population in Bandiagara, Mali and that the prevalence of vivax malaria is underestimated. Blood samples were collected from 300 children aged 0-6 years during quarterly surveys in Bandiagara, Mali. Polymerase chain reaction diagnosis of *P. vivax* was carried out using DNA samples extracted from filter paper. We will report the prevalence of *P. vivax* infection at five quarterly time points from 2009 to 2010, with confirmatory testing with microsatellites. In addition, we will characterize the Duffy antigen status in each child that was *P. vivax* positive, and we will assess the presence of *P. vivax* sporozoites in mosquitoes caught in the study areas.

381

SEROLOGICAL DYNAMICS OF MULTIPLE *PLASMODIUM* SPECIES CO-INFECTIONS IN A HUMAN POPULATION

Seth A. Amanfo[1], Francisca Mutapi[1], Nicholas Midzi[2], Takafira Mduluza[3], David Cavanagh[1]

[1]Centre for Immunity, Infection and Evolution, Institute of Immunology and Infection Research, University of Edinburgh, Edinburgh, United Kingdom, [2]Department of Medical Microbiology, University of Zimbabwe, Harare, Zimbabwe, [3]Department of Biochemistry, University of Zimbabwe, Harare, Zimbabwe

Malaria is an important parasitic disease posing major public health challenges in developing countries. Infection with malaria parasites elicits immune responses, and after drug treatment antibodies to *Plasmodium*-specific antigens are often detectable in serum. The dynamics of antibody responses to *Plasmodium* spp. co-infection in populations from Zimbabwe where malaria is endemic were determined by ELISA. Antibody (Ab) responses to the C-terminal region of merozoite surface protein 1 (MSP 1_{19}) were determined for all four human *Plasmodium* species. A panel of other antigens, derived from MSPs of *P. falciparum*, were used to determine the serological complexity of each individual's recent *P. falciparum* exposure. Preliminary analysis of this data shows that IgG responses to MSP 1_{19} of *P. falciparum* (PfMSP 1_{19}) were the most frequently observed, followed by responses to *P. malariae* and *P. ovale*, with responses to *P. vivax* MSP 1_{19} rarely detected. Interestingly, all sera positive for *P. malariae* MSP 1_{19} were also positive for PfMSP 1_{19}, indicating that co-infection with these two species is a common occurrence, and that monospecies *P. malariae* infections are rare. Competition ELISA showed that Abs to PfMSP 1_{19} and/or PmMSP 1_{19} did not cross-react with each other, indicating Ab specificity. Serum samples with antibody reactivities to PfMSP 1_{19} often also showed reactivity to PfMSP2A and PfMSP2B derived antigens, but the patterns of reactivity with these polymorphic antigens varied markedly between individuals. This study reinforces the importance of diagnosing not only the presence of *Plasmodium* parasites, but also the species of *Plasmodium* infecting a patient for optimal treatment of malaria co-infections. Further understanding of the dynamics of multiple *Plasmodium* species infection is important, as co-infections may exacerbate the pathology of the disease or alternately decrease the severity of clinical episodes. Measurement of species-specific antibody responses may provide new insights into the extent (or indeed existence) of cross-species immunity that exists in individuals infected with multiple malaria parasite species.

382

NATURAL IMMUNE RESPONSE IN ACUTE *PLASMODIUM VIVAX* INFECTED PATIENTS LIVING IN A SOLE *P. VIVAX* INFECTION COHORT IN CENTRAL CHINA

Kulachart Jangpatarapongsa[1], Hui Xia[2], Qiang Fang[2], Jetsumon Sattabongkot[3], Liwang Cui[4], Baiqing Li[5], Rachanee Udomsangpetch[6]

[1]Faculty of Medical Technology, Mahidol University, Bangkok, Thailand, [2]Department of Parasitology, Bengbu Medical College, Anhui, China, [3]Mahidol Vivax Research Center, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [4]Department of Entomology, The Pennsylvania State University, University Park, PA, United States, [5]Department of Immunology, Bengbu Medical School, Anhui, China, [6]Faculty of Science, Mahidol University, Bangkok, Thailand

Plasmodium vivax infection possesses a characteristic of relapsing fever indicating the re-infection by previously hidden parasites in the host. The

relapsed infection can lead to activation of memory T cells pool which might bring up protective immunity. This study aims to characterize natural immune responses in acute *P. vivax* infected patients living in a sole *P. vivax* infection cohort in Central China. We conducted the cross-sectional immune-phenotypic analysis from three recruitments: patients infected with *P. vivax*, malaria-immune and malaria-naive controls. Using flow cytometry, we showed memory T cells were elevated in blood during acute infection. The level of γΔ T cells was two fold higher than that of naïve controls. This suggested that two populations, memory and γΔ T cells, responded specifically to the *P. vivax* parasites. On contrary, B, NK and NKT cells were decreased during acute infection. In addition, regulatory T cells were reduced, suggesting the non-immune suppressive role of *P. vivax* parasites. Interestingly, *P. falciparum* antigens cross-stimulated T cells obtained from these *P. vivax*-infected patients. These results provided a further insight into interaction between *P. vivax* parasites and host cell-mediated immunity in the exclusive *P. vivax* endemic area that could be important for future development of a successful vaccine designation.

383

IMPACT OF AGE OF FIRST EXPOSURE TO *PLASMODIUM FALCIPARUM* ON ANTIBODY RESPONSES TO MALARIA IN CHILDREN: A RANDOMIZED, CONTROLLED TRIAL IN MOZAMBIQUE

Augusto J. Nhabomba

CISM, Maputo, Mozambique

Background: Malaria continues to be an important cause of morbidity and death among children younger than 5 years old. Nevertheless, The impact of the age of first *Plasmodium falciparum* infection on the rate of acquisition of immunity to malaria and on the immune correlates of protection has proven difficult to elucidate. Previous studies have explored in-depth the contribution of age and exposure in the development of protective immune response. Such that their findings suggested that more a mature immune systems like those of older children should benefit the development of protective immune systems. Moreover, previous work hypothesized a key age period where exposure to malaria parasite should occur in a way that would exalt the development of naturally acquired immune (NAI) response without jeopardize the heath of infants from endemic areas. The notable need of discerning the exact period for adequate development of NAI was the rational for the present study Methods: Participants (n=349) were enrolled at birth to one of three groups: late exposure, early exposure and control group, and were followed up for malaria morbidity and immunological analyses at birth, 2.5, 5.5, 10.5, 15 and 24 months of age. Total IgG, IgG subclasses and IgM responses to MSP-119, AMA-1, and EBA-175 were measured by ELISA, and IgG against variant antigens on the surface of infected erythrocytes by flow cytometry. Factors affecting antibody responses in relation to chemoprophylaxis and malaria incidence were evaluated. Results: Generally, antibody responses did not vary significantly between exposure groups except for levels of IgM to EBA-175, and seropositivity of IgG1 and IgG3 to MSP-119. Previous and current malaria infections were strongly associated with increased IgG against MSP-119, EBA-175 and AMA-1 ($p<0.0001$). After adjusting for exposure, only higher levels of anti-EBA-175 IgG were significantly associated with reduced clinical malaria incidence (IRR 0.67, $p=0.0178$). Conclusions:Overall, the age of first *P. falciparum* infection did not influence the magnitude and breadth of IgG responses, but previous exposure was critical for antibody acquisition. IgG responses to EBA-175 were the strongest correlate of protection against clinical malaria

384

IDENTIFICATION OF NOVEL HLA CLASS I-RESTRICTED T CELL EPITOPES IN THE *PLASMODIUM FALCIPARUM* VACCINE CANDIDATE ANTIGENS CSP AND AMA1 THAT INDUCE IFN-RESPONSES IN A NATURALLY EXPOSED POPULATION

Kwadwo A. Kusi

Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana

Malaria eradication requires a concerted approach involving all available control tools and an effective vaccine would complement these efforts. For T cell multi-epitope vaccines, immunodominant epitope selection could be based on the identification of protected individuals in subunit/whole sporozoite vaccine studies with naïve persons or on the identification of persons who show resistance in endemic areas. While the former may help identify protection-associated epitopes in a few antigens, the latter is representative of a field challenge with multiple malaria antigens and may identify immunodominant epitopes within these antigens. We therefore investigated whether naturally exposed individuals had HLA-restricted T cells against *Plasmodium falciparum* CSP and AMA1 epitopes. Eleven volunteers between 18 and 49 years were recruited from an urban community of Ghana in 2011. Volunteer PBMCs were tested against 12 overlapping 15mer peptide pools spanning the entire AMA1sequence and 9 such CSP pools by *ex vivo* Elispot. Criteria for IFN-γ positivity was a stimulation index > 2 and a spot difference > 10, both with respect to spot forming cells per million PBMCs for corresponding unstimulated cells. Volunteers were HLA-typed and their PBMCs subsequently tested against mixtures of previously identified HLA Class I-restricted epitopes within AMA1 (HLA A01, A02, A03, A24, B07 and B44) and CSP (HLA A01, A02, A03, A24 and B15). These were selected because they contained at least one epitope from the positive 15mer pools or that they contained epitopes that matched volunteer HLA types. For AMA1, 10 of the 11 volunteers responded to some 15mer peptide pools while all volunteers responded to some HLA-restricted epitope mixtures. For CSP, all volunteers responded to some peptide pools whilst 10 volunteers responded to some HLA-restricted epitope mixtures. The data demonstrates the potential of T cells from naturally exposed individuals to make parasite-specific IFN-γ responses and point to the need to include field assessment of HLA-restricted epitopes in vaccine design strategies.

385

OPSONIZING ANTIBODIES TO *PLASMODIUM FALCIPARUM* MEROZOITES ASSOCIATED WITH IMMUNITY TO CLINICAL MALARIA IN PAPUA NEW GUINEA

Danika L. Hill[1], Emily M. Eriksson[1], Danny W. Wilson[1], Connie S. Li Wai Suen[1], Peter M. Siba[2], Diana S. Hansen[1], Alan F. Cowman[1], Ivo Mueller[1], Louis Schofield[1]

[1]Walter and Eliza Hall Institute of Medical Research, Parkville, Australia, [2]Papua New Guinea Instititute of Medical Research, Goroka, Papua New Guinea

Naturally acquired humoral immunity to the malarial parasite *Plasmodium falciparum* can protect against disease, although the precise mechanisms remain unclear. Although antibody levels can be measured by ELISA, few studies have investigated functional antibody assays in relation to clinical outcomes. We have developed a flow cytometry based functional assay to measure antibody-mediated phagocytosis of merozoites, and have applied this to a longitudinal cohort study conducted in a malaria endemic region of Papua New Guinea (PNG). Opsonising antibody responses to 3D7 merozoites were found to: i) increase with age, ii) be enhanced by concurrent infection, and iii) correlate with protection from clinical episodes and high-density parasitemia. Stronger protective associations were observed in individuals with no detectable parasitemia at baseline. When opsonising antibody responses were measured against merozoites from various isolates, stronger or weaker associations with protection from clinical disease were observed. This study presents the first evidence for

merozoite phagocytosis as a correlate of acquired immunity and clinical protection against *P. falciparum* malaria, and demonstrates that using a geographically diverse parasite strains can significantly influence the statistical associations detected.

386

DENDRITIC CELLS SUBSETS MEDIATED IMMUNE RESPONSE DURING *PLASMODIUM BERGHEI* ANKA AND *P. YOELII* INFECTION

Tarun Keswani, Arindam Bhattacharyya

University of Calcutta, Kolkata, India

Immune response responsible for the induction and regulation of this response are poorly understood during malaria infection. As immunity is initiated by dendritic cells (DCs), we compared their phenotype and function during *Plasmodium berghei* ANKA (PbA) and *P. yoelii* 17NXL (P.yoelii) infection in Swiss mice individually. PbA infected mice developed a greater number of myeloid and mature DCs on 8 dpi spleen, which were fully functional and capable of secreting IL-12. In contrast, nonlethal *P. yoelii* infected mice produced more plasmacytoid and less mature DCs, resulting in higher levels of IL-10. The mice infected with PbA resulted in an increase in expression of stimulatory (MHCII) and co-stimulatory (CD80 CD86 and CD40) molecules, whereas opposite result was observed during *P. yoelii* infection. Correlating with expression of splenic DCs subsets from PbA and *P. yoelii* infected spleen, our immunoblot assays confirmed the difference in expression of FoxP3, IL-17, TGF-β and IL-6. Thus, results from this study indicate that the subset, the phenotype and the type of inflammatory and anti-inflammatory signals of splenic DCs are critical factors responsible for the irregularity in the ability to induce or regulate Th1 immune responses on host pathogenesis during malaria infection.

387

PROTECTIVE IMMUNITY AGAINST BLOOD-STAGE MALARIA INDUCED BY VACCINATION WITH *PLASMODIUM* MIF

Alvaro Baeza Garcia[1], Tiffany Sun[1], Exler Valerie[1], Scott J. Roberts[1], Andrew J, Geall[2], Rick Bucala[1]

[1]Yale University, New Haven, CT, United States, [2]Novartis Vaccines and Diagnostics, Cambridge, MA, United States

A major obstacle to a malaria vaccine is the inability of the host to establish an effective memory T cell response. All *Plasmodium* species encode a close ortholog of the innate cytokine macrophage migration inhibitory factor, termed PMIF, which regulates the IL-12/IFNγ response during blood-stage infection to promote the differentiation and exhaustion of effector T cells and reduce the development of memory precursor CD4 T cells. We hypothesized that PMIF may be an attractive vaccine target since its neutralization may reduce deleterious cytokine expression and increase protective memory T cell responses. Using a novel, non-viral self-amplifying RNA vaccine, mice (BALB/c) immunized with PMIF and infected with blood-stage *P. berghei* showed reduced parasitemia and a 37% prolongation in mean survival time when compared to a group vaccinated with control antigen (p=0.0016). This effect was associated with a greater number of antigen-experienced CD4 T cells and an 80% increase in $CD62L^{+}IL7R\alpha^{+}$ memory T cells (Tmem). Notably, PMIF-immunized mice that were infected, cured with chloroquine, and re-challenged with *P. berghei* showed 50% expansion of their *Plasmodium*-responsive Tmem populations and complete protection from re-infection when compared to a control vaccine group. Protection in this model of malaria infection also was associated with a 50% increase in plasmablasts ($CD19^{lo}B220^{lo}CD138^{hi}IgD^{-}$) and a 15-fold higher titer of an anti-*Plasmodium* antibody response (IgG2a and IgM). An enhancement in both protective humoral and cellular immunity could be demonstrated by PMIF immunization. Passive transfer of immune IgG from infected and cured mice conferred 33% (p<0.0001) protection in lethal cerebral malaria (*P.berghei* infected C57BL/6 mice) and adoptive transfer of CD4 T cells from PMIF-vaccinated mice into naive congenic BALB/c mice conferred complete protection (p<0.0001) against subsequent infection. These data suggest that targeting an active mechanism by which *Plasmodia* interfere with immunologic memory holds promise for a novel vaccine approach.

388

DIFFERENT LATE STAGES OF *PLASMODIUM FALCIPARUM* STIMULATE IFN-Γ PRODUCTION IN CULTURED PBMCS FROM SYMPTOMATIC AND ASYMPTOMATIC INDIVIDUALS BY ELISPOT

Katherine J. Torres[1], Elizabeth Villasis[1], Pamela Rodriguez[1], Paola Larrauri[1], Romina Pacheco[1], Katherin Peñaranda[1], Claudia Carrera[1], Joseph Vinetz[2], Dionicia Gamboa[1]

[1]Instituto de Medicina Tropical Alexander von Humboldt, Lima, Peru, [2]University of California San Diego, San Diego, CA, United States

Plasmodium infections trigger complex immune reactions from their hosts against several life stages of the parasite. These immune responses are highly variable, depending on age, genetics, and the condition of the disease in the host as well as species and strain of parasite. Interferon (IFN)-gamma ELISPOT was used to monitor the magnitude of cellular immune response to *P. falciparum* antigens. Production of IFN-γ from cryopreserved PBMCs collected by leukapheresis procedure from Asymptomatic (Asym), Symptomatic (Sym) individuals infected with *P. falciparum* and Endemic Controls (C) was measured against different antigens (Ag): Hemozoin (Hz) and DNAse-treated Hz and synchronized-purified parasites at different stages (mature trophozoites, schizonts in developing and mature) from referential (ITG) and native strain (F06) of *P. falciparum* parasites cultured *in vitro*. In the Sym group we observed an increased number of Spot Forming Cells (SFC) when we stimulate with stages of developing and mature schizonts; being this response more increased with the reference strain compared to the native strain. A lower response was observed when PBMCs from these individuals was stimulated by mature trophozoite. The Asym individuals showed increased response of IFN-γ when stimulated with the native strain of *P. falciparum*, and this response was higher when PBMCs were exposed to mature stage parasites (trophozoites or schizonts). The responses in the three groups were greater when the PBMCs were stimulated with any stage of the parasite in compare to the response of the stimulation with the hemozoin. The response with this last antigen (Hz) was high in the purified hemozoin of referential strain and such response was greater in the Sym group compared to the Asym and control groups. DNAse-treated Hz presented a lower IFN-γ SFC in all groups. In conclusion; based on these results and experimental conditions, it seems that PBMCs could be stimulated by different developing stages of schizonts of *P. falciparum* in ex-vivo experiments, however; in Asym individuals the response seems to be higher when PBMCs are exposed to parasites from native strain in terms of IFN-γ response.

389

EPITOPE MAPPING OF *PLASMODIUM FALCIPARUM* APICAL MEMBRANE ANTIGEN 1 USING AN *E. COLI* AUTOTRANSPORTER SYSTEM ON HIGH-THROUGHPUT PROTEIN MICROARRAYS

Andrea A. Berry[1], Mary Sedegah[1], Jason Bailey[1], Mark A. Travassos[1], Matthew Adams[1], Philippe Azimzadeh[1], Drissa Coulibaly[2], Jozelyn Pablo[3], Rie Nakajima[3], Algis Jasinskas[3], Sheetij Dutta[4], Fernando Ruiz[5], Shannon Takala Harrison[1], Mahamadou A. Thera[2], Ogobara K. Doumbo[2], Philip L. Felgner[3], James P. Nataro[5], Christopher V. Plowe[1]

[1]Howard Hughes Medical Institute/Center for Vaccine Development, Baltimore, MD, United States, [2]Malaria Research and Training Center, Faculty of Medicine and Dentistry, Bamako, Mali, [3]Division of Infectious Diseases, Department of Medicine, Irvine, CA, United States, [4]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [5]Department of Pediatrics, Charlottesville, VA, United States

Despite extreme diversity, apical membrane antigen 1 (AMA1) is a leading blood stage vaccine candidate antigen in part because anti-AMA1 antibodies inhibit erythrocyte invasion *in vitro*. Although the protein is known to be immunogenic, there is limited information on which epitopes of AMA1 are associated with cross-protective antibody responses. Autotransporters are virulence factors of gram negative bacilli that can be modified to become bacterial expression systems with advantages over other systems such as phage display: autotransporters are easily manipulated in the laboratory using standard cloning techniques, and they allow the presentation of relatively large epitopes. To identify cross-reactive and cross-protective AMA1 epitopes, we engineered EspP, an *E. coli* autotransporter, to display regions of AMA1 and variants of those regions. Sera from rabbits immunized with 3D7- and FVO- AMA1 ectodomains were reactive with the resulting recombinant AMA1-autotransporters. When p-distance and Grantham variation scores were calculated by comparing rabbit immunogen/AMA1-autotransporter sequence pairs, we found that sera were cross-reactive with heterologous haplotype constructs when p-distance and Grantham variation scores were smaller. We optimized expression and purification of AMA1-autotransporters for use in high throughput protein microarrays. We will present results of protein microarray reactivity with human sera from children and adults pre- and post- malaria season and pre- and post- receipt of AMA1 vaccine FMP2.1/AS02A. Our hypothesis is that seroreactivity to AMA1-autotransporters will be higher in adults than in children and increase in magnitude and breadth over the course of a malaria season, reflecting acquisition of immunity to AMA1 epitopes and ultimately allowing for the identification of cross protective epitopes. A protein expression system that can readily express conformational peptides in a variety of sizes will be helpful in determining AMA1 epitopes important to a broadly protective AMA1-based malaria vaccine.

390

PLASMODIUM FALCIPARUM, BUT NOT *P. VIVAX*, CAN INDUCE ERYTHROCYTIC APOPTOSIS

Maria de Fatima Ferreira-da-Cruz[1], Paulo Renato R. Totino[1], Eliana B. Alves[2], Monica R. Costa[3], Claudio Tadeu Daniel-Ribeiro[1], Marcus Vinicius G. Lacerda[3]

[1]Fundação Oswaldo Cruz, Rio de Janeiro, Brazil, [2]HEMOAM, Manaus, Brazil, [3]FMTAM-HD, Manaus, Brazil

Apoptosis can occur in red blood cells (RBC) and this process could play a role in the pathogenesis of many diseases. In malaria it is well known the participation of parasitized RBC in anemia and thrombotic processes. However, non-parasitized RBC (npRBC) apoptosis could amplify the hematologic disorders associated to malaria. In fact, in experimental malaria, increased levels of apoptosis were observed in npRBC during *Plasmodium yoelii* infection, but in human malaria erythrocytic apoptosis has never been studied. The present study was performed to investigate if npRBC apoptosis also occurs in *P. vivax* and *P. falciparum* infections. Apoptosis of npRBC was evaluated in blood samples of *P. vivax* malaria patients and clinical health individuals living in Manaus, Brazil, both *ex vivo* and after incubation of RBC for 24h. Additionally, it was tested the capacity of *P. vivax* or *P. falciparum* plasma patients to induce *in vitro* apoptosis of normal RBC from a clinical health individual living in Rio de Janeiro, a non-endemic malaria region. Apoptosis was detected by flow cytometry using annexin V staining. Contrasting with experimental malaria that significantly increase the levels of apoptotic npRBC both ex-vivo and after 24h of incubation, no significant alteration on apoptotic npRBC rates was detected in *P. vivax* infected patients, when compared with non-infected control individuals. Similar results were observed when plasma of these *P. vivax* patients was incubated with normal RBC. Conversely, plasma from *P. falciparum*-infected subjects induced significant apoptosis of these cells. It was concluded that apoptosis of normal RBC can be induced by plasma from individuals with *P. falciparum* (but not with *P. vivax*) malaria. This finding could reflect the existence of erythrocytic apoptosis during the course of falciparum malaria that could contribute to the pathogenesis of hematological and vascular complications associated to *P. falciparum* malaria.

391

STUDY ON ASSOCIATION BETWEEN GENETIC POLYMORPHIMS OF TUMOR NECROSIS FACTOR-A, INTERLEUKIN-10, INTERFERON-Γ, AND MALARIA VIVAX IN BRAZIL

Adriana A. Furini[1], Gustavo C. Cassiano[2], Franciele M. Tomaz[1], Marcela P. Capobiano[2], Marinete M. Póvoa[3], Michele Encinas[4], Diego L. Madi[4], Pamella C. Trindade[1], Valeria D. Fraga[1], Luciana M. Conceição[1], Ricardo L. Machado[3]

[1]Faculdade de Medicina de São José do Rio Preto, São José do Rio Preto, Brazil, [2]Universidade Estadual Paulista, São José do Rio Preto, Brazil, [3]Instituto Evandro Chagas, São José do Rio Preto, Brazil, [4]Centro Universitário de Rio Preto, São José do Rio Preto, Brazil

Malaria is a major cause of morbidity and mortality in many tropical and subtropical countries. In brazil, the *Plasmodium vivax* has been the most prevalent species, accounting for approximately 83% of malaria cases in the brazilian amazon region. Despite of the clinical descriptions of the disease caused by *P. vivax* is well described, standards regarding humoral and cellular immune response, as well as the pattern of cytokines are scarce and not fully understood. Polymorphisms in genes cd28, ifnγ, tnfα and il10, that are encoder molecules that interact in modulating pathways of the cellular and humoral immune response, may influence the resistance or susceptibility to malaria. We had analyzed 90 blood samples from patients with vivax malaria diagnosed by molecular and non-molecular techniques and 51 from non-malarial and all from goianésia of pará city, pará state, brazil. Polymorphisms in genes IL10 (-819 c>t, -592 c>a), ifnγ (-183 g>t), tnfα (-238g>a), and cd28 (-372g>a, +17t>c) were analyzed by PCR-RFLP. All subjects were genotyped with 48 ancestry informative insertion-deletion polymorphisms to determine the proportion of African, European and Amerindian ancestry to avoid bias due to differences in ancestry contributions in malaria and non-malaria groups. We used the fisher exact test to measure association between genotypes and malaria infection. All polymorphisms tested were in hardy-weinberg equilibrium. The African, European and native American admixture did not differ among cases and controls. No significant association was found between the polymorphisms tested and vivax malaria and non-malarial individuals. P-values in co-dominant, dominant and recessive models were also calculated and no significant association was found. These findings make us to believe that the analyzed polymorphisms are not associated with susceptibility or resistance with vivax malaria in the studied population.the results will be finalized by june 2016.

392

CONCURRENT MALARIA PARASITES AND CHIKUNGUNYA VIRUS CO-INFECTION ABATES NEUROPATHOLOGY OF EXPERIMENTAL CEREBRAL MALARIA

Teck Hui Teo

Singapore Immunology Network, Singapore, Singapore

Malaria and Chikungunya fever (CHIKF) are arthropod borne diseases that share the same geographical distributions and recent reports in human shows that co-infections do occur. With the increase in the spread of Chikungunya virus (CHIKV) in the tropics, the potential risk of co-infection in malaria patients is heightened. However, little is known if malaria-associated pathologies are modified during co-infections. In this project, we studied the effect of the viral infection on the pathologies in non-lethal and experimental cerebral malaria (ECM) using *Plasmodium yoelii* cl1.7x (Py17x) and *Plasmodium berghei* ANKA (PbA) respectively. No effect was observed in sequential or concurrent co-infection of CHIKV and Py17x. Interestingly, although no effect was seen in sequential co-infection of CHIKV and PbA, protective effects were observed during concurrent co-infection. Concurrent co-infection reduced ECM mortality with little effects on the parasitemia. We found that the reduction of ECM were associated with reduced parasite load in the brain, improved blood-brain-barrier integrity, reduced pathogenic T cells in the brain and reduced cross-presentation of pathogenic epitopes by brain endothelial cells. Leukocytes profiling in the spleen early in the infection suggests that the lack of pathogenic T cells in the brain is not a result of T cells induction but of migration. Taken together, this study demonstrates that host pathogenic immune response towards *Plasmodium* parasites was altered during CHIKV co-infection leading to reduced neuropathology.

393

IL4 GENE POLYMORPHISMS ARE NOT ASSOCIATED WITH *PLASMODIUM VIVAX* MALARIA IN BRAZIL

Franciele M. Tomaz[1], Gustavo C. Cassiano[2], Adriana A. Furini[1], Marcela P. Capobiano[2], Pamella C. Trindade[1], Marinete M. Póvoa[3], Valéria D. Fraga[1], Lucas R. Azevedo[2], Sônia M. Oliani[2], Luciana M. Conceição[1], Ricardo L. Machado[3]

[1]Faculdade de Medicina de São José do Rio Preto, São José do Rio Preto, Brazil, [2]Universidade Estadual Paulista, São José do Rio Preto, Brazil, [3]Instituto Evandro Chagas, Belém, Pará, Brazil

Interleukin 4 (IL-4) is an anti-inflammatory cytokine, which regulates balance between Th1 and Th2 immune response, immunoglobulin class switching and humoral immunity. The present study investigated the influence of polymorphisms in IL-4 gene related to the immune system in patients with malaria caused by *Plasmodium vivax* in Brazilian endemic area. A total of 83 individuals infected by *P. vivax* were genotyped by pcr/rflp for two (-590 c/t, -33 c/t) single nucleotide polymorphisms (snps) and the intron 3 vntr polymorphism pcr method in IL4 gene. The density of parasitemia in the infected individuals was recorded and expressed as the number of asexual *P. vivax* per microliter of blood assuming a count of 100 fields per slide. The serum levels of IL-4 were detected by milliplex map kit (human cytokine/chemokine magnetic bead panel-hcytomag-60k) using magpix/luminex®. Analyses were performed using r version 2.8.1 statistical software. For the polymorphism at position - 590 in the il4 gene, the c/t genotype had the highest frequency (55.4%). For the polymorphism at position -33, the c/t genotype had the highest frequency (51.8%) for the polymorphism at vntr the b1b2 genotype had the highest frequency (50.6%). The genotype frequencies were according to the Hardy-Weinberg equilibrium. The il-4 serum level ranged from 0,61 to 9,32 pg/ml. The parasitemia on the thick blood films ranged from 5 to 15.000 parasites/mm3. There were no statistically significant differences either in parasitaemia, serum il4 level among individuals with different genotypes and haplotypes. Our findings suggest that il4 gene polymorphisms were not associated with serum cytokine and peripheral *P. vivax* parasitaemia in brazilian amazon region. The present findings reinforce and increase our understanding about the role of the immune system on the clinical course of the no severe malaria vivax.

394

POTENTIAL IMPACT OF INTERMITTENT PREVENTIVE TREATMENT (IPT) ON THE ACQUISITION OF ANTIBODIES TO MALARIA ANTIGENS GLURP-R0 AND AMA-1 IN SENEGALESE CHILDREN

Magatte Ndiaye[1], Khadime Sylla[1], Roger C. Tine[1], Babacar Faye[1], Jean Louis Ndiaye[1], Ami Colle Lo[1], Annie Abiola[1], Yemou Dieng[1], Doudou Sow[1], Badara Cissé[1], Michael Theisen[2], Oumar Gaye[1], Michael Alifrangis[2]

[1]Faculty of Medicine, Parasitology Department, University Cheikh Anta Diop, Senegal, Senegal, [2]Centre for Medical Parasitology, Department of International Health, Immunology and Microbiology, University of Copenhagen; Department of Infectious Disease, Copenhagen University Hospital, Copenhagen, Denmark

Intermittent preventive treatment of infants and children (IPTi/IPTc), currently implemented in several sub-Saharan countries with endemic malaria, may disturb natural acquisition of malaria immunity in infants and children. This study aimed to determine the possible impact of IPTi/IPTc on IgG responses to Glutamate-rich Protein-R0 (GLURP-R0) and Apical Membrane Antigen 1 (AMA1) antigens, as markers of acquisition of malaria immunity in Senegalese children living in Saraya district, where IPTi/c has been implemented since 2007 (IPT+), and in Tambacounda district without IPTi/c (IPT-). Sera from 372 randomly selected blood samples (186 from IPT+ and 186 from IPT- communities) blotted onto filter paper, were obtained tree years after IPT was introduced in IPT+ communities and tested by ELISA for IgG response to *AMA1* and *GLURP-R0* antigens. Malaria prevalence by microscopy was 7.5% (176/2353) and 10.1% (81/804) in the IPT+ and IPT- districts respectively. For both antigens, total IgG prevalence and concentrations were higher in IPT- than in the IPT+ group. Among *P. falciparum* negatives samples; the mean antibody response (OD) against *GLURP-R0* was significantly higher (0.089 vs. 0.025 OD) in the IPT- as compared to IPT+ group. Likewise, for the *Plasmodium falciparum* positives samples, the IgG response was significantly higher in IPT- group (*GLURP-R0*=0.258; *AMA-1*=0.089) compared to IPT+ group (*GLURP-R0*=0.128; *AMA-1*=0.043). The low level of IgG antibody response to AMA-1 and GLURP-R0 antigens observed in the IPT+ group indicate that the IPT strategy could delay acquisition of antimalarial immunity. However, other factors could also explain these findings such as marginal differences in the intensity of malaria transmission in the two localities and/or genetic differences between the two populations in their response to the antigens.

395

REGULATORY T CELLS AND IL-10 KINETICS DURING ACUTE UNCOMPLICATED MALARIA AND CONVALESCENCE IN MALAWIAN CHILDREN RESIDING IN HIGH AND LOW MALARIA TRANSMISSION AREAS

Wilson L. Mandala[1], Tonney S. Nyirenda[2], Malcolm E. Molyneux[1], Rupert Kenefleck[3], Lucy Walker[3], Calman A. MacLennan[4], Robert S. Heyderman[2]

[1]College of Medicine, University of Malawi, Blantyre, Malawi, [2]MLW, University of Malawi, Blantyre, Malawi, [3]University of Birmingham, Birmingham, United Kingdom, [4]Novartis Vaccines Institute for Global Health, Siena, Italy

Malaria still infects most Malawian children causing mortality in about 5% of those infected living in both high and low transmission areas. Several factors contribute towards why some children develop the more severe form of malaria which causes death one of which is the host's own immune response to the infection. Regulatory T cells (Tregs) are known to play some role in negating immune-related pathology but also

favour multiplication and maturation of malaria parasites. High levels of Tregs during convalescence would essentially predispose the infected children to more infections in subsequent months and also increase the chance of them developing the more severe, and often lethal, clinical form of the disease. In a prospective cohort study, we recruited children aged 6-60 months presenting to hospital with uncomplicated malaria (UCM) at the point confirmed parasitemia and scores 5 on Blantyre Coma Score (BCS) in Blantyre (low malaria transmission area) and Chikwawa districts (high malaria transmission area). Children were followed after a month and three months during convalescence. A total of 5ml venous blood was collected prior to treatment with LA and we determined the proportion of Tregs CD4+ T cells:CD4+CD25intCD127lowFoxP3+ by immuno-phenotying (IPT), and quantified inflammatory cytokines : IFN-γ, TNF-α and anti-inflammatory cytokine: IL-10, TGFβ using ELISA. Children presenting with UCM had mild lymphopenia and spleenomegaly which normalised in convalescence. Children with UCM had higher levels of Tregs, IL-10 and IFN-γ which also normalised in convalescence. Tregs levels were associated with concentrations of IL-10 but not of TGF-β. As high as 15% of the children recruited when presenting with UCM were still *P. falciparum* parasiteamic during convalescence but none of the participants developed any form of symptomatic malaria during the three months follow-up period. In conclusion, recovery from UCM does not translate to total parasiteamia clearance in infected children in both low and high transmission areas. Therefore, children recovering from UCM should be strongly advised to sleep under ITNs and should be screened for parasiteamia during convalescence and referred for antimalarial prophylaxis where possible.

396

POLYMORPHISM ANALYSIS OF THE CTLA-4 GENE IN *PLASMODIUM VIVAX* MALARIA PATIENTS FROM BRAZILIAN AMAZON REGION

Pamella C. Trindade[1], Gustavo C. Cassiano[2], Franciele M. Tomaz[1], Adriana A. Furini[1], Marcela P. Capobianco[2], Marinete M. Póvoa[3], Valéria D. Fraga[1], Luciana M. Conceição[1], Ricardo L. Machado[3]

[1]Faculdade de Medicina de São José do Rio Preto, São José do Rio Preto, Brazil, [2]Universidade Estadual Paulista, São José do Rio Preto, Brazil, [3]Instituto Evandro Chagas, Belém, Brazil

Plasmodium vivax has been the most common cause of the human malaria parasite in the brazilian amazon region. Cell-mediated immunity requires costimulatory activity to initiate or inhibit antigen-specific t-cell responses. CTLA-4 is an inhibitory receptor expressed by activated and regulatory t cells. The aim of this study was to analyze two snps located on promotor region of CTLA4 gene in *P. vivax* patients and correlated it with parasitaemia and IL-4 levels. A total of 188 *P. vivax* malaria patients were enrolled in the study. Dna was extracted from blood samples using dna-easy kit (invitrogen). A PCR-RFLP protocol was used to analyze the genotype and allele frequencies of these polymorphisms. The density of parasitemia in the infected individuals was recorded and expressed as the number of asexual *P. vivax* per microliter of blood assuming a count of 100 microscopy fields and estimated before treatment. The serum levels of IL-4 were detected by milliplex map kit using magpix/luminex®. Analyses were performed using r version 2.8.1 statistical software. For the polymorphism at position -1577 g>a, the g/a genotype had the highest frequency (49.4%), followed by the g/g genotype (41%) and the a/a genotype (9.6%).for the polymorphism at position -1722 t>c, the t/t genotype had the highest frequency (86.7%), followed by the T/C genotype (12.3%) with the least frequent being the C/C genotype (1%). The IL-4 plasma level ranged from 0,61 to 9,32 pg/ml. There were no statistically significant differences either in parasitaemia and plasma il4 levels among individuals with different genotypes. This study showed that there was no association between the CTLA-4 snps with the development of malaria vivax, serum cytokine and peripheral *P. vivax* parasitaemia in brazilian amazon region. The CTLA -4 snps may be associated with malaria vivax in other endemic areas, but it appears to have no such effect in this studied population. The study also highlights the importance of conducting genetic association studies in different ethnic populations.

397

ACQUIRED IMMUNITY AND RISK OF RECURRENCE FOLLOWING RADICAL TREATMENT AGAINST *PLASMODIUM VIVAX*

Julio A. Ventocilla[1], Salomon Durand[1], Meddly L. Santolalla[1], Karina P. Leiva[1], Cesar Cabezas[2], Carlos Alvarez[3], Andres A. Lescano[1], G. Christian Baldeviano[1]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Instituto Nacional de Salud del Peru, Lima, Peru, [3]Direccion Regional de Salud de Loreto, Lima, Peru

Plasmodium vivax recurrence after supervised Primaquine/Choloquine (CQ/PQ) radical cure is due to interactions between parasite and host factors controlling recrudescence, relapse or re-infection. Since immunity to symptomatic malaria is primarily mediated by antibodies, we hypothesized that high antibody titers against merozoite surface proteins (MSP) during acute malaria infection would have decreased risk of recurrence following radical cure. A clinical trial aimed at assessing the efficacy of three different primaquine regimes in the Peruvian Amazon enrolled 540 acute *P. vivax* monoinfections between 2006-8. Patients were followed for 210 days after treatment and 90 recurrent cases were identified while 395 individuals did not experienced malaria recurrence. Antibodies against selected merozoite proteins were measured using ELISA assays and six putatively neutral microsatellite markers was genotyped to distinguish homologues vs. heterologous recurrence. The relationship between age-dependent antibody responses during acute infection and the risk of recurrence in this population was analyzed using survival analysis. The median titers of anti-*P. vivax* MSP-1 IgG antibodies (PvMSP-1 IgG) was 3.5 times higher among recurrent cases versus recurrence-free individuals ($p<0.001$). Only 2% (4/194) of subjects in the two upper antibody level quintiles experienced recurrence while 36% (69/194) and 17% (17/97) had recurrences in the second/third and lower quintile, respectively. This effect was confirmed by Cox hazard regression analysis and was found to be independent of patient age, parasitemia, PQ dose and other covariates likely to influence recurrence rate. Our findings suggest that the intensity of anti PvMSP-1 antibodies during acute infection is strongly associated with symptomatic recurrence independently of the dose of primaquine used in the radical cure treatment.

398

ACUTE *PLASMODIUM FALCIPARUM* MALARIA EXPANDS T FOLLICULAR HELPER CELLS IN MALIAN CHILDREN

Nyamekye Obeng-Adjei, Peter Crompton, Susan Pierce

Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Immunity to clinical malaria is only acquired after years of repeated *Plasmodium falciparum* infections in children living in malaria endemic areas. Antibodies have been shown to be protective against clinical malaria, but the acquisition and maintenance of long-lived plasma cells and memory B cells in response to *P. falciparum* appear inefficient in response to natural infection. The generation of long-lived plasma cells and memory B cells requires the function of follicular helper $CD4^+$ T (T_{FH}) cells in germinal center reactions. To better understand the cellular basis of the inefficient acquisition of protective antibody immunity in malaria, we conducted a longitudinal study to determine whether T_{FH} cells are generated in response to natural *P. falciparum* infections in children. We found that T_{FH} cells as defined as $CD4^+$, $CD45RO^{+/-}$, $CXCR5^+$, $PD1^+$ and $CXCR3^{+/-}$, increase in the peripheral circulation of children during acute *P. falciparum* malaria compared to the children's pre-infection baselines. ICOS, a costimulator that is essential for T_{FH} development and function, was markedly up-regulated during infection suggesting that these T_{FH} may

be highly functional. Work is in progress to directly measure the capacity of these T_{FH} cells to provide help to memory B cells *in vitro*. In addition we are examining whether the magnitude and quality of malaria-induced T_{FH} correlate with the magnitude and quality of *P. falciparum*-specific antibody responses and clinical malaria outcomes.

399

A CROSS-SECTIONAL SURVEY OF ANTIBODY RESPONSES TO *PLASMODIUM FALCIPARUM* ANTIGENS IN A REGION OF DECLINING MALARIA TRANSMISSION IN SOUTHERN ZAMBIA

Ben Katowa[1], Tamaki Kobayashi[2], Harry Hamapumbu[1], Jailos Lubinda[1], Philip E. Thuma[1], William J. Moss[2]

[1]Macha Research Trust, Choma, Zambia, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Malaria transmission has declined in southern Zambia during the past decade. With reduced transmission, malaria reservoirs become increasing difficult to identify using conventional diagnostic methods such as rapid diagnostic test (RDT). Serology could be a useful tool to detect areas with ongoing transmission. Households were randomly selected using satellite imagery and participants were enrolled into a cross-sectional survey in 2012. A blood sample was collected by finger prick for RDT and dried blood spots (DBS) following written, informed consent. The DBS were collected on Whatman, Protein Saver card 903 and dried overnight. The DBS were later assayed for immunoglobulin G (IgG) antibodies to *P. falciparum* whole asexual stage lysate using an enzyme immunoassay (EIA). The threshold for seropositivity was determined using serum from US residents never exposed to malaria (which was defined as three standard deviations from ten samples). In 2012, 686 DBS were collected and analysed. The parasite prevalence by RDT was 0.2%. The mean EIA optical density (OD) values were 0.65, 0.71, 0.83, 0.90 and 1.1 for age less than 5 years, 5-10 years, 10-15 years, 15-20 years and older than 20 years, respectively. Similarly, seropositivity increased with age and was 12%, 22%, 45%, 57% and 75% for age less than 5 years, 5-10 years, 10-15 years, 15-20 years and older than 20 years, respectively. The low seropositivity and mean EIA OD values in younger age groups are consistent with a reduction in exposure to *P. falciparum*. The household locations of seropositive children younger than five years of age were mapped using ArcGIS to identify areas of malaria transmission. Serology can complement information obtained from RDTs to identify hot spots of recent malaria transmission.

400

ELEVATED LEVELS OF IL17 AND ITS ASSOCIATION WITH LOW HAEMOGLOBIN IN SEMI-IMMUNE MICE INFECTED WITH *PLASMODIUM BERGHEI* ANKA

Gideon K. Helegbe[1], Nguyen T. Huy[2], Tetsuo Yanagi[2], Mohammed N. Shuaibu[2], Mihoko Kikuchi[2], Mahamoud S. Cherif[2], Kenji Hirayama[2]

[1]University for Development Studies, School of Medicine and Health Sciences, Tamale, Ghana, [2]Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki, Japan

Malaria anaemia is still a major public health problem and its pathogenesis still unclear. Interestingly, the progression of anaemia is at relatively low parasitaemia with some mortality in the semi-immune individuals in the endemic areas. A recent study has observed variation in IL17 levels in different mice strains, thus implicating autoimmune mechanism in RBC loss due to *Plasmodium* infection. The objective of this study was to evaluate IL17 levels in anaemic condition and to determine if genetic factors are involved. To address this objective, we crossed two mice strains (Balb/c of low parasitaemia) and CBA (moderately high parasitaemic) to get the progeny, called the F1. Balb/c (8), CBA (8) and F1 (12) were taken through 6-7 cycles of infection (with *Plasmodium berghei* ANKA) and treatment (with chloroquine/pyremethamine) to generate semi-immune status. Parasitaemia and haematological parameters were monitored. Kinetics of antibody production, cytokine levels (in serum and cultured supernatant of stimulated spleen cells) and CD4+CD25+T regulatory cells were evaluated by ELISA, bead-based multiplex assay kit and FACs respectively; at days 0, 16, 28 for Balb/c and F1, and days 0, 8 and 12 for CBA. Similar survival (>70%), mean %Hb loss (45%) and mean parasitaemia (5%) was observed in Balb/c and F1, while 0% survival, mean %Hb loss (80%) and mean parasitaemia of 15% was observed in CBA. IgG subtypes were two times higher in Balb/c and F1 than CBA. While IL1a, IL4, IL10, IL12a, IFNγ and TNFα were similar in the three mice strains, IL17 was 3 times higher in Balb/c and F1 than CBA. Maximum cytokine level was observed at D16 (point at which recovery from parasitaemia occurs, with lowest Hb) in the Balb/c and F1. CD4+CD25+ Treg cells in CBA were lower than those of Balb/c and F1. In conclusion, autoimmune is implicated in Hb loss due to high IL17 levels and may be controlled by a genetic factor. Further studies of F2 between the F1 and Balb/c will be informative to evaluate if these genes are segregated or further apart

401

THE MALARIA VACCINE CANDIDATE GMZ2 ELICITS FUNCTIONAL ANTIBODIES IN INDIVIDUALS FROM MALARIA-ENDEMIC AND NON-ENDEMIC AREAS

Larissa Bouyoukou Hounkpatin

Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon

GMZ2 is a hybrid protein consisting of the N-terminal region of the glutamate-rich protein fused in frame to the C-terminal region of merozoite surface protein 3 (MSP3). GMZ2 formulated in Al(OH)3 has been tested in 3 published phase 1 clinical trials. The GMZ2/alum formulation showed good safety, tolerability, and immunogenicity, but whether antibodies elicited by vaccination are functional is not known. Serum samples prior to vaccination and 4 weeks after the last vaccination from the 3 clinical trials were used to perform a comparative assessment of biological activity against *Plasmodium falciparum*. We showed that the maximum level of immunoglobulin G (IgG) antibodies obtained by GMZ2 vaccination is independent of ethnicity, time under malaria-exposure, and vaccine dose and that GMZ2 elicits high levels of functionally active IgG antibodies. Both, malaria-naive adults and malaria-exposed preschool children elicit vaccine-specific antibodies with broad inhibitory activity against geographically diverse *P. falciparum* isolates. Peptide-mapping studies of IgG subclass responses identified IgG3 against a peptide derived from MSP3 as the strongest predictor of antibody-dependent cellular inhibition. These findings suggest that GMZ2 adjuvanted in Al(OH)3 elicits high levels of specific and functional antibodies with the capacity to control parasite multiplication.

402

FREQUENCY AND PHENOTYPE OF ΓΔ T CELL SUBSETS IN AN AREA OF HIGH MALARIA TRANSMISSION INTENSITY

Felistas N. Namirimu[1], Prasanna Jagannathan[2], Katherine Bowen[2], Ann Auma[1], Michelle Boyle[2], Lila Farrington[2], Mayimuna Nalubega[1], Samuel Wamala[1], Esther Sikomyu[1], Kate Naluwu[1], John Rek[1], Ronald Senoga[1], Emmanuel Arinaitwe[1], Grant Dorsey[2], Moses R. Kamya[3], Margaret E. Feeney[2]

[1]Infectious Diseases Research Collaboration, Kampala, Uganda, [2]University of California San Francisco, San Francisco, CA, United States, [3]Makerere University, Kampala, Uganda

Plasmodium falciparum infection is thought to induce potent immunoregulatory responses, but the precise mechanisms in humans are unclear. Prior studies have shown that a subset of γΔ lymphocytes, VΔ2$^+$ T cells, become rapidly activated upon stimulation with *P. falciparum* antigens, suggestive of an innate-like immune response. We have found that repeated exposure to malaria is associated with loss and dysfunction of VΔ2$^+$ T cells in 4-year-old children living in a high endemicity setting. To test the hypothesis that repeated exposure to malaria also leads to increased expression of immunoregulatory markers on this γΔ subset,

we measured the frequency and phenotype of γΔ T cell subsets in children and their caregivers (n=171) enrolled in a longitudinal cohort study in Nagongera, a rural area in Eastern Uganda with high perennial transmission intensity (EIR 315). We measured frequencies of VΔ1$^+$ and VΔ2$^+$ T cells and evaluated cell surface expression of Tim-3, PD1, ILT2, CD57, CD56, CD16, and NKG2A by multiparameter flow cytometry using freshly obtained peripheral blood mononuclear cells. The mean age of children in the cohort was 6.6 yrs (range 10 months-11 years, n=128), and was 37 yrs in their caregivers (range 20.2-67.7 years, n=43). The mean incidence of malaria was 4 episodes ppy in children (range 0-11 ppy) and 1 episode ppy in their caregivers (range 0-5 ppy). In multivariate linear regression, we observed a significant inverse association between the frequency of VΔ2$^+$ T cells and the prior incidence of malaria (P<0.001), independent of age. We further found significantly increased expression of Tim-3, CD57, NKG2A, and CD16 on VΔ2$^+$ T cells with increasing age. Our findings are consistent with the hypothesis that, in high endemicity settings, cumulative exposure to malaria may be associated with increased expression of regulatory markers on VΔ2$^+$ T cells. These findings have important implications in understanding the immunopathogenesis of malaria in childhood.

403

THE ASSOCIATION BETWEEN MATERNAL HOOKWORM INFECTION IN PREGNANCY AND ANTIMALARIAL ANTIBODY RESPONSES IN THE OFFSPRING

Juliet Ndibazza[1], Chris Drakeley[2], Alison Rand[2], Gyaviira Nkurunungi[1], Angela Nalwoga[1], Lawrence Muhangi[1], Birgitte Vennervald[3], Alison Elliott[1]

[1]Medical Research Council/Uganda Virus Research Institute, Entebbe, Uganda, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]University of Copenhagen, Copenhagen, Denmark

Several studies have shown that helminth infections have adverse effects on malaria. However, the immunological mechanism by which helminths modify the host response to malaria is uncertain. In a trial of anthelminthics in 2, 507 pregnant women [ISRCTN32849447], maternal hookworm infection was associated with an increased risk of clinical and asymptomatic malaria in their offsprings. We hypothesised that helminth infection in pregnancy modifies the fetus' initial response to malaria and, consequently, their antimalarial immune responses in early childhood. Stored blood samples from children of study women were tested for IgG antibody concentrations (µg/ml) to Apical Membrane Antigen-1 (AMA1) and Merozoite Surface Protein-1 (MSP1) by enzyme immunoassay (EIA). Overall, antibody concentrations to anti-AMA1 and anti-MSP1 were significantly higher (p<0.001) among children with asymptomatic parasitaemia compared to children without parasitaemia. However, at the age of one year, parasitaemic children of hookworm infected mothers had a significantly lower concentration of anti-AMA1 antibody (mean $\log_{10}$ (95% CI): 3.51 (3.03-4.07)) compared with parasitaemic children of uninfected mothers (4.07 (3.58-4.63)), (p for interaction = 0.003). Similarly at five years of age, parasitaemic children of hookworm infected mothers had a significantly lower concentration of anti-AMA1 antibody (4.48 (3.67-5.48)) compared with parasitaemic children of uninfected mothers (5.84 (5.01-6.80)), (p for interaction = 0.01). Maternal hookworm infection did not modify anti-MSP1 responses in children. These results highlight the importance of controlling for helminth co-infections in the assessment of malaria vaccine efficacy. Further immunological studies to understand the role of helminth co-infections on antimalarial immune responses are essential, and could subsequently lead to improved results for the malaria vaccine effort.

404

PHAGOCYTOSIS AND OPSONIZATION OF *PLASMODIUM FALCIPARUM* GAMETOCYTES

Corey S. Weinstein, Dibyadyuti Datta, Nirbhay Kumar, **Geetha P. Bansal**

Tulane University, New Orleans, LA, United States

During natural infection malaria parasites are injected into the bloodstream of a human host by the bite of an infected female *Anopheles* mosquito. Immune responses are generated against various life cycle stages of the parasite that have important roles in resistance to malaria and in reducing natural transmission. A better understanding of the early immune response against mature gametocyte stage of the parasite will inform transmission blocking vaccine strategies. The asexual and sexual erythrocytic life cycle forms of *Plasmodium* are stages that pass through humans into *Anopheles* mosquitoes during a blood meal. The sexual stages develop into competent parasites in the mosquitoes and are later injected back into a human host to complete a transmission cycle. There is a fairly good understanding of the adaptive cellular and humoral immune responses, but little is clearly known about the innate immune response. Several published studies suggest that phagocytosis and opsonization of asexual, immature sexual and free merozoite stages occur. We hypothesize that circulating mature gametocytes are phagocytosed directly or after opsonization with specific antibodies present in immune sera from people with previous exposure to malaria. We evaluated phagocytosis of various developmental forms of erythrocytic stage parasites in the absence of immune sera, and opsonization in the presence of immune sera. Results focusing on the uptake of mature gametocytes by phagocytic cells in order to differentiate the opsonic and phagocytic responses will be presented. Identification of phagocytic receptors and targets of opsonizing antibodies will enhance understanding of the role of phagocytosis in the clearance of circulating mature gametocytes, and the link to humoral arm of adaptive immunity. Phagocytosis and opsonization mediated immune activation together with presentation of gametocyte specific antigens will contribute to natural transmission blocking immunity. Specific strategies may also be developed to induce similar antibodies by vaccines aimed at overall transmission reduction.

405

ASYMPTOMATIC MULTICLONAL *PLASMODIUM FALCIPARUM* INFECTIONS CARRIED THROUGH THE DRY SEASON PREDICT PROTECTION AGAINST CLINICAL MALARIA IN THE FOLLOWING HIGH TRANSMISSION SEASON

Klara Sondén[1], Safiatou Doumbo[2], Ulf Hammar[1], Manijeh Vafa-Homann[1], Aissata Ongoiba[2], Boubacar Traoré[2], Matteo Bottai[1], Peter D. Crompton[3], Anna Färnert[1]

[1]Karolinska Institutet, Stockholm, Sweden, [2]Mali International Center of Excellence in Research, Bamako, Mali, [3]National Institutes of Health, Rockville, MD, United States

Immunity to the antigenically diverse *Plasmodium falciparum* parasite is acquired gradually after repeated exposure. Infections often consist of several concurrent parasite clones and the diversity of infections in asymptomatic individuals has predicted a reduced risk of malaria during follow up in high transmission settings. In areas of continuous transmission, the number of clones might be a marker of exposure and it is difficult to distinguish between persistent and transient infections. Therefore, we assessed the genetic diversity of *P. falciparum* infections in a longitudinally followed cohort in an area of highly demarcated seasonal malaria transmission in Mali. During the one-year study period, cases of febrile malaria were detected by passive surveillance among 225 individuals aged 2-25 years of age.. Genotyping of the highly polymorphic merozoite surface protein 2 gene (MSP2) was performed on blood samples collected at five cross-sectional surveys. The genetic diversity was highest in children 5-10 years old and increased during the peak of transmission in all age groups. Detection of multiclonal infections before the rainy season

conferred a decreased risk of malaria, compared to being parasite negative or having one clone detected, when assessing time to first malaria episode The results suggest that persistent multiclonal infections carried through the dry season protect against subsequent clinical malaria, probably by maintaining otherwise waning protective immune responses.

406

IDENTIFYING MRNA TARGETS OF *PLASMODIUM FALCIPARUM* RNA BINDING PROTEINS USING DNA MICROARRAY

Chayaphat Wongsombat

National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailand

Although resistance has rendered the once standard antimalarial Pyrimethamine ineffective against *Plasmodium falciparum* across the world, it is hope that new compounds can be developed to combat the pyrimethamine resistant strains of *P. falciparum*. Although much is known about the drug-resistant variant enzymes in terms of their structures and biochemical characteristics, little is known of how the DHFR-TS enzyme is regulated in the parasite cell. Through greater understanding of the cellular regulation, antifolates could be employed in a more effective manner, e.g. avoiding the known phenomenon of drug-induced expression of DHFR-TS. Intringuingly, DHFR-TS has nucleic acid binding activity *in vitro*, and is thought to be involved with post-transcriptional regulation of gene expression, including itself. In an attempt to characterize its putative mRNA targets and identify conserved regulatory elements amongst the targets, we use the recombinant DHFR-TS to separate its targets from total parasite RNA. The selected RNAs are then interrogated by DNA microarray analysis and identified en masse. In order to validate the approach, the RNA targets of the *P. falciparum* Bruno-like putative RNA binding proteins will be characterized initially and compared by bioinformatics with known Bruno-like mRNA targets in other species.

407

RAPID GENE REPLACEMENTS IN *PLASMODIUM FALCIPARUM* USING A NEW APPLICATION OF ATTP X ATTB TECHNOLOGY

Udayaprakash Murugesan[1], Savitha Chellappan[1], Michael W. Mather[2], Sanjay Desai[3], **Praveen Balabaskaran Nina**[1]

[1]PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India, [2]Drexel University College of Medicine, Philadelphia, PA, United States, [3]National Institutes of Health, Rockville, MD, United States

Allelic exchange transfection in *Plasmodium falciparum* is an arduous, low efficiency process that often takes 6 - 12 months; many experiments do not work and are presumed to fail because the desired changes may be lethal to the haploid blood-stage parasite. We have found that Bxb1 integrase mediated attP x attB technology could be successfully used to overcome limitations in manipulating the endogenous copy of clag3, recently shown to be essential for parasite nutrient acquisition. The attB sequence was successfully introduced into a native clag3 intron by conventional allelic exchange transfection. Introduction of this sequence did not adversely affect intron splicing and yielded an intact, unmodified protein. Subsequent transfection with attP-containing plasmid that carries desired downstream changes in the clag3 gene yielded rapid introduction of desired site-specific integrants that came up as early as 3 weeks in culture. The major limitation of this strategy of introducing attB into an intronic sequence is that nearly half of the genes in the *Plasmodium* genome are devoid of introns To overcome this limitation, we are adapting this strategy to study the function of endogenous copies of nonintronic genes such as Dynamin-2 (PfDyn2), Cardiolipin synthase (PfCLS) and MSP7. We are introducing an intron that contains the attB sequence, into the coding (exon) regions of PfDyn2, PfCLS and MSP7. For integration into the ORF of PfDyn2, we have made a construct that comprises a 2 Kb gene fragment from the 3' end of PfDyn2, which incorporates a 200bp intron that includes a central 40bp attB site. The distal 800bp of the 2 kb construct has been re-coded (using E.coli codon frequencies) to ensure integration occurs upstream of the intron. A similar approach has been followed for making the PfCLS construct. Transfection studies are underway, and upon successful introduction of introns with the attB sequence into the coding regions, we will be able to rapidly manipulate the PfDyn2, PfCLS and MSP7 loci by Bxb1 integrase mediated, rapid site specific recombination of attP-containing constructs into the attB sequence. This technology will help us manipulate endogenous copies of nonintronic genes and is highly cost-effective. It has the potential to open up new avenues for investigations of malaria parasite molecular biology.

408

GENETIC CHARACTERISTICS OF PARASITES IN PREGNANCY-ASSOCIATED MALARIA IN COLOMBIA

Eliana M. Arango Florez[1], Roshini Samuel[2], Olga M. Agudelo Garcia[1], Jaime Carmona Fonseca[1], Amanda Maestre[1], Stephanie K. Yanow[2]

[1]University of Antioquia, Medellin, Colombia, [2]University of Alberta, Edmonton, AB, Canada

This study wanted to analyze the genetic diversity and frequency of resistant-sensitive genotypes of *Plasmodium falciparum* and *P. vivax* isolated from peripheral and placental blood of pregnant women, and non-pregnant population of Colombia, as well as to explore associations between the plasmodial infection in peripheral and placental blood with placental histology, maternal anemia and low birth weight. Study populations: a) 96 pregnant women at delivery, independently of malaria symptoms; b) 130 patients with acute malaria (about half per each species), corresponding to 50 pregnant women attending antenatal care, 40 non pregnant women and 40 men. Plasmodial infection was detected by thick smear and quantitative real time PCR (qPCR). Parasite genotypes in compartments (periphery vs. placenta) and populations (pregnant vs. non pregnant) were compared using five molecular markers per each species, which were analyzed by nested PCR and capillary electrophoresis. Point mutations in the genes dhps, dhfr and mdr1 of both species were analyzed by direct sequencing. In pregnant women at delivery, qPCR detected significantly more infections (130/288) than microscopy (20/288); 65% of women had at least one positive compartment and the placenta exhibited the highest frequency of infection (57%). *P. falciparum* was detected in 63% (82/130) of infections at delivery and most of them (65%; 84/130) had <2 DNA copies/µL. Both *P. vivax* and *P. falciparum* microscopic and submicroscopic placental infections were associated with villitis and intervillitis. Placental malaria infections were not associated with maternal anemia or low birth weight. *P. falciparum* placental isolates had significant genetic differentiation compared to peripheral isolates from pregnant and non-pregnant patients with acute malaria. On the contrary, all the *P. vivax* isolates were genetically similar independently of the population or compartment. More than 90% of the analyzed isolates of both species had the triple mutation genotype in genes dhfr-dhps associated with sulfadoxine/pyrimethamine resistance. All *P. falciparum* isolates had the mutation 86Y in Pfmdr1, while only one *P. vivax* isolate had the double mutation 976F-1076L in Pvmdr1, associated with chloroquine resistance.

409

ANTI-PLASMODIAL PROPERTIES OF SOME SELECTED GHANAIAN MEDICINAL PLANTS; IDENTIFICATION OF NOVEL ACTIVE COMPOUNDS AGAINST *PLASMODIUM FALCIPARUM*

Jeffrey Agyapong[1], Mitsuko Suzuki[2], Gordon A. Awandare[3], Michael Ofori[1], Irene Ayi[1], Eric H. Frempong[4], Daniel A. Boakye[1]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Tokyo Medical and Dental University, Tokyo, Japan, [3]University of Ghana, Accra, Ghana, [4]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Malaria, caused by *Plasmodium* spp has been considered as a major disease of public health importance affecting multitudes of people worldwide, particularly in the tropics and sub-tropics. Due to increasing drug resistant parasites to existing drugs, development of new anti-malarial drugs are eagerly awaited. In Africa, there is extensive use of traditional medicinal plants for treatment of various diseases. Many research studies into medicinal plants have shown their significant potentials for anti-plasmodial properties but a few of the active ingredients have been studied.. The present study aimed at screening selected medicinal plants used in Ghana for activity against *Plasmodium falciparum* to determine their active compounds. A high-throughput 96 wells flow-cytometry screening system was established using the method reported by Smilkstein and others, with modification. 50% Et-OH crude extracts of medicinal plants were prepared and applied to synchronized *P. falciparum* (3D7 strains) culture and FACS analysis was carried out to determine the IC_{50} of the extracts. Although screening is still ongoing, one active crude extract, JJNC008L, was found possessing activities against *P. falciparum* and identified two novel compounds from this extract, compound #1 and #2. These compounds have significantly high anti- *P. falciparum* activities with IC_{50} values of 3.89 µM and 1.52 µM respectively. Novel compounds identified in this study could be candidates to develop new chemotherapy for malaria. Furthermore, our high-throughput FACS screening system could be useful tool for malaria drug assay.

410

PRODUCTION AND CHARACTERIZATION OF A LIBRARY OF FULL-LENGTH *PLASMODIUM VIVAX* MEROZOITE PROTEINS

Jessica Hostetler[1], Sumana Sharma[2], Gavin J. Wright[2], Julian Rayner[2], Rick Fairhurst[1]

[1]National Institutes of Health, Rockville, MD, United States, [2]Wellcome Trust Sanger Institute, Hinxton, United Kingdom

A vaccine targeting the illness-inducing blood stage of parasite development is an essential component of any worldwide malaria eradication campaign, but major gaps in our understanding of *Plasmodium vivax* biology, including the protein-protein interactions that mediate erythrocyte invasion, hinder the search for an effective vaccine. Only a single parasite ligand-host receptor interaction is presently known, that between *P. vivax* Duffy Binding Protein (PvDBP) and Duffy Antigen Receptor for Chemokines (DARC), and strain-specific immune responses to PvDBP make this antigen a challenging vaccine target. We are carrying out a comprehensive study of *P. vivax* proteins that mediate erythrocyte binding and invasion in order to identify additional vaccine candidates. As a first step, we produced a library of 39 full-length recombinant *P. vivax* proteins to test for erythrocyte binding and immunoreactivity. To our knowledge, this represents the largest full-length *P. vivax* antigen set ever assembled. Candidates were selected based on predicted localization to the merozoite surface or invasive secretory organelles, and on homology to *P. falciparum* vaccine candidates. 37/39 *P. vivax* recombinant proteins were expressed in the HEK293E cell system, which has been successfully used for expression of full-length *P. falciparum* invasion ligands such as PfRH5. Known or predicted functions, such as the interaction between merozoite surface proteins Pv12 and Pv41, were confirmed and several novel parasite protein-protein interactions were identified. Pilot immunoreactivity screens using sera from Cambodian patients with *P. vivax* malaria showed that IgG variously recognize the majority of antigens tested. The large-scale initial screenings of this library will be presented through protein expression, protein interaction and seroreactivity data, as well as immuno-epidemiological studies.

411

GENETIC VARIABILITY AND POPULATION STRUCTURE OF KENYAN *PLASMODIUM FALCIPARUM* ISOLATES

Luicer I. Olubayo

United States Army Medical Research Unit-Kenya, Kisumu, Kenya

Malaria parasite genetic variability and population structure varies among areas of differing endemicity and are key factors in malaria control strategies. Unlike previously used antigen-encoding loci under strong selection, *Plasmodium falciparum* microsatellite markers are an attractive target for population structure studies. In Kenya, molecular studies on drug resistant malaria and antigenic molecules have been inadequate in providing accurate genetic profiles of parasite populations in the country. Understanding the genetic structure of malaria parasites is essential to predict how fast phenotypes of interest, such as novel antigenic variants or drug resistant alleles, originate and spread in populations. Twelve polymorphic *P. falciparum* microsatellite loci were genotyped in 250 parasite isolates from five locations in Kenya using capillary electrophoresis. Analysis of the generated fragments was performed to determine proportions of mixed genotype infections, genotype diversity among isolates, multilocus standardized index of association, and inter-population differentiation. The data revealed dramatic differences in parasite population structure in different geographical locations. An 80% multiplicity of infection was detected in parasites circulating in Kenya. Samples from Kisumu, a high malaria endemic region had a high diversity (He _ 0.73) revealing genetic crossing. Results have shown a *P. falciparum* population structure as well as significant linkage disequilibrium in Kenyan parasites. Regional diversity was observed in range of population structures. These results could be related to geographic difference and low flow of parasites between sites. These data reveal a range of population structures within a single pathogen species and suggest intimate links between patterns of epidemiology and genetic structure.

412

GENETIC DIVERSITY OF *PLASMODIUM VIVAX* IN AREAS OF HIGH RISK OF MALARIA IN CÓRDOBA-COLOMBIA

Carlos J. Castro, Virginia Rodriguez, Maria Fernanda Yasnot

Universidad de Córdoba, Monteria, Colombia

The aim of this work was to study the genetic diversity of natural populations of *Plasmodium vivax* in areas of high malaria risk in Córdoba-Colombia. Molecular confirmation of infection by *P. vivax* DNA extraction using Chelex-100 was made, thereafter a nested PCR protocol was used to amplify the gene encoding 18S ribosomal small subunit (ssrARN) *P. vivax* and *P. falciparum*. A sample confirmed as *P. vivax* underwent a PCR -RFLP for Pvmsp - 3α gene, restriction enzymes used were Alu I and Hha I, which generated an electrophoretic profile that identified different haplotypes for Pvmsp - 3α gene.Of the 125 samples analyzed by nested PCR for Pvmsp - 3α gene, 116 successfully amplified. The size of the PCR products allowed to demonstrate the movement of three different genotypes for the Pvmsp - 3α gene: A (1900 bp), type B (1500 bp) and type C (1100 bp), being the most frequent genotype A (88%). 97.4 % (113 /116) of the samples showed simple infections and 2,6% (3/116) polyclonal infections, two by types A and B and one by types A and C. Besides finding a band is reported in the electrophoretic profile of the amplification products Pvmsp -3α gene with an approximate size of 800 bp which does not correspond with the sizes reported to date, which may represent a new allele of Pvmsp-3α gene however, these results should be interpreted cautiously, pending the results of sequencing. Digestion of PCR products obtained for the Pvmsp - 3α gene with enzyme Alu I showed ten different restriction patterns while the with the enzyme Hha I produced nine. The results of

the restriction enzyme digestion of the 113 samples tested revealed that 40/113 (35.3%) and 47 /113 (41.6%) of these samples showed polyclonal infections when they are digested with the enzyme Alu I and Hha I, respectively. There is a high degree of genetic variability in Pvmsp-3α gene of *P. vivax* parasite population circulating in the department of Córdoba.

413

THE ROLE OF TEMPORALLY REGULATED RNA-BINDING PROTEINS IN *PLASMODIUM* INFECTION

Elyse E. Munoz, Scott E. Lindner

Pennsylvania State University, University Park, PA, United States

Translational repression of specific mRNAs allows the temporal modulation of gene expression by *Plasmodium* species. This mechanism enables regulated preparation for the vector/host and host/vector transmission events and perhaps the infection of a new red blood cell. To further our understanding of this mechanism, we have employed genetic manipulation of *P. yoelii*, a rodent-infectious parasite, to investigate RNA-binding proteins that may play critical roles in this process. Our previous studies have shown that Puf2, a member of the Pumilio-FBF family of RNA-binding proteins, plays an integral role in maintaining the infectivity of salivary gland sporozoites, as well as regulating RNA homeostasis and translational repression. In the absence of Puf2, salivary gland sporozoites become less infectious with prolonged salivary gland residence time, and undergo premature developmental changes, resembling liver stage parasites while still in the salivary gland. In the absence of Puf2, the transcript abundance of ALBA4 increases by approximately 120-fold. Proteins containing the ALBA domain (acetylation lowers binding affinity) are functionally important in several parasite species, including *Leishmania, Trypanosoma*, and *Plasmodium*, where it has been shown to bind DNA and RNA. Because of their ability to bind RNA, we are interested in the potential role of ALBA proteins in translational repression. In the human-infectious species *Plasmodium falciparum*¸ ALBA4 protein localization changes from perinuclear to cytoplasmic during blood stage progression. In *P. yoelii*, we also have evidence for cytoplasmic localization of ALBA4 in blood stage schizonts as seen by live fluorescence microscopy. To fully understand the implications of this finding, we are extending these studies by genetically deleting ALBA4 to determine its essentiality, and examining ALBA4's expression profile in other life cycle stages to determine its function and importance.

414

GENETIC VARIATIONS OF *PLASMODIUM VIVAX* IN THE PERUVIAN AMAZON: DIVERSITY ASSOCIATED WITH POPULATION GENETICS AND VACCINE CANDIDATES GENES AGAINST MALARIA

Manuel M. Fasabi[1], Raul Chuquiyauri[2], Maritza Calderón[3], Robert H. Gilman[4], Joseph M. Vinetz[5]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Institute of Tropical Medicine Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Laboratory of Molecular Biology, LID, Universidad Peruana Cayetano Heredia, Lima, Peru, [4]Asociación Benéfica Prisma, Lima, Peru, [5]Unit of Infectious Diseases, University of California San Diego, La Jolla, CA, United States

Knowledge of both genetic variability of *Plasmodium vivax* and polymorphism of its main vaccine candidate genes is of great importance to complement the strategies of control, treatment and development of vaccines against this parasite. The current study had two main objectives, first, to determine the genetic variability of *P. vivax* in the Peruvian Amazon among years 2006-2009 using the merozoite surface protein gene (PvMSP-3α) as a molecular marker, and second, to determine the genetic polymorphism of both the Circumsporozoite protein (PvCSP) and major transmission blocking vaccine candidates, Pvs25 and Pvs28. A total of 1780 samples were obtained from the communities of Santo Tomas, San Jose de Lupuna, Padrecocha and Mazan. By PCR, we found 3 variants of PvMSP-3α gene named type A of 1.9 kb (93.4%), type B of 1.5 kb (6.1%) and type C of 1.2 kb (0.3%); besides, a mixture of two types of amplified fragments (A/B or A/C) were showed in 0.2% of the samples. Also by PCR-RFLP, we identified 11 different alleles of PvMSP-3α. To know the Circumsporozoite protein genetic polymorphism, 42 samples were analyzed; we found 11 types of PvCSP which were composed of 5 different nonapeptides, all belonging to VK210 type. On the other hand, the genetic polymorphism of Pvs25 and Pvs28 were studied by sequencing 51 and 49 samples, respectively; each result were compared to reference strain Sal I. For Pvs25, a non synonymous mutation (Q87K[cag/aac] was identified giving two haplotypes. For Pvs28, four non synonymous mutation were reported (M52L[atg/ctg], D87N[gat/aat], N110Y[aat/tat] y T140S[acc/agc]) resulting on four new haplotypes. All Pvs25 haplotypes and just one Pvs 28 haplotype were reported for all the studied Peruvian Amazon Communities. Our results confirm there are different genotypes of P vivax in the Peruvian Amazon and that polymorphism of major transmission blocking vaccine candidates, Pvs25 and Pvs28, is more limited than polymorphism of PvCSP; the implications of these genetic diversity in vaccine development should be studied.

415

INHIBITION OF RETICULOCYTE MATURATION TO INCREASE *PLASMODIUM VIVAX* PROLIFERATION *IN VITRO*

Kathryn Shaw Saliba[1], Caeul Lim[1], Fiona Raso[1], Elsa Hansen[1], Caroline Buckee[1], Carlo Brugnara[2], Dyann Wirth[1], Manoj Duraisingh[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Children's Hospital Boston, Boston, MA, United States

Plasmodium vivax is restricted to the reticulocyte for both growth and invasion. One of the major obstacles to successful continuous *in vitro* culture of *P. vivax* is that reticulocytes rapidly mature to erythrocytes *in vitro*. This steady maturation effectively dilutes the culture, as constant replenishment of reticulocytes is essential for sustained parasite propagation. We hypothesize that inhibition of reticulocyte maturation would increase the number of viable cells for invasion and would enhance *P. vivax* proliferation overall. Reticulocyte maturation involves hallmark cellular processes: (i) membrane remodeling and (ii) autophagy of organelles, which can be targeted by small molecules. We initially undertook a small-scale screen to show that reticulocyte maturation can be inhibited and the inhibited cells can support parasite invasion and growth. On going research includes a large-scale screen with a panel of bioactive small compounds and a genetic approach using RNAi with cultured reticulocytes from hematopoietic stem cells.

416

PLASMODIUM VIVAX: A GENOTYPIC INTERPRETATIONAL PROBLEM

Miles B. Markus

University of the Witwatersrand, Johannesburg, South Africa

It is conventionally assumed that in the absence of either reinfection, or recrudescence following persistence of merozoites in the bloodstream, the hypnozoite stage (this term was coined by me 3.5 decades ago) is the source of recurrent *Plasmodium vivax* malaria, including recurrences caused by parasites that are genetically similar to those which were responsible for the initial clinical manifestations. How frequently (if ever) this is the case, though, is uncertain, partly because hypnozoites are never, as far as is known, generated by the prior blood-stage infection. Consequently, the source of homologous recurrences is on genetic grounds conceptually not easily ascribable to hypnozoites. This parasite form seems to be directly sporozoite-derived. If so, it has yet to be shown that genotypically homologous sporozoites inoculated by the mosquito can behave in two different ways, i.e. involving some sporozoites initiating early hepatic schizogony but others becoming dormant as hypnozoites. This might indeed happen, of course. However, there are

also other reasons (partly resulting from recent research findings) for asking the question: "Could it normally or sometimes be non-hepatic, non-bloodstream, quiescent parasites (as opposed to hypnozoites) that give rise to recurrent homologous *P. vivax* malarial episodes?" Attention will be drawn via the poster to both new and hitherto overlooked, indirect evidence for this possibility (if not probability). That aside, uncertainty concerning whether *P. vivax* recurrences have a dual origin (sometimes hypnozoites and other times non-hypnozoites) could complicate molecular identification of drug-resistant parasites, for reasons that are perhaps not readily apparent.

417

DNA DOUBLE STRAND BREAK AND REPAIR IN ANTI-MALARIAL ACTION OF ARTESUNATE

Anusha Gopalakrishnan, **Nirbhay Kumar**

Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States

Artemisinin-based combination therapy (ACT) is the recommended first line treatment for *Plasmodium falciparum* malaria. Mechanisms involved in the anti-malarial action of artemisinin are poorly understood, and it has been suggested that the cytotoxic effect of artemisinin is mediated by free radicals followed by alkylation of malarial proteins1-3. The endoperoxide bridge, the active moiety of artemisinin derivatives (ART), is cleaved in the presence of ferrous iron generating reactive oxygen species (ROS) and superoxide anions4,5. However, the emergence of resistance to artemisinin in *P. falciparum* underscores the need for new insights into the molecular mechanisms of anti-malarial activity of artemisinin. Here we show that artesunate (ART) functions by causing DNA double strand breaks in malaria parasites in a pharmacologically relevant dose and time-dependent manner. We found that DNA damage induced by ART was accompanied by an increase in intracellular ROS in the parasites. Presence of mannitol, a ROS scavenger, during the first 60 minutes reversed the cytotoxic effect of ART and reduced DNA damage with minimal effect on parasite growth. Accumulation of DNA damage resulting from ROS was accompanied by an anti-parasite effect, suggesting a causal relationship between ROS, DNA damage and parasite death. Furthermore, we show that modulating glutathione (GSH) levels impacts ROS and DNA damage induced by ART. Finally, we also show that ART-induced ROS production involves a potential role for NADPH oxidase, an enzyme involved in the production of superoxide anions. Our results provide novel insights into the molecular mechanisms underlying the anti-malarial activity of artemisinin. These studies will provide enhanced understanding of the molecular mechanism involved in resistance to artemisinin and help in the design of new anti-malarials against the most virulent *Plasmodium* species.

418

HEME-INDUCED ENDOTHELIAL CELL APOPTOSIS IS MEDIATED BY MULTIPLE SIGNALING PATHWAYS

Mingli Liu

Morehouse School of Medicine, Atlanta, GA, United States

Recent studies in our laboratory and by others demonstrate the increasing importance of elevated serum concentrations of free heme, a by-product of parasite proliferation, as a key inducer of inflammation and damage to the host microvascular endothelium as well as the mortality associated with fatal cerebral malaria (CM). Previous results indicate that heme induced human brain vascular endothelial cells (HBVEC) apoptosis in a dose dependent fashion. Since brain microvascular endothelial cells are key components of the BBB, which are severely disrupted during CM pathogensis, we hypothesized that heme (at physiologically relevant levels) induced inflammation and apoptosis in HBVEC are mediated by independent pro-inflammatory and pro-apoptotic signaling pathways. Our goal was to investigate the mechanism underlying the apoptotic and inflammatory effects of heme in HBVEC *in vitro*. We used real time RT^2 Profile polymerase chain reaction (PCR) array for apoptosis to analyze apoptotic gene expression profiles of HBVEC cells 24h after heme treatment. ***2)*** The involvement of selected pro-apoptotic, anti-apoptotic and inflammatory genes was further analyzed by quantitative PCR (qPCR). Heme causes cell death in HBVEC by increasing apoptotic rate ***2)*** The results of real time RT^2 Profile PCR array analysis are expressed as the fold changes in expression obtained by comparing HBVEC treated with heme or with vehicle as control. The up-regulated genes (with fold-change greater than 2) include ABL1, BAG1, BCL2, BCL2L10, BIRC3, BNIP3, BNIP3L, CASP3, CASP4, CASP5, CD40LG, CD70, CIDEA, CYCS, FASLG, MCL1, TNFRSF9, TNFSF10, TP73, and TRAF3. The down regulated genes consist of BAD, BAK1, CASP2, CASP6, CD27, CRADD, GADD45A, LTA, TNFRSF1A, BAG3 and BCL2A1. ***3)*** Several genes with prominent pro-apoptotic function were up-regulated in HBVEC cells after treatment with heme and confirmed by validation of qPCR, including BCL2L10, CD40LG, CD70, CIDEA, FASLG, TNFRSF9, TNFSF10 and TP73. Some genes for anti-apoptosis were down-regulated, such as BIRC3, BAG3 and BCL2A1. In conclusion, heme induces endothelial cell apoptosis through multiple signaling pathways. This indicates that reducing or inhibiting production of excess free heme may potentially reduce the adverse outcomes associated with CM and other hemolytic diseases.

419

QUANTITATIVE EXPRESSION OF MCHERRY-LUCIFERASE IN ALL LIFE CYCLE STAGES OF *PLASMODIUM FALCIPARUM*

Shulin Xu[1], Alison E. Roth[1], Steven P. Maher[1], Hitoshi Otsuki[2], John H. Adams[1]

[1]*University of South Florida, Tampa, FL, United States,* [2]*Division of Medical Zoology, Department of Microbiology and Immunology, Faculty of Medicine, Tottori University, Tottori, Japan*

Live cell imaging and sensitive quantitative assays are valuable tools in the study of *Plasmodium falciparum* life cycle, especially for stages in the mosquito vector and in the liver. The objective of the project is to generate a stable *P. falciparum* line with high levels of luciferase and mCherry expression for drug discovery in liver stages or other life cycle stages. Since the emergence of GFP several fluorescent proteins have been employed for live imaging of *Plasmodium* parasites. Relative to GFP mCherry has improved photostability and less background fluorescence, resulting in better signal-to-noise ratios. In contrast, luciferase provides a more sensitive measure in quantitative assays and as consequence has been widely used for high throughput screening (HTS) in drug discovery. To create stable transgenic parasites expressing mCherry-luciferase we used the TTAA-specific transposon *piggy*Bac genetic engineering of *P. falciparum*. Luciferase and PbDHFR3' UTR gene was amplified from existing expression cassette and inserted it into a mCherry-hDHFR *piggyBac* vector pL-BacII-bEDMH to obtain pL-BACII-bEDMH-Luc plasmid vector. The vector was designed to express both mCherry and luciferase driven by *P. berghei* EF1α, which is a constitutive promoter active in all developmental stages. Promega Luciferase Reporter System was used to detect luciferase activity and mCherry was checked by live cell imaging. Seven clones carrying integrated transposon reporter cassettes were confirmed. Theses integrated clones express high level of luciferase and mCherry signals through all stage of parasite blood cycle. The clone PfKF7G4 was confirmed to infect mosquito, form sporozoites and express both luciferase and mCherry in mosquito stage. The luciferase-mCherry expressing cassette was successfully integrated in the genome of *P. falciparum* KF7 by *piggyBac* transposon system. In blood cycle and mosquito stage, a stable high level expressing luciferase and mCherry reporter gene *P. falciparum* parasite line-PfKF7G4 has been developed. PfK7FG4 can be used for parasite visualization and quantitative analysis. It may offer more efficient drug assays for vector and liver stage studies of *P. falciparum*.

420

INTRAGENIC PROMOTER HAPLOTYPES OF IL-10 AND TNF-α INFLUENCE SUSCEPTIBILITY TO *PLASMODIUM FALCIPARUM*-INDUCED SEVERE MALARIAL ANEMIA

Zachary Karim, Prakasha Kempaiah, Gregory Davenport, Evans Raballah, Samuel Anyona, John M. Ong'echa, Douglas J. Perkins

University of New Mexico School of Medicine, Albuquerque, NM, United States

Plasmodium falciparum associated severe malarial anemia [SMA, hemoglobin (Hb)<5.0g/dL] is a leading cause of morbidity and mortality in African children. Examination of the host immune response and underlying genotypic traits that condition SMA can offer an improved understanding of malaria pathogenesis. Pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) serve as early response signaling molecules that activate the innate immune response. Discordantly, anti-inflammatory mediators, such as interleukin-10 (IL-10) act as secondary modulators to regulate and balance the pro-inflammatory response. Dysregulation of both TNF-α and IL-10 have been shown to influence clinical outcomes in human malarial infections. To investigate the role of polymorphic variation in the *IL-10* and *TNF*-α genes on susceptibility to SMA, haplotypes were constructed using six promoter variants [IL-10 -627A/C, IL-10 -854C/T, IL-10 -1117A/G, TNF-α -238G/A, TNF-α -308G/A and TNF-α -1031T/C]. Selection of variants was based on minor allelic distribution of >10% in African populations and potential transcription factor binding elements. Acutely infected children (n=1220, aged 3-36 mos.) from western Kenya were investigated in this study. Logistic regression was performed [controlling for age, gender, nutritional status, co-infections (HIV-1, bacteremia), G6PD deficiency, alpha-thalasemmia and HbS]. These analyses revealed that presence of the IL-10-627AC/-854CT genotypes were associated with protection against SMA (average OR, 0.68; 95% CI, 0.47-0.91; *P<0.05*), whereas the TNF-α -308GG genotype enhanced susceptibility to SMA (OR, 1.23; 95% CI, 1.08-1.54; *P<0.05*). Similarly, several extended haplotypes between the two promoters (i.e., list) were associated with enhanced susceptibility to SMA (average OR 1.39; 95% CI 1.02-1.94; *P<0.05*), while others (list) showed protection against SMA (average OR 0.88; 95% CI 0.35-0.97; *P<0.05*). Taken together, these results demonstrate that promoter variation in *IL-10* and *TNF*-α are important in conditioning susceptibility to SMA.

421

SCHOOL-BASED TYPHOID VACCINATION PROGRAM IN KARACHI: POLICY IMPLICATIONS FOR TYPHOID CONTROL

Rehman Tahir[1], Ghulam Mustafa[1], Waqaas Bhutta[1], Mohammad I. Khan[2], R. L. Ochiai[2], Zulfiqar A. Bhutta[3]

[1]Trust for Vaccines and Immunization, Karachi, Pakistan, [2]International Vaccine Institute, Seoul, Republic of Korea, [3]Aga Khan University, Karachi, Pakistan

Population-based surveillance conducted from 2002-2003 as part of the Diseases of the Most Impoverished (DOMI) Program indicated high burden of typhoid fever in the slums of Karachi-Pakistan. Incidence of typhoid fever was 573 per 100,000 persons per year in children aged 2-4 years and 423 per 100,000 in children aged 5-15 years. Adding to the available evidence providence for information for decision making, all parties involved in the typhoid control including International Vaccine Institute, Aga Khan University and Trust for Vaccines & Immunization as part of Vi-Vaccines for Asia (ViVA) Initiative piloted a program of Vi-Vaccine in schools and madrasahs of two Towns of Karachi. The goal of the program was to introduce typhoid Vi-polysaccharide vaccine through school-based campaigns and demonstrate to policymakers that a school-based typhoid vaccination campaign is feasible and desired by the population. Prior to vaccination campaigns, social mobilization activities were conducted to create awareness of typhoid fever and its prevention. The main focus of social mobilization was to build and enhance awareness of the risks, signs, symptoms and preventive measures of typhoid fever. Schools were provided with multiple informational tools on typhoid fever and vaccination campaign. In order to ensure quality, clarity and relevance of messages, formative research was also conducted prior to social mobilization activities. Vaccination campaigns were conducted according to international guidelines. Approximately 120,000 school and madrasah going children were given typhoid vaccine. AEFI cases were reported and documented among children who were vaccinated accounting for about 0.28% of children vaccinated. 30 % increase in vaccination coverage was found in Jamshed Town as compared to Gulshan Town as result of implementation of modified communication strategy. The initial phase of the project has sensitized the population for Typhoid prevention. The collaborative efforts of the use of typhoid vaccine in the schools of Karachi added to existing information on the safety and feasibility of injectable typhoid vaccine. A routine vaccination program in schools will increase the use of the vaccine for prevention of typhoid. Policy advocacy is need of hour for inclusion of Typhoid vaccine in routine EPI program.

422

CHOLERA OUTBREAK IN BAKURA LOCAL GOVERNMENT AREA, ZAMFARA STATE-NIGERIA. SEPTEMBER-OCTOBER 2013

Aishatu Abubakar Sadiq[1], Ahmed Sufyan[1], Anthony Suleiman[1], Patrick Nguku[1], Kabir Sabitu[2], Gabriele Poggensse[1]

[1]Nigeria Field Epidemiology and Laboratory Training Programme, Abuja, Nigeria, [2]Department of Community Medicine, Ahmadu Bello University, Zaria, Nigeria

On 22nd October 2013, 150 cases of watery diarrhea were reported to the Federal Ministry of health from Bakura, Zamfara state. We investigated to identify the causative organism, infection source, implement public health interventions and propose recommendations. We defined a case as any person aged 1 year or more with acute watery diarrhea with or without vomiting, living in Bakura between 22nd September to 22nd October 2013. Twelve stool specimens were sent for laboratory testing. Cases were selected using the case definition and confirmed using laboratory tests. A descriptive study and a case control study were conducted to identify the source of infection. We conducted additional investigations to assess the sanitation practices and water source in the area. We identified 274 cases that met the case definition among the 100,125 residents of Bakura (Overall attack rate: 0.3%). There were 4 deaths (Case fatality rate: 1.5%). Males were more affected than females (67.9% versus 32.1%). The first case-patient developed an illness during the third week of September, 2013. The median age of cases: 17 years (range: 2- 42 years), while median age of controls: 31 years (range: 5- 43 years). Compared with 28 controls, the 28 cases did not differ in terms of residential location and environmental exposure.Cases were more likely to use well as a source of drinking water (OR: 1.15; 95% CI: 0.35-3.76), pit latrine as a means of sewage disposal (OR: 67.50; 95% CI: 7.3-15.74), practice poor hand washing (OR:10.8; 95% CI: 2.52-50.62)and hence develop disease. Of the 12 stool specimens from case-patients tested for Vibrio cholerae ,12(100 %) were positive for serotypes 01 and 0139. 2(100%)Water samples from communal wells also yielded growth of Vibrio cholerae. The outbreak in Bakura was confirmed to be cholera infection. The plausible risk factors associated with cholera infection during the outbreak were: Poor hand washing practices, use of contaminated wells as water source and use of pit latrines. We provided health education using community health teams, drugs, intravenous fluid therapy, disinfection packs and treated water wells. We recommended continued health education by health workers for community members within the affected communities on the importance of person hygiene and environmental sanitation.Findings from the outbreak investigation were forwarded to the state and Federal ministry of health.

423

GUT HOMING MUCOSAL RESPONSE TO ORAL VACCINES IN INFANTS: A COMPARISON BETWEEN UNDERNOURISHED AND WELL NOURISHED

Ayan Dey[1], Anuradha Sinha[2], Ranjan K. Nandy[2], Deok Ryun Kim[1], Suman Kanungo[2], William A. Petri[3], Thomas F. Weirzba[1], Cecil Czerkinsky[4]

[1]International Vaccine Institute, Seoul, Republic of Korea, [2]National Institute of Cholera and Enteric Diseases, Kolkata, India, [3]University of Virginia, Charlottesville, VA, United States, [4]IPMC Nice-Sophia, Antipolis, France

Oral polio vaccine (OPV) and rotavirus vaccines are less effective in children in the developing world. The reasons for this poor response to these oral vaccines are not well understood. In the present study we investigated the association of malnutrition with the mucosal response to Polio and Rotavirus vaccine in infants from an urban slum of Kolkata, India. An ELISPOT assay was utilized to measure Polio and Rotavirus vaccines induced immune response. The assay was used for simultaneous detection of mucosal derived gut homing IgA producing antibody secreting cells (ASCs) expressing mucosal integrin $\alpha4\beta7$ against Polio Virus 1, 2 and 3 and Rotavirus. Systemic responses to Poliovirus vaccines were also measured by positive selection of ASCs expressing HLA-DR and CD19 antigen. HLA-Dr and CD19 markers are expressed during maturation and development of B-cells. In infants 4 fold rise in gut mucosal ASC count were observed to Polio vaccine. The percentage of subject with 4 fold rise in ASC count to Polio type1, 2 and 3 were 50%, 30% and 50% respectively. In the case of Rotavirus vaccine the 4 fold rise in gut mucosal ASC count was observed in approximately 26% of subjects. Infants with moderate to severe malnutrition (HAZ<–2) compared to better-nourished infants had significantly lower polio type 1 (Number of Positive respondents in HAZ -2, P=0.01) and type 3 (Number of Positive respondents in HAZ -2, P=0.03) ASCs response. However, in case of polio type 2 (Number of Positive respondents in HAZ -2, P=0.25) and rotavirus vaccine (Number of Positive respondents in HAZ <-2versus HAZ> -2, P=0.47), no association of vaccine response and malnutrition was observed. The ELISPOT data suggested a poor mucosal response to oral vaccines in infants. The mucosal immune response to Polio vaccine was affected by malnutrition. However, there was no effect of malnutrition on Rotavirus vaccination in this population at the measured time points. This study has also demonstrated the role of ELISPOT in measuring mucosal response to oral vaccines. However, this data needs to be correlated with other vaccine measures to determine whether these blood ASC responses could provide proxy markers of the mucosal immune response to oral vaccine.

424

DIARRHEAGENIC *ESCHERICHIA COLI* IN MOTHER-CHILD PAIRS IN ILE-IFE, SOUTHWESTERN NIGERIA

Babatunde W. Odetoyin[1], Oladipo A. Aboderin[1], Iruka N. Okeke[2]

[1]Obafemi Awolowo University, Ile-Ife, Nigeria, [2]Haverford College, Haverford, PA, United States

Diarrhoeagenic *Escherichia coli* are among the most common bacterial causes of morbidity and mortality particularly in young children. These pathogens are not sought routinely and capacity in detection is still grossly limited in developing countries. We investigated the distribution and dissemination of diarrhoeagenic *E. coli* (DEC) in children paired with their mothers in a Nigerian community. A total of 252 stool samples obtained from 126 children with diarrhea paired with their mothers at a state hospital in Ile-Ife, Nigeria were screened for DEC by multiplex polymerase chain reaction. DEC were identified in specimens from 35.7 % of individuals. The most common DEC pathotype in children with diarrhoea as well as their mothers was shiga toxin producing *E. coli* (STEC) (42, 16.7%). Identical pathotypes were found in 13 (10.3%) of the mother-child pairs. These consisted of enterotoxigenic *E. coli* (ETEC) (5, 4.0%), enteroaggregative *E. coli* (EAEC) (5, 4.0%) and STEC (3, 2.4%). The DEC isolates were commonly resistant to ampicillin (121, 96.8%), sulphonamide (118, 94.4%), tetracycline (119, 95.2%), streptomycin (115, 92.2%) and trimethoprim (113, 90.4%), but less commonly resistant to ciprofloxacin (9, 7.2%). This study suggests that healthy mothers may be asymptomatic reservoirs of multiply resistant strains of *E. coli* that are pathogenic in their children.

425

POPULATION-BASED INCIDENCE OF ROTAVIRUS AMONG CHILDREN AGED < 5 YEARS IN THE CATCHMENT AREAS OF TWO DIARRHEA SURVEILLANCE HOSPITALS IN DHAKA, BANGLADESH

Repon C. Paul[1], Abu S. Faruque[1], M. Abdul Malek[1], Stephen P. Luby[2]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]Stanford University, Stanford, CA, United States

Rotavirus is the most common cause of severe diarrhea in children worldwide. It is a vaccine-preventable disease. However, introduction of a vaccine in a resource constrained country like Bangladesh depends on its cost-effectiveness which, in turn, depends on incidence. icddr,b conducts diarrhea surveillance in its Dhaka and Mirpur hospital, but hospital-based incidence underestimates the real burden because many patients seek treatment elsewhere. We estimated the incidence of rotavirus among children aged < 5 years in the catchment area of Dhaka and Mirpur hospital by adjusting the hospital-based incidence by the proportion of severe diarrheal children in the hospital catchment areas who were admitted to surveillance hospitals. In Dhaka Hospital every 50th admitted patient and in Mirpur Hospital every 10th admitted patient was enrolled in surveillance and tested for rotavirus using a stool enzyme immunoassay. We extrapolated the total number of rotavirus cases in surveillance hospitals among all children admitted from the hospital catchment areas. We defined the catchment areas of the surveillance hospitals as those neighborhoods where more than two-thirds of admitted patients resided. To estimate the proportion of severe diarrhea cases among children aged < 5 years in the hospital catchment area who were admitted to surveillance hospitals, we conducted a house-to-house survey in randomly selected areas. We considered diarrhea in the preceding 12 months to be severe if a child with diarrhea was admitted to a healthcare facility, or received intravenous rehydration, or died following frequent loose or watery stools. Among the surveillance enrolled children aged < 5 years from January 2011 through December 2013, 1,247 (42%) in Dhaka Hospital and 626 (33%) in Mirpur Hospital were positive for rotavirus. According to survey in hospital catchment areas, the proportion of severe diarrheal children who were admitted to surveillance hospitals was 0.69 (95% CI: 0.62-0.75) in Dhaka Hospital and 0.77 (0.70-0.83) in Mirpur Hospital catchment areas. The population-based incidence of rotavirus among children aged < 5 years was estimated to be 28 (95% CI: 25-31) in Dhaka Hospital and 38 (95% CI: 35-42) in Mirpur Hospital catchment areas per 1,000 population. This study provides a credible estimate of rotavirus incidence in Dhaka, which can be used to assess the cost effectiveness of rotavirus prevention activities including vaccination.

426

DETECTION AND CHARACTERIZATION OF ESBL AND PLASMIDIC AMPC BETALACTAMASES IN *SALMONELLA* SPP. FOODBORNE ISOLATES FROM LIMA, PERU

Sandra Martínez-Puchol[1], Maria Jesus Pons[1], Cláudia Gomes[1], Lidia Ruiz-Roldán[1], Noemí Palma[1], Maribel Riveros[2], Karen Ocampo[2], Alfredo Corujo[3], Theresa J. Ochoa[2], Joaquim Ruiz[1]

[1]Barcelona Centre for International Health Research (CRESIB), Barcelona, Spain, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Nutreco, Toledo, Spain

The role of food as carrier of pathogens is a major public health problem being *Salmonella enterica* infections, mostly isolated in chickens or derived products, the leading cause of foodborne human infections in many countries. The problem is exacerbated by the increasing levels of antibacterial resistance, including resistance to cephalosporins by the presence of extended-spectrum beta-lactamases (ESBL) and AmpC beta-lactamases. The aim of this study was to analyse the presence of ESBL and AmpC, the general susceptibility and the mechanisms of resistance to beta-lactams in *Salmonella* spp. isolated from meat samples from Peru. Twenty-one strains of *Salmonella* spp. isolated from meat from 5 markets of Lima were serotyped by microarray. Antimicrobial susceptibility was tested by disk diffusion. The presence of ESBL was determined by double synergy test and the presence of AmpC was assessed by disk antagonism. The presence of blaSHV, blaTEM, blaCARB, blaOXA1-like, blaOXA2-3-like, blaOXA5-7-like, blaCTX-M, blaCTX-M-9-group and class I integron was analysed by PCR and sequencing. The serotyping resulted in 17 *S. enterica* serovar Infantis, 2 *S. enterica* serovar Enteritidis, 1 *S. enterica* serovar Kentucky and 1 *S. enterica* serovar Anatum. The isolates were completely or highly resistant to rifampicin, ampicillin, cotrimoxazole, amoxicillin and nalidixic acid amongst others. The ESBL phenotype was found in 3 isolates, identified as blaCTX-M-65. The presence of AmpC beta-lactamases was confirmed in 7 isolates. Class I integron carrying the aadA1 gene was found in 18 isolates, conferring resistance to streptomycin. The most prevalent serotype found was *S.* Infantis. The presence of these mechanisms, and the multiresistance shown, involves a high risk of transmission to humans since it has already been reported outbreaks caused by *S.* Infantis ESBL-producers. Moreover it is necessary to further characterize the AmpC beta-lactamases found. All this shows the high importance of adequate sanitary control in animal production to prevent foodborne human infections and complications derived from antimicrobial resistance.

427

INCIDENCE AND SEVERITY OF TRAVELERS' DIARRHEA AMONG STUDENTS AT A SPANISH LANGUAGE SCHOOL IN CUSCO, PERU

Emily L. Deichsel[1], Miguel M. Cabada[2], Karen Mozo[3], Maria Alejandra Caravedo[3], Italo Novoa[3], Kelsey O'Brien[1], Drake H. Tilley[1]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Infectious Disease Division, University of Texas Medical Branch, Galveston, TX, United States, [3]Universidad Peruana Cayetano Heredia, Lima, Peru

Travelers' diarrhea (TD) is the most common illness among travelers to low and middle income countries, yet there are few prospective incidence studies evaluating the full spectrum of disease. To evaluate the incidence and causes of TD, we conducted a prospective cohort study among travelers in Cuzco, Peru from June 2012 to January 2014. Participants provided 1 stool sample at enrollment, then weekly, and when sick with diarrhea. Two definitions of diarrhea were used: 1) the WHO definition of 3 or more loose stools in a 24 hour period and 2) any self-reported diarrhea by participants. Subjects reporting diarrhea were evaluated daily to record signs and symptoms. In total, 249 participants were enrolled accounting for 663 observed patient-weeks. Participants were 65% female with a mean age of 28.1 years (SD 11.4 years) and represented 22 different countries principally from North America and Europe. Of the 711 samples collected, 168 (24%) represent diarrheal episodes and 543 (76%) represent asymptomatic baseline or follow-up samples. We recorded 174 episodes of diarrhea representing 131 (53%) participants and an incidence rate of 1.14 episodes per patient per month, or a 26% chance of having diarrhea each week while in Cusco. Seventy two percent of diarrhea episodes met the WHO definition, however 28% still self-reported problems with diarrhea. Those meeting the WHO definition lasted on average, 1.18 days longer (95% CI 0.53-1.82) than those not meeting the definition. However, subjects in both groups lost a workday (45% WHO; 37% non-WHO) and experienced abdominal pain (6% WHO; 8% non-WHO), excessive thirst (43% WHO; 40% non-WHO), decreased urination (18% WHO; 15% non-WHO) and dark urine (19% WHO; 20% non-WHO) each observation day. Non-WHO cases were more likely to report nausea (OR: 2.54; 95% CI: 1.29, 5.02) and bloating (OR: 2.02; 95% CI: 1.03, 3.96) per observation day. In conclusion, the WHO definition misses ¼ of TD episodes which still have similar symptoms and impact among travelers. A better diarrhea definition for travelers should be considered.

428

DIVERSITY OF ANTIBIOTIC RESISTANCE GENES AND STAPHYLOCOCCAL CASSETTE CHROMOSOME *MEC* ELEMENTS IN FAECAL ISOLATES OF COAGULASE-NEGATIVE STAPHYLOCOCCI FROM NIGERIA

Ezekiel O. Akinkunmi

Obafemi Awolowo University, Ile-Ife, Nigeria

Coagulase-negative staphylococci (CoNS) are opportunistic pathogens found as colonisers of the human gut. This study was carried out to examine the genetic drug resistance mechanisms in faecal isolates of CoNS. The study investigated 53 non-duplicate CoNS isolates obtained from the fresh stool samples of apparently health subjects in the community of Ile-Ife, South-Western Nigeria. Phenotypic antibiotic susceptibility testing was assessed by the disc diffusion test while antibiotic resistance genes were analysed by PCR. Isolates with *mecA* were subjected to Staphylococcal Chromosome Cassette *mec* and cassette chromosome recombinase *ccr* complex type determinations. Resistance genes were detected only in isolates that showed resistance by phenotypic screening. The *aac(6')-aph(2")* gene was detected in all the three isolates resistant to gentamicin. Four out of five erythromycin resistant isolates were positive to *ermC,* the remaining carried the *msrA*. The *tetK* gene was detected in 6 of the 7 tetracycline resistant isolates while 4 possessed the *tetM* gene. Three of the isolates (*S. haemolyticus*, *S. xylosus* and *S. capitis*) had both genes. SCC*mec* types found were: SCC*mec* I- *ccrAB*β2-α2 (4 isolates: 3 *S. epidermidis*, 1 *S. warneri*), SCC*mec*IVb- *ccrAB*β2-α3 (1 isolate: *S. epidermidis*), SCC*mec*IVd- *ccrAB*β2-α3 (8 isolates: 3 *S. epidermidis*, 2 *S. xylosus*, 1 *S. saprophyticus*, 1 *S. warneri*, 1 *S. capitis*), and untypable (2 isolates: *S. epidermidis*). This genetic background could be a reservoir for interspecies gene transfer among CoNS and *S. aureus* in the intestinal tract.

429

PERSISTENT TYPHOID FEVER EPIDEMICS IN INTERNALLY DISPLACED PERSONS CAMPS IN UGANDA

Scholastica A. Okui

AHPC Ministry of Health, Uganda, Kampala, Uganda

Typhoid fever has continued to cause recurrent epidemics in some districts in Uganda from 2009 to date. According to the epidemiological data from the Ugandan Ministry of Health (MOH) from 2009 to 2013, there has been an increase in the number reported cases of typhoid fever particularly from districts with people living in internally displaced peoples camps (IDPs) where some patients are even admitted with complications such as intestinal perforations. The factors leading to this increase have not yet been established and worse still, is the fact that the reported typhoid fever cases were diagnosed using the Widal test which is non-specific

and may lead to over diagnosis of the disease. The study therefore was to attempt to establish factors leading to the increase of typhoid fever cases and to confirm all such reported cases with culture methods. The specific objectives were to study the socio-demographic profiles of the residents of the four most affected districts, their environmental sanitation situations as well as the water sources used. Both Widal and culture methods were used to establish the typhoid fever causative organisms, establish the carrier status of recovered cases, and establish antibiotic sensitivity of the salmonella organisms isolated. A survey was conducted to study the situations of residents of four camps in the four districts. Follow-up interviews and Focus group discussions were carried out. Observations of the homesteads and latrines for sanitation and hygiene were also conducted. Water samples from sources and households were analysed for faecal contamination. Similarly blood samples obtained from admitted patients and previously confirmed cases who had recovered from typhoid fever were cultured and the isolated salmonella typhi organisms, tested for antibiotic sensitivity. The main findings why typhoid fever is recurrent in four camps in the four districts included: poor hygienic practices, poor sanitation. Low latrine coverage (40%), unsafe water sources, contaminated with faeces (both *E. coli* and *Salmonella typhi* organisms were isolated. *Salmonella typhi* was isolated from 27/81(33%) patients by blood culture, while 58/81(71.6%) were positive with the Widal test. 76% of the cultured *S.typhi* organisms were resistant to amp, sulf, tetr and cotri. which are first line drugs in Uganda, but susceptible to chloral, na. and cipro. carrier status was 36%.

430

SPATIOTEMPORAL TRENDS AND IMPORTANT LOCAL INFLUENCES ON HOSPITALIZED PEDIATRIC DIARRHEA IN HO CHI MINH CITY, VIETNAM

Corinne Thompson[1], Jon Zelner[2], Tran Do Hoang Nhu[1], Phan Vu Tra My[1], Hoang Le Phuc[3], Nguyen Minh Ngoc[4], Lu Lan Vi[5], Bryan Grenfell[2], Stephen Baker[1]

[1]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, [2]Princeton University, Princeton, NJ, United States, [3]Children's Hospital 1, Ho Chi Minh City, Vietnam, [4]Children's Hospital 2, Ho Chi Minh City, Vietnam, [5]Hospital for Tropical Disease, Ho Chi Minh City, Vietnam

Diarrheal disease in Ho Chi Minh City (HCMC), Vietnam remains a significant cause of morbidity in young children. Distinct seasonal patterns are present in diarrheal disease reporting to hospital in this setting, with large peaks occurring in the colder and drier months. The reasons for these trends are unclear. In order to explain these observed patterns, we analyzed a set of hospitalized pediatric diarrheal admissions from three large hospitals in HCMC from 2005-2010. We developed a mixed effects model to explore important influences in reported diarrhea, examining time series trends and associations with a variety of environmental covariates including flood level, temperature and rainfall. Additionally, we used Empirical Bayesian Kriging to map smoothed rates of reported diarrhea stratified by season and age-group to investigate the spatiotemporal distribution of reported diarrhea cases across HCMC. Our results highlight the continuing burden of pediatric diarrheal disease in this setting: of ~480,000 hospital admissions, gastroenteritis was the second most common reason for hospitalization in children under 16, accounting for 12% of all admissions. From our mixed effects model, we found a strong epidemic component that was spatially synchronized across HCMC, suggesting citywide epidemics, probably due to viral pathogens. We speculate that these patterns are due to the entrance of new seasonal variants or related to the seasonal birthrate across the city. We additionally identified a strong positive association with temperature, a relationship which strengthened significantly with distance from the city center. Furthermore, an evaluation of pairwise correlations between the time series of the 24 districts of HCMC and the distance between district centroids identified a spatial dependence radius of only 5km, suggesting the presence of both city-wide and local effects. Finally, visualization of smoothed rates of reported diarrhea highlight substantial differences in diarrheal hotspots by season and age, suggesting varying pathogens are present at different times in different areas amongst different ages. Although several crucial limitations are present in such a dataset, the results still provide insight for public health practitioners and clinicians working in HCMC, Vietnam and may also reflect trends present in other industrializing, densely populated settings.

431

HOST GENE POLYMORPHISMS AND SUSCEPTIBILITY TO BACTERIAL ENTEROPATHOGENS IN CHILDREN LIVING IN GAMBIA AND KENYA

Roshni G. Daver[1], Jitesh Shewale[2], Brianna Lindsey[3], Joseph Oundo[4], Benjamin Ochieng[5], Martin Antonio[6], Sandra Panchalingam[7], Kelly Volcik[1], Megan Grove-Gaona[2], O Colin Stine[3], James P. Nataro[8], James Kaper[3], Myron M. Levine[3], Karen L. Kotloff[3], Pablo C. Okhuysen[9]

[1]University of Texas, Medical School at Houston and M.D. Anderson Cancer Center, Houston, TX, United States, [2]University of Texas, School of Public Health, Houston, TX, United States, [3]The University of Maryland School of Medicine, Baltimore, MD, United States, [4]Ministry of Public Health and Sanitation, Nairobi, Kenya, [5]Kenya Medical Research Institute/ Centers for Disease Control and Prevention, Kisumu, Kenya, [6]The Medical Research Council Unit, Banjul, Gambia, [7]University of Maryland, School of Medicine, Baltimore, MD, United States, [8]The University of Virginia School of Medicine, Charlottesville, VA, United States, [9]M.D. Anderson Cancer Center, Houston, TX, United States

The Global Enteric Multicenter Study (GEMS) is a prospective collaborative study to determine the pathogen specific, diarrhea associated attributable disease burden of diarrhea in Africa and Asia in children less than 5 years of age. Using samples from children that participated in this case-control study and were seen at in the GEMS clinics in The Gambia and Kenya, we conducted a host gene candidate study that examined the association of the host DNA single nucleotide polymorphisms (SNPs) and specific bacterial enteropathogens causing diarrhea. Cases of diarrhea were matched with healthy controls from the same area. Stool was collected and examined comprehensively for the presence of enteropathogens and host DNA polymorphisms. We studied de-identified fecal DNA for the presence of SNPs (N=144) in 26 genes that code for host proteins involved in pathogen attachment, inflammation, innate and acquired immune responses to enteropathogens. We analyzed the distribution of host genotypes and compared cases vs. controls according to enteropathogens identified. Comparisons were made using SNPSTATs software following an additive model. A mixed logistic regression model was used to adjust for potential confounding factors including site, age, weight for height and weight for age scores. Microbiological and genotype data were available in 1,164 subjects. Diarrhea was associated with 6 SNPs located in *SELPLG, CD55, LPLUNC, IL12 B*, and *CORO1C*. In The Gambia, diarrhea due to *Shigella* was associated with a SNP in *SELPLG*, diarrhea from enterotoxigenic *E. coli* was associated with SNPs in *DAF (CD55), LPLUNC, SELPLG*, diarrhea from enteroaggregative *E. coli* was associated with SNPs in CORO1C. In Kenya, diarrhea from enteroaggregative *E.coli* was associated with SNPs in *IL12B* and *CORO1C* Distinct SNPs were associated with pathogen specific diarrhea in children under 5 years of age living in two African countries.

432

GUT MICROBIAL SUCCESSION AFTER ENTEROTOXIGENIC *ESCHERICHIA COLI* INFECTION IN BANGLADESH

Ana A. Weil[1], Yasmin Begum[2], Daniel T. Leung[2], Richelle C. Charles[1], Jason B. Harris[1], Edward T. Ryan[1], Firdausi Qadri[2], Stephen B. Calderwood[1], Lawrence David[3], Regina C. Larocque[1]

[1]Massachusetts General Hospital, Boston, MA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]Duke University, Durham, NC, United States

Enterotoxigenic *Escherichia coli* (ETEC) is a common cause of bacterial enteritis in the developing world. Recent human and animal studies

suggest that alterations in commensal flora influence recovery from enteric infections. We examined serial rectal swabs from patients after ETEC infection to observe microbial succession during the recovery period. DNA was extracted from rectal swabs and analyzed using 16S ribosomal RNA gene sequencing. Over a three-month follow-up period, 75 samples were analyzed from 18 individuals in Dhaka, Bangladesh. To simplify the analysis of these complex communities, we investigated bacterial dynamics at the genus level. The bacterial family including E. coli species was most dominant on the day of patient enrollment when ETEC stool culture was positive. The day after antibiotic treatment for ETEC, bacteria including skin flora such as Gram positive organisms were most dominant, with a decrease in bacteria from the family including E. coli species. Compared to day 10 of follow up, the skin flora group was predominant on Day 2 (Day 2, 7/16 44% versus Day 10, 0/17, P=0.003 by Fisher's exact test). On day 10 of follow up, anaerobic flora such as Prevotella species and other common enteric anaerobes were predominant (Day 10 12/17 71%, Day 2 4/16 25%, P=0.011 by Fisher's exact test). The predominant bacterial populations on Day 10 and Day 30 were similar to gut microbiome studies in healthy controls in the developing world. The gut microbiome following ETEC infection rapidly recovers to baseline after a dramatic perturbation and progression through distinct phases of recovery. Understanding the pattern of microbial succession in recovery from enteric disease is a foundation for studying factors of susceptibility and protection from disease and could potentially lead to interventions to hasten recovery from infection.

433

MEMORY B CELL RESPONSES TO *VIBRIO CHOLERAE* 01 O-SPECIFIC POLYSACCHARIDE (OSP) IN PATIENTS WITH CHOLERA IN DHAKA, BANGLADESH

Amena Aktar[1], M. Arifur Rahman[1], Sadia Afrin[1], Aklima Akter[1], Taher Uddin[1], Daniel T. Leung[2], Fahima Chowdhury[1], Ashraf I. Khan[1], Meagan K. Bufano[2], Md. Abu Sayeed[2], Richelle C. Charles[2], Regina C. LaRocque[2], Jason B. Harris[2], Peng Xu[3], Stephen B. Calderwood[2], Pavol Kováč[3], Edward T. Ryan[2], **Qadri Firdausi**[1]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]Massachusetts General Hospital, Boston, MA, United States, [3]National Institute of Diabetes and Digestive and Kidney Diseases, LBC, National Institutes of Health, Bethesda, MD, United States

Cholera is a severe, dehydrating diarrheal illness that in the last two decades has been caused largely by *Vibrio cholerae* O1. There is a growing body of evidence that protective immunity against cholera targets lipopolysaccharide (LPS), especially the O-specific polysaccharide (OSP) component of LPS. Immunity following wild-type cholera persists for at least 3 to 10 years, even in young children. Immunity following oral cholera vaccination is of lesser magnitude and shorter duration, especially in young children under the age of five years. Following wild-type cholera, protection against cholera persists despite decreases in effector immune responses, suggesting that memory responses may play a critical role in mediating long-term protection against cholera. We took advantage of the recently described ability to purify *V. cholerae* O1 OSP, and assessed memory B cell responses targeting OSP in patients recovering from wild type cholera in Bangladesh. The Ogawa serotype was the primary circulating serotype at the time of this study, and we assessed immune responses out to 6 months after presentation with illness. We found that serum OSP IgA and IgG responses were evident within 7 days of presentation, and that these values decreased toward baseline over the follow-up period. We found that IgA and IgG memory B cell responses targeting OSP were also present within 30 days of presentation, and persisted through the follow-up period. When we analyzed the data by age of patients, we found IgG and IgA memory responses targeting OSP were detectable in adults (>18 years of age, n=15), older children (6-17 years of age, n=20) and children (2-5 years of age, n=11). Our results suggest that memory B cell responses following wild-type cholera are induced, that they persist in time, and that these responses are comparable among adults, older children, and younger children in this cholera endemic area. These results suggest that memory B cell responses targeting OSP could play a role in mediating long-term immunity against cholera.

434

ASSESSING SERUM LEPTIN LEVELS IN CHILDREN HOSPITALIZED WITH CHOLERA IN BANGLADESH

Brie W. Falkard[1], Taher Uddin[2], M. Arifur Rahman[3], Amena Aktar[3], Taufiqur R. Bhuiyan[3], Daniel T. Leung[3], Firdausi Qadri[3], Edward T. Ryan[1]

[1]Massachusetts General Hospital, Boston, MA, United States, [2]University of Melbourne, Melbourne, Australia, [3]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Cholera is a severe watery diarrhea caused by the noninvasive gram-negative bacterium *Vibrio cholerae*. Leptin is a critical hormone in human metabolism that plays a role in developing adequate immune responses. Malnourished children with low leptin levels have reduced T cell responses. In order to evaluate the potential role of leptin during cholera, we measured leptin levels on day 2, 7 and 30 in cholera patients 5 years of age and younger at the International Centre for Diarrhoeal Disease Research in Dhaka, Bangladesh. We compared these levels with respect to age, gender and nutritionally-matched healthy controls, as well as day 180 levels within the cholera patients themselves. We found that patients at the acute stage of cholera had significantly lower serum leptin levels than matched healthy controls, and that these acute-phase leptin levels rose through convalescence. We also evaluated the association between leptin on day 2 and immunological responses that developed at later time points to cholera toxin B subunit (CtxB), a T cell-dependent antigen, and *V. cholerae* lipopolysaccharide (LPS), a T cell-independent antigen. We found a significant association of acute phase leptin levels to immune responses to CtxB. Our results suggest that leptin may play a role during cholera, especially in maturation of immune responses to T cell-dependent antigens. Our results also suggest that malnourished children that have low leptin levels may be impaired in their ability to develop such responses.

435

CAMPYLOBACTER JEJUNI CAPSULAR TYPE DISTRIBUTION IN THE AMAZON AND COASTAL REGIONS IN PERU

Drake H. Tilley

U.S. Naval Medical Research Unit - 6, Lima, Peru

The enteric pathogen *Campylobacter jejuni* constitutes one of the major causatives agents of diarrhea worldwide. *C. jejuni* expresses 47 different capsule polysaccharides (CPS) and constitutes a major factor in *C. jejuni*-mediated pathogenesis. Recent published findings demonstrate a CPS-mutant diphtheria toxin conjugate vaccine as protective against CPS-homologous *C. jejuni* infection in an *Aotus nancymaae* non-human primate diarrheal model. World-wide surveillance describing CPS type distribution is necessity to determine regional vaccine efficacy of CPS-specific vaccines against *C. jejuni*. To this end, two regionally distinct pediatric prospective community based studies were considered to evaluate CPS distribution in Peru. Santa Clara, a rural community located in the Peruvian Amazon was compared to Pampas de San Juan, a shanty town located in the peripheral area of Lima. Both populations were analyzed by a multiplex PCR capable to detect the 47 CPS types of *C. jejuni*. Santa Clara study included 131 symptomatic and asymptomatic individuals of which 201 *Campylobacter jejuni* isolates were acquired from stool samples. The Pampas de San Juan study included 95 samples obtained from 51 symptomatic and asymptomatic children. Only 7% of the isolates from Santa Clara were untypable by this technique. The most prevalent CPS types detected from Santa Clara were HS8/HS17 (15%), HS15 (13%) and HS3 complex (12%). In Pampas de San Juan, a higher percentage of isolates, 25%, were untypable by this new technique while HS15 (9%), HS4 complex A (7%) and HS41 (6%) were the most

prevalent CPS types. In addition, tracking of CPS types in individuals over time demonstrate different CPS types present from *C. jejuni* re-infection suggesting a possible acquired immunity to distinct CPS. The results herein describe differential CPS type distribution in two distinct regions of Peru, with HS8/HS17 (15%) and HS15 (9%) being the most prevalent in Santa Clara and Pampas de San Juan, respectively.

436

A HIGH PREVALENCE OF HYDATIDOSIS IN SLAUGHTERED GOATS AT A SOUTHWESTERN ABATTOIR, NIGERIA: IMPLICATIONS ON LIVESTOCK PRODUCTION AND HUMAN HEALTH

Hezekiah K. Adesokan[1], IfeOluwapo O. Akanbi[2], Ibikunle M. Akanbi[1], Doyinsola A. Sule[2]

[1]University of Ibadan, Ibadan, Nigeria, [2]Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Nigeria

Hydatid disease is an important zoonosis globally particularly in developing countries like Nigeria where backyard slaughter is rampart and carcass wastes are easily accessible to scavenging dogs. Adult goats raised on extensive system of management slaughtered at a tropical abattoir in south-western Nigeria were inspected for the presence of hydatid cysts. In all, a total of 2,140 goats were slaughtered from which 214 were randomly selected and inspected over the study period. The results show a prevalence of 53.27% with 50.88%, 38.6%, 6.14% and 4.39%, respectively located in the lungs, liver, heart and intestines. In addition, 33.33% of the hydatid cysts were fertile with a significantly higher proportion (51.72%) from the lungs. Our findings reveal a high prevalence of hydatidosis in slaughtered goats in south-western Nigeria and this portends serious economic implications on livestock production through reduced productivity and even death. The prevalence of fertile cysts obtained in this study suggests a high potential for human infection with hydatidosis through contacts with exposed dogs or consuming water contaminated with infected dogs faeces. The current results suggest that a thorough investigation that leads to a disease control strategy is required to reduce the economic and public health consequences of hydatidosis.

437

KAP (KNOWLEDGE, ATTITUDE AND PRACTICE) OF ECHINOCOCCOSIS - FIRST REPORT FROM CENTRAL SUDAN

Mohamed Elamin Ahmed[1], Osama Ahmed Hassan[2], Abdelrahman K. Khalifa[2], Eyhab Elobied[2], Ahmed A. Osman[2], Martin Grobusch[3], Peter Kern[4]

[1]Alneelain University, Khartoum, Sudan, [2]Sudan Hydatid Research Society, Khartoum, Sudan, [3]Amsterdam Medical College, Amsterdam, Netherlands, [4]Ulm University, Ulm, Germany

In Sudan, Echinoccosis is a chronic neglected disease caused by *Echinoccocus granulosus* (EC). It affects animals and humans can be accidentally infected through direct contact with dogs or ingestion of contaminated water or food. Studies have shown infection rate as follows: Dogs 50-70% , camels 35% , sheep,goats and cattle10-11%,where as humans in central Sudan 0.3-1.0%. Hydatid cyst in humans might lead to serious complications if left untreated. Treatment is expensive and sometime is cumbersome where advanced surgery can be needed. The study area is known by its large livestock with camels prepondance and where previous surveys in dogs, camels and human has been performed. Screening in humans has led to successful medical treatment only, using portable ultrasound in all asymptomatic cases. In Sudan regarding Human EC genotyping, G6 (camel strain) is the cause for almost all human samples collected. Therefore, the objective of this study is to explore knowledge, attitude and practice (KAP) of people living in three villages in Tambool area, central of Sudan. A cross sectional study using close ended structured questionnaire covering the thematic areas of Hydatid KAP was administered to 312 household (full coverage). The data was entered and analyzed by SPSS. Results showed that participants mean age was 37.5 ±16.3 years. Substantial number of the respondents (73.7%) never heard about the disease. Only 26% heard about it, however they reflect low knowledge about cause, mode of transmission, prevention and control of Echinococcosis. In terms of attitude, half of the participants who heard about the disease believe Hydatid cyst patients should be isolated. This might indicate potential stigma. Participants practice showed some behavioral risk as 81.7% slaughter animals inside home without veterinary supervision and 70 % throw the offal outside home where stray dogs eat it. In conclusion, the study mapped the baseline for KAP of the community of the study area. It revealed that the population had poor knowledge and attitude. Their practices might help circulating the parasite in the area. Consequently health education is highly needed to prevent and control the parasite and disease.

438

OVALBUMIN-INDUCED AIRWAY INFLAMMATION IS REDUCED BY *ECHINOCOCCUS GRANULOSUS* INFECTION WHICH DOWN-REGULATED IL17 IN MICE

Hui Wang[1], Jun Li[1], Hongwei Pu[1], Bilal Hasan[2], Jinfeng Ma[3], Malcolm K. Jones[4], Xue Zhang[1], Chuanshan Zhang[1], Donald Peter McManus[5], Renyong Lin[1], Hao Wen[1], Wenbao Zhang[1]

[1]The First Affiliated Hospital of Xinjiang Medical University, Urumqi, China, [2]Laboratory of Respiratory Physiology and Pathology, Traditional Chinese Medicine Hospital Affiliated with Xinjiang Medical University, Urumqi, China, [3]Department of Epidemiology and Health Statistics, School of Public Health Xinjiang Medical University, Urumqi, China, [4]School of Veterinary Sciences, The University of Queensland, Queensland, Australia, [5]Molecular Parasitology Laboratory, Infectious Diseases Division, QIMR Berghofer Medical Research Institute, Brisbane, Australia

Cystic echinococcosis (CE) is a cosmopolitan zoonosis caused by the larval stage of the dog tapeworm *Echinococcus granulosus*. The infection induces a polarized T-helper type 2 (Th2) response in its intermediate hosts. Here, we examined the effects of *E. granulosus* infection on mouse ovalbumin (OVA)-induced asthma. BALB/c mice were intraperitoneally transplanted with 50 small Eg cysts cultured *in vitro* 3 months prior to being sensitized and challenged with OVA. The mice with cysts transplanted harbored an average of 34.8 cysts per mouse, with a mean diameter of 4.4 mm. Histological staining of lung tissues showed that *E. granulosus* infection significantly reduced the severity of OVA-induced airway inflammation including reduction of eosinophil cell accumulation and mucus production. Airway hyperresponsiveness of OVA-challenged mice after *E. granulosus* infection was significantly suppressed as compared to the OVA-only challenged mice. The infection significantly increased both Th1 and Th2 cytokine expression in PBMC and also in lung tissue, but significantly down-regulated IL-17a expression. When the mice were challenged with OVA, the expressions of IFN-γ, IL-2, IL-4 and IL-5 in lung were down-regulated,whereas, IL-2 and IFN-γ were expressed in high level. In conclusion, *E. granulosus* infection markedly reduced the severity of OVA-induced airway inflammation. Down-regulation of Th17 responses in lung tissue may be a potential therapeutic treatment against allergic asthma.

439

RIEC: THE FIRST REGISTRY OF CYSTIC ECHINOCOCCOSIS, FROM ITALIAN TO EUROPEAN

Francesca Tamarozzi[1], Patrizia Rossi[2], Fabio Galati[3], Mara Mariconti[4], Giovanni Jacopo Nicoletti[1], Francesca Rinaldi[1], Adriano Casulli[2], Edoardo Pozio[2], Enrico Brunetti[5]

[1]University of Pavia, Department of Clinical Surgical Diagnostic and Paediatric Sciences, World Health Organization Collaborative Centre for Clinical Management of Cystic Echinococcosis, Pavia, Italy, [2]Istituto Superiore di Sanità, Department of Infectious, Parasitic and Immunomediated Diseases, Rome, Italy, [3]Istituto Superiore di Sanità, SIDBAE, Information Technology, Rome, Italy, [4]IRCCS San Matteo Hospital Foundation, Pavia, World Health Organization Collaborative Centre for Clinical Management of Cystc Echinococcosis, Pavia, Italy, [5]University of Pavia, Department of Clinical Surgical Diagnostic and Paediatric Sciences, IRCCS San Matteo Hospital Foundation, Pavia, World Health Organization Collaborative Centre for Clinical Management of Cystic Echinococcosis, Pavia, Italy

Cystic Echinococcosis (CE) is endemic in Eastern and Southern Europe, Italy included. However, its real burden is largely unknown due to the lack of efficient reporting systems designed to take into account the peculiar features of this disease. In Italy, only a yearly summary of regional data is required by the health authorities and the prevalence and incidence of human CE are hugely underestimated because only hospitalized cases are registered, while the majority of CE cases are diagnosed and managed on an outpatient basis. Furthermore, no official data are transmitted to European authorities. The neglect of CE also results in general lack of knowledge on its diagnosis and clinical management outside referral centres, with consequent heterogeneity in clinical practices and often unnecessary procedures with associated risks and costs. In 2012 the Istituto Superiore di Sanità (ISS - Italian National Health Institute - Rome) in collaboration with the University of Pavia, WHO Collaborative Centre for the Clinical management of Cystic Echinococcosis, implemented the Italian Registry of Cystic Echinococcosis (RIEC). This is a prospective multicenter registry of CE patients visited from January 2012 in Italian health centres that adhered voluntarily. RIEC is accessible on the website of ISS since October 2012 with the aims of: indicating the burden of CE in Italy; bringing the importance of this infection to the attention of health authorities; encouraging public health policies geared toward its control; stimulating research on CE. Moreover, it provides an useful tool for patients follow-up and evaluation of therapeutic interventions. As of February 2014, 346 patients were enrolled in 11 centres, figures largely outnumbering the national reports of many endemic European countries. We will discuss updated results and challenges of RIEC, that is the template for the European Registry of CE, to be implemented within the FP7-HERACLES project.

440

IDENTIFICATION OF FUNCTIONAL MITOGEN-ACTIVATED PROTEIN KINASE KINASES (MAPKK) HOMOLOGUES FROM *ECHINOCOCCUS GRANULOSUS*: PROTOSCOLICIDAL ACTIVITY OF MAPK SIGNALING PATHWAY INHIBITORS

Zhang Chuanshan[1], Li Jing[1], Wang Junhua[1], Lü Guodong[1], Lu Xiaomei[1], Zhang Wenbao[1], Dominique A. Vuitton[2], Wen Hao[1], Lin Renyong[1]

[1]The First Affiliated Hospital of Xinjiang Medical University, Urumqi, China, [2]University of Franche-Comté, Besançon, France

Cystic echinococcosis (CE) treatment urgently needs a new drug. Understanding molecular regulation is essential for identification of drug targets. In the present study, we identified two new members of the MAPKKs, MEK3/6 and MEK1/2 homologues (termed as EgMKK1 and EgMKK2, respectively) from *Echinococcus granulosus*, the causative agent from CE. Both EgMKK1 and EgMKK2 were co-expressed in the larval stages. EgMKK1 encoded protein was localized in the subtegumental and tegumental layer and EgMKK2 was localized in the tegumental and sucker of the parasite. As shown by yeast two-hybrid and Co-immunoprecipitation analysis, EgMKK1 strongly interacted with the p38-like MAP kinase Egp38. EgMKK2, on the other hand, not only interacted with a member of the parasite's 14-3-3 protein family, but also with the Erk-like MAP kinase EgERK. Recombinant EgMKK1 and EgMKK2 displayed kinase activity towards myelin basic protein substrate. When Sorafenib Tosylate (Bay 43-9006), an inhibitor of MAPKKK (Raf-1/B-Raf), and U0126 and PD184352, inhibitors of MAPKK (MEK1/2), were added to the medium for *in vitro* cultivation of *E. granulosus* protoscoleces, these inhibitors resulted in a marked dephosphorylation of EgERK for 20 and 30 days, then had actually an inhibitory and cytolytic effect on the larval stage of the parasite. In addition, Sorafenib Tosylate killed protoscoleces more effectively. *In vitro* culture of protoscoleces in the presence of 25 μM Sorafenib Tosylate or 100 μM U0126 for a period of 30 days was parasiticidal, as determined by murine bioassays, while treatment with 100 μM PD184352 was not. Our study indicates that small inhibitors of MAPK signaling pathway may be potential drugs for the treatment of CE.

441

TRANSCRIPTIONAL PROFILES OF PROTOSCOLECES OF *ECHINOCOCCUS GRANULOSUS* IN RESPONSE TO TGF-Β REVEAL INCREASED EXPRESSION OF GENES INVOLVED IN GROWTH OR DEVELOPMENT

Guodong Lü, Yunfeng Xiao, Hao Wen, Renyong Lin

Xinjiang Medical University, Urumuqi, China

TGF-β is a crucial cytokine participate in the interplay between the intermediate host and helminthes. TGF-β receptors were discovered in many cestode, and could bind the human TGF-β. However, the function of host TGF-β on the *Echinococcus* is still not elucidated, and this paper aim to explore the question at transcription level. Microarray analysis was used to investigate differential expression genes in protoscolices of *Echinococcus granulosus* cultured in the presence or absence of human TGF-β at different time points (4h, 8h and 24h) *in vitro*. A total of 523 genes were up- or down-regulated in response to TGF-β, compared with control group, 390 genes were up-regulated and 47 genes were down-regulated at 8h, and 376 genes were up-regulated and 19 genes were down-regulated at 12h, including 310 differential genes were regulated at both time point. Gene ontology (GO) analysis showed that the biological process of the up-regulated genes in protoscolex were predominantly involved in DNA packaging, nucleosome assembly, chromatin assembly, etc. And the cellular component gene were located in cell nucleus. TGF-β appeared to promote growth or development of the protoscolex by up-regulated the gene related with mitosis. In addition, the study also indicated that TGF-β has a multiple influence on the protoscolex, as reflected in the increased stimulation of gene expression of the ErbB signaling pathway, MAPK signaling pathway, Notch signaling pathway and VEGF signaling pathway.

442

IDENTIFICATION OF FUNCTIONAL SMAD8 AND SMAD4 HOMOLOGUES FROM *ECHINOCOCCUS GRANULOSUS*: PROTOSCOLICIDAL ACTIVITY OF TGF-Β/BMP SIGNALING PATHWAY INHIBITORS

Lin Renyong[1], Zhang Chuanshan[1], Wang Limin[1], Wang Hui[1], Pu Hongwei[1], Wang Junhua[1], Lü Guodong[1], Lu Xiaomei[1], Zhang Wenbao[1], Dominique A. Vuitton[2], Wen Hao[1]

[1]The First Affiliated Hospital of Xinjiang Medical University, Urumqi, China, [2]University of Franche-Comté and University Hospital, Besançon, France

Cystic echinococcosis (CE) treatment urgently needs a new drug. Understanding molecular regulation is essential for identification of drug targets. In the present study, we identified two new members of the Smad proteins, Smad8 and Smad4 homologues (termed as EgSmad8 and EgSmad4, respectively) from *Echinococcus granulosus*, the causative

agent from CE. Both EgSmad8 and EgSmad4 were co-expressed in the larval stages and their encoded proteins were localized in the subtegumental and tegumental layer of the parasite. As shown by yeast two-hybrid and pull-down analysis, EgSmad8 displayed a positive binding interaction with EgSmad4 and a previously identified EgTβR I. In addition, EgSmad8 localized in the nuclei of Mv1Lu cells (mink lung epithelial cells) upon treatment with human TGF-β1 or human BMP2, indicating that translocation of EgSmad8 into nuclei depends upon signals initiated by the ligands of human origin. When LDN193189, an inhibitor of TGF-β/BMP type receptors, and SIS3, an inhibitor of R-Smads, were added to the medium for *in vitro* cultivation of *E. granulosus* protoscoleces, these inhibitors had actually an inhibitory and cytolytic effect on the larval stage of the parasite. Our study indicates that small inhibitors of TGF/BMP signaling pathway may be potential drugs for the treatment of CE.

443

NEW ULTRASONOGRAPHIC CLASSIFICATION OF HEPATIC ALVEOLAR ECHINOCOCCOSIS

Tilmann Graeter, Wolfgang Kratzer, Suemeyra Oeztuerk, Tanja Kaltenbach, Mark Martin Haenle, Peter Kern, Beate Gruener

University Hospital Ulm, Ulm, Germany

Ultrasonography provides one of the most important diagnostic tools in suspected alveolar echinococcosis. The aim of the study was to establish a new sonographic classification based on a large patient population with confirmed hepatic alveolar echinococcosis. In 225 patients, ultrasound morphology of liver lesions due to an alveolar echinococcosis was retrospectively examined. The findings were grouped into the new classification scheme. The following classification have been established: storm and hail pattern, pseudo hemangioma-like pattern, ossification pattern and metastasis-like pattern. The respective classification patterns are demonstrated by imaging examples. The proposed ultrasonographic classification improves the diagnosis of hepatic alveolar echinococcosis. This makes it possible to interpret different clinical courses better and helps in the context of scientific studies to improve the comparability of ultrasonographic findings.

444

HYDATID DISEASE IN IRAQ: A RETROSPECTIVE STUDY OF 1,980 PATIENTS IN BAGHDAD

A Hadi Al Khalili

Department of Neurosurgery, Baghdad University, Baghdad, Iraq

Human hydatid disease caused by *Echinococcus granulosus* has been endemic in Iraq for many years. Migration of people and intermediate hosts over time can impact the distribution and epidemiology of this disease, especially in developing countries and in times of conflict. Observations made on the clinical services that care for hydatid patients in Baghdad posed the question of whether there is an age-related association with the location of cysts in various organs. Therefore to address this question we conducted a retrospective chart review of 1,980 patients with hydatid cyst admitted to three major hospitals in Baghdad, Iraq. In particular we noted the age at which the patient presented and the location of hydatid cysts diagnosed radiographically or surgically. The majority of patients with hydatid disease of the body were between 20 and 40 years old. In our series, we observed that abdominal hydatid was seen in 66.7%, liver (54%), spleen (3.2%), kidney (3.2%), peritoneum (3.2%), retroperitoneal (1.1%), pelvis (0.8%), and pancreas (0.3%). The chest was involved in 29%, lungs 28%, pleura 0.2%, pericardium 0.1%, mediastinum 0.4%, bone and spine 0.8%, soft tissue 3.1% and brain and orbit 0.5%. When we analyzed patients by age groups, we noted an apparent association of certain ages with the highest risks of particular organ involvement. Hydatid disease of the liver was most common in the 20-39 year age group, whereas lung involvement was most common in a slightly younger subset. Kidney cysts were most commonly observed in the 40-49 year age group. Other frequencies were noted for bone and spine (30-39 years). Brain and orbit disease was most common in the 5-19 year old age group. These observations suggest that several possible explanations for the majority of brain/orbit disease in young people. The age and/or immune status when hydatid disease is acquired by humans may impact the ultimate location of cysts in the body. These observations may lead to new insights into the pathogenesis, diagnosis and treatment of human hydatid disease.

445

PREDICTION AND IDENTIFICATION OF ANTIGENIC EPITOPES IN EG95 OF *ECHINOCOCCUS GRANULOSUS*

Jianging Ding

Xinjiang Medical University, Urumqi, China

This study was undertaken 1) to predict the T and B union epitope of the Eg95 antigen of *Echinococcus granulosus* , 2) to obtain the different peptide fragment of T-B union epitope via Phage display technology, 3) toidentify and screen the peptide fragment via Western Blot and ELISA, and 4) to provide the basis for the T and B union epitope vaccine's manufacture of Echinococcosis. Online networks BCEpred and LEPS predicted the B cell epitope of Eg95 antigen. Using SYFPEITHI software predicted the MHC I nonamers T cell epitope of HLAA 0201 and H2-Db. To analyzes the secondary structure, transmembrane structure, B cell epitope, T cell epitope of humans and mice. Finally, determine the area of B cell and T cell union epitope.Using DNAman software design primers, amplified amino acid sequence of T-B cell union epitope,then restruct the gene and M13KE Phage carrier using T4 Ligase. Constructed M13KE/Eg95-1 M13KE/Eg95-2 and M13KE/Eg95-3 plasmid then transformed into E.coliER2738 respectively, identified the correct sequence using PCR. Purified the recombinant phage by PEG/NaCl way of precipitation. Identified the expression level of recombinant protein rPIII by SDS-PAGE electrophoresis analysis.Prepared the rEg95 patient serum and antiserum as antibody, confirmed the correctness of epitope peptide antigen by Western Blot. Finally,compared with epitope peptide antigen's reactivity by ELISA, screened and comfirmed better of antigen epitope peptide as candidate epitopes of epitope vaccine of Echinococcosis. Predicted the advantage Eg95 antigen epitope by line through using the bioinformatics method, and confirmed 3 T-B union epitope. To successfully cloned and constructed the prokaryotic expression plasmid M13KE/Eg95-1, M13KE/Eg95-2 and M13KE/Eg95-3, Purified the recombinant phage by PEG/NaCl way of precipitation. SDS-PAGE assay showed that Eg95-1,Eg95-2 and Eg95-3 protein III was successfully expressed. Echinococcosis patient and anti-rEg95 serum can identify the 3 T-B union epitope, but the signal intension was differences.

446

HUMAN CYSTIC ECHINOCOCCOSIS IN SUDAN: A SILENT HEALTH THREAT

Rihab A. Omer[1], Arwid Daugschies[1], Mohammed Ahmed[2], Ayman Elnahas[3], Peter Kern[4], Thomas Romig[5]

[1]Institute of Parasitology, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany, [2]Elshaab Teaching Hospital, Khartoum, Sudan, [3]Department of Surgery and Anaethesia, Faculty of Veterinary Medicine, University of Khartoum, Khartoum, Sudan, [4]Faculty of Medicine, University of Ulm, Ulm, Germany, [5]Institute of Parasitology, Department of Zoology, University of Hohenheim, Stuttgart, Germany

Cystic echinococcosis (CE) is a zoonosis caused by cestodes of the genus Echinococcus. The adult tapeworms are intestinal parasites of dogs and some other carnivores which require a larval tissue stage (hydatid cyst) in other animal species (intermediate hosts). This larva usually grows in the form of a large, fluid-filled cyst ('cystic echinococcosis'), which can affect many organs, most often liver and lungs. In humans, CE causes significant morbidity, largely depending on the size and localization of the cyst(s). Treatment requires abdominal or pulmonary surgery or other invasive procedures (percutaneous treatment techniques) in combination with

prolonged chemotherapy, which is costly and often not available in remote areas. The economic burden of CE is caused by human mortality and disability. The transmission of the parasite to humans is facilitated by the presence of dogs and livestock in conjunction with slaughtering practices (feeding of raw offal to dogs) and poor hygiene. CE has a particular impact in developing countries and in terms of global disease burden (in DALYs), it is close to African trypanosomosis and schistosomosis, zoonoses which receive far more attention in terms of research and control. Prevalence levels of CE can be >5% in the nomadic or semi-nomadic ethnicities of northern Kenya or southern Sudan and yet not exactly determined in other parts. The access to therapy for this particular part of the population is usually sporadic, and established procedures for treatment are not always feasible under the local conditions. Preventive programs are difficult to implement and sustain over sufficiently long periods due to the lack of infrastructure and/or financial resources. It is important to have a clear picture about the disease in the country to enable its control regardless of all these difficulties, thus study aims at highlighting the prevalence, diagnosis and distribution of cystic echinococcosis in patients distributed in different parts of the Sudan since the disease was first reported in 1908.

447

TH1 /TH2 AND TH17/TREG IMMUNE RESPONSE IMBALANCE IN PATIENTS WITH HEPATIC ALVEOLAR ECHINOCOCCOSIS

Tuerhongjiang Tuxun, Hao Wen, Ren-Yong Lin, Hai-Zhang Ma, Jin-Hui Zhang, Jin-Ming Zhao

State Key Lab Incubation Base for Xinjiang Major Diseases Research and Xinjiang Key Laboratory of Echinococcosis, First Affiliated Hospital of Xinjiang Medical University, Urumqi, China

Alveolar echinococcosis (AE), caused by the larval stage of *echinococcus multilocularis (E.m),* continues to be a worldwide public health problem. During the infection, *E.m* interacts with and modulate host's immune system for its successful survival. Herein, we have comprehensively studied T cell subsets immune response profile in patients with AE. Total number of 55 subjects were enrolled and divided into three groups: AE group, (n=15), AE with albendazole treatment (AE+ABZ, n=14), and healthy controls (HC, n=26). Th17 and Treg cells frequencies in peripheral blood mononuclear cells (PBMCs) were measured by flow cytometry. The mRNA expression levels of T-bet, GATA3, RORrt, and FoxP3 were measured by RT-PCR. Plasma level of IFN-r (Th1), IL-5 and IL-6 (Th2), IL-17A, IL-17F, and IL-23 (Th17); and IL-10 (Treg) were detected by ELISA. Result showed that the Th17 cell frequency significantly decreased in AE group, however it is remarkably increased in AE+ABZ group than that in HC group; T-bet mRNA expression in AE group is lower than HC group, while, higher in AE+ABZ group; GATA3 mRNA expression is slightly increased in AE group, however, it is significantly decreased in AE + ABZ group; RoRrt mRNA expression level decreased in both group than that in HC group, moreover, a slight increase showed in AE +ABZ group than AE group; Foxp3 mRNA expression level slightly increased in AE group than HC group, however, it is decreased in AE + ABZ group. Plasma level of IFN-r slightly decreased in AE, and increased in AE + ABZ group compared to HC group; Levels of plasma IL-5 and IL-6 slightly increased in AE group, whereas, it decreased in AE +ABZ group than that in HC group; IL-17A, IL-17F, and IL-23 significantly increased in both group than that in HC group, however, the levels are lower in AE + ABZ group; Plasma levels of IL-10 are increased in both AE and AE + ABZ group, however, the level was lower in AE +ABZ group. Our results demonstrated that there is Th1 / Th2, and Th17 / Treg immune response imbalance exist during the infection, this imbalance may play a potential role in parasite immune evasion and immune-pathogenesis of hepatic alveolar echinococcosis.

448

EXPRESSION OF TOLL-LIKE RECEPTOR 2, 4 AND 7 IN PERIPHERAL BLOOD MONONUCLEAR CELLS IN PATIENTS WITH HEPATIC CYSTIC AND ALVEOLAR ECHINOCOCCOSIS

Hai-Zhang Ma, Tuerhongjiang Tuxun, Hao Wen, Ren-Yong Lin, Jin-Ming Zhao, Jin-Hui Zhang

State Key Lab Incubation Base for Xinjiang Major Diseases Research and Xinjiang Key Laboratory of Echinococcosis, First Affiliated Hospital of Xinjiang Medical University, Urumqi, China

This study aims at to explore expression of Toll-like receptors (TLRs) and related cytokines in patients with cystic echinococcosis (CE) and alveolar echinococcosis (AE). 55 subjects were enrolled and divided into three groups: AE group (N=15), CE group (N=14), and healthy controls (HC, N=26). The mRNA expression levels of TLR2, TLR4 and TLR7 in peripheral blood mononuclear cells (PBMCs) were measured by using real-time fluorescent quantitative reverse-transcription polymerase chain reaction (RT-PCR). Plasma levels of INF-γ, IL-5, IL-6, IL-10 and IL-23 were detected by using ELISA. Values are expressed as mean ± SD and the data were analyzed by ANOVA. If significance was found, Newman-Keuls test was performed for post-hoc analysis to detect the difference among groups. TLR2 mRNA expression was significantly increased in the CE and AE groups compared to the HC group (P<0.05). TLR4 mRNA expression was higher in the CE and AE groups and statistical significance was only shown in CE group (P<0.05). However, TLR7 mRNA expression was remarkably decreased both in CE and AE groups with statistical significance. Plasma levels of IL-10 and IL-23 in patients with CE and AE were significantly higher than those in controls (P<0.05). Levels of serum IL-5 and IL-6 CE and AE group were higher than those in HC group with no statistical significance (P>0.05), IFN-γ level was slightly decreased in AE group, oppositely, increased in CE group with no statistical significance. Expression pattern of TLR 2, 4 and 7 in PBMCs in patients with AE and CE might be involved in the cytokine modulation, which allowed the parasite to escape, which seems to be stronger in AE, host immune-surveillance and promoted chronic infection.

449

NEUROCYSTICERCOSIS: AN UNKNOWN, A FORGOTTEN, OR A NEGLECTED PARASITIC ZOONOSIS IN NIGERIA?

Ikenna O. Ezeh, Ekene V. Ezenduka, Denchris N. Onah

University of Nigeria Nsukka, Nsukka, Nigeria

Epilepsy or seizure is among the most common neurological disorders all over the world, including Nigeria and the aetiology may vary. However, the International League Against Epilepsy recognizes neurocysticercosis as a growing problem in many tropical countries and as a leading cause of epilepsy and seizures. Neurocysticercosis (NCC) is an infection of the central nervous system (CNS) caused by the metacestode stage (*Cysticercus cellulosae*) of the pig tapeworm of man *Taenia solium*. It is probably the most common helminth parasite incriminated in central nervous system parasitic infections in human beings. *T. solium* taeniasis and therefore, NCC are common in many of the world's poorer countries especially where the environmental hygiene is poor and families raise free-roaming pigs that have easy access to and consume human faeces. In Nigeria, *T. solium* cysticercosis is common in pigs but there is little information on human *T. solium* taeniasis and virtually none on human *T. solium* cysticercosis (ocular- and neuro-cysticercosis). Conversely, epilepsy is the commonest neurological condition diagnosed in adults in most Teaching Hospitals in Nigeria but whether it could be attributed to neurocysticercosis is not clear. This paper examines neurocysticercosis vis a vis epilepsy and seizures and draws attention to this important zoonosis which it seems in Nigeria is an unknown, a neglected or a completely forgotten major causative agent of neurological conditions.

450

RE-EVALUATION OF PORCINE CYSTICERCOSIS IN NSUKKA AREA OF ENUGU STATE, NIGERIA

Idika K. Idika, Ugochi J. Agbo, Ikenna O. Ezeh, Ekene V. Ezenduka, Denis N. Onah

University of Nigeria, Nsukka, Nsukka, Nigeria

The prevalence of porcine cysticercosis in Nsukka Area of Enugu State, Nigeria was re-evaluated between February and June, 2013, using structured questionnaire and post mortem examination of slaughtered pig carcasses at Ibagwa, Orba and Nsukka slaughter slabs. Questionnaires were distributed to willing butchers and pig marketers and completed copies retrieved and analysed. The slabs were visited every other week and pig carcasses examined in accordance with standard meat inspection procedures. Briefly, carcasses were examined visually under natural light and palpated before longitudinal incisions were made on the heart, tongue, masseter, neck, intercostal, shoulder and thigh muscles and examined. Carcasses containing cysticerci were recorded as positive for *Cysticercus cellulosae*. The sex, age and breed of all animals examined were determined. Results showed cysticercosis prevalence of 3.3%, 4.3% and 0% for Ibagwa, Orba and Nsukka slaughter slabs respectively and an overall prevalence of 2.4% for the three study areas during the period. Moreover, the type of pig husbandry practiced was the most important factor influencing the prevalence of the infection. Analysis of the questionnaire responses showed that the majority of the respondents were not aware of the zoonotic implication of porcine cysticercosis irrespective of their educational background and that epilepsy in the family could be associated with the infection in man. It is concluded that *Taenia solium* cysticercosis remains endemic in Nsukka area of Enugu State, Nigeria and poses a major health hazard that must be addressed by coordinated control programs.

451

EVALUATION OF TRICLABENDAZOLE AGAINST *TAENIA SOLIUM* METACESTODE IN NATURALLY INFECTED PIGS

Ana M. Vargas-Calla[1], Luis A. Gomez-Puerta[1], Juan F. Calcina[1], Teresa Lopez-Urbina[1], Hector H. Garcia[2], Armando E. Gonzalez[1]

[1]Universidad Nacional Mayor de San Marcos, Lima, Peru, [2]Universidad Peruana Cayetano Heredia, Lima, Peru

Taenia solium cysticercosis is an important zoonotic disease in developing countries. Many studies have been conducted to evaluate the efficacy of different antiparasitic drugs against the larval stage (cysticercus) of *T. solium*. Currently, oxfendazole (OFZ) is the best drug against porcine cysticercosis, with excellent efficacy using a single oral dose. On the other hand, triclabendazole (TCZ) is the most common drug used to treat livestock helminthiases. Therefore, the aim of this study was to evaluate the efficacy of TCZ in naturally cysticercosis-infected pigs. Eighteen pigs were divided into 3 groups of 6 individuals each. The groups were treated as follows: a first group was treated orally with TCZ at a single dose of 30 mg/kg of body weight, the second group was treated orally with OFZ at a single dose of 30 mg/kg of body weight and the third group was left untreated (control). All animals were kept under the same management conditions. The pigs were humanely killed 17 weeks post-treatment and the number of surviving cysts in muscles was assessed. Cysts in pigs treated with TCZ had a normal appearance, not different from the control group ($p>0.05$). All pigs treated with OFZ had only degenerated cysts in their carcasses ($p<0.05$ for parasiticidal efficacy compared with the control group). TCZ is not efficacious to treat porcine cysticercosis.

452

TAENIA SOLIUM GENOTYPES IN A RURAL COMMUNITY: RELATIONSHIP BETWEEN PORCINE CYSTICERCOSIS AND HUMAN TAENIOSIS

Monica J. Pajuelo[1], Maria Eguiluz[1], Armando Gonzalez[2], Patricia Sheen[1], Héctor García[1], Robert Gilman[3], Mirko Zimic, for the Cysticercosis Working Group in Peru[1]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Universidad Nacional Mayor de San Marcos, Lima, Peru, [3]Johns Hopkins University, Baltimore, MD, United States

Taenia solium is a cestode that infects pigs and humans in different regions of the world and is cause of neurocysticercosis in humans. The understanding of the association between the genetic variability of the parasite with geographic origin and phenotypic characteristics like infectivity, pathogenicity and response to treatment, will be a critical tool in the efforts to prevent and control this disease. After analyzing the entire *T. solium* genome, we demonstrated that microsatellite markers are widely distributed throughout of the genome. In addition these markers have shown to have enough variation to differentiate strains. The objective of this study is to use microsatellite markers and to compare the genetic profiles between cysts recovered from naturally infected pigs and tapeworms from human hosts in a rural community in Northern Peru. We performed a cross sectional study where four tapeworms were genotyped and showed different profiles. All pigs in the community were screened for cysticercosis, using the tongue test. We identified 8 cysticercosis-tongue-positive pigs. Animals were humanely sacrificed and 30 individually cysts were recovered from the carcass of each pig. Cysts were genotyped with the same microsatellite markers and compared to the genotypes of the previously identified tapeworms. We found genetic variation among this population. Two pigs had about 30% of cysts with a genotype that exactly matched to the tapeworm corresponding to the geographically closest human host. Interestingly, one pig showed 50% of cysts with a genotype different to any of the tested tapeworms. We propose microsatellites as promising genetic markers with which it would be possible to study the genetic epidemiology of this disease, in particular the transmission from tapeworm carrier to pig and the reintroduction of foreign cysts/tapeworms into a community. We are conducting additional studies to verify the source of genetic variation of microsatellites in *T. solium* cysts.

453

PURIFICATION OF VESICULAR FLUID ANTIGEN OF CYSTICERCI *TAENIA SOLIUM*, FOR HUMAN DIAGNOSIS IN THE ANDEAN TRAPEZIUM AND UNASUR COUNTRIES

Eduardo R. Ayala[1], Eduardo F. Miranda[1], DIRESA: Huanuco, Junin, Ayacucho, San Martin, Ucayali Health Regional Direction MINSA-Peru[2]

[1]National Institute of Health, Lima, Peru, [2]Ministry of Health, Lima, Peru

Neurocysticercosis (NCC) is a neglected parasitic disease and a leading cause of seizures and epilepsy in developing countries. Serological assays detecting *T. solium* antigens complement standard imaging techniques to establish NCC diagnosis. Crude antigens extracted from the vesicular fluid of *T. solium* cysticerci (*Tsol*.VFAg) have been used for this purpose; however, the availability of technology and cost required to purify is limited in developing countries. We compared and evaluated four purification methods to obtain *T. solium* antigens for serological assays. Cysticerci were collected from different endemic areas of Peru and *Tsol*. VFAg were purified using the following methods: i) ammonium sulphate, ii) lentil-lectin sepharose (affinity chromatography), iii) sephadex G-75, and iv) electro-elution. The analytic sensitivity and specificity were evaluated using patient serum from individuals with NCC and other parasitosis. The crude antigenic protein concentration was 3.6µg/µL which is within the range as suggested by CDC and also SDS-PAGE electrophoresis revealed seven glycoproteins (GP50, GP42-39, GP24, GP21, GP18, GP14 and GP13kDa) with affinity/lentil-lectin method. Our research identified eight

bands diagnostics with affinity/lentil-lectin method (GPs.35, 31, 24, 23, 18, 17, 14 and 13kDa.), while the other three methods detected different fractions of those proteins (ammonium sulphate: no band, electro-elution: 6 bands of 97-GP12kDa, and sephadex G-75: 17 bands of 100-GP6KDa). Western blot using purified glycoprotein (affinity/lentil-lectin method) identified 50/50 patients with NCC (100% sensitivity), 50/50 healthy control individuals (100% specificity), 20 individuals with other parasitosis (*Hymenolepis nana, Echinococcus granulosus, Fasciola hepatica*) give cross-reacted with the GP42-39Kda.protein. Affinity chromatography (lentil-lectin sepharose) is the method of choice to purify glycoproteins from *T. solium* cysticerci for the development of a reliable and affordable immunodiagnostic kit for NCC which can be applied in the Andean trapezium and UNASUR countries.

454

EPILEPSY AND CYSTICERCOSIS IN THE HIGHLANDS OF MADAGASCAR: IS SEROLOGY RELEVANT?

Thierry Franchard[1], Ismael Chakir[2], Falinirina Raharinjanahary[3], Mahenintsoa Rakotondrazaka[2], Anjanirina Rahantamalala[2], Angeline Rasoarimanana[4], Alain Kapisy[5], Romy Razakandrainibe[2], Benedicte Contamin[6], **Ronan Jambou**[7]

[1]Institut Pasteur de Madagascar, Paris, Madagascar, [2]Institut Pasteur de Madagascar, Antananarivo, Madagascar, [3]Service de Santé du district de Moramanga, Moramanga, Madagascar, [4]Hopital Luthérien d'Antsirabe, Antsirabe, Madagascar, [5]Service Médical Inter Entreprises, Anstirabe, Madagascar, [6]Centre d'Infectiologie Charles Mérieux, Faculté de médecine d'Antananarivo, Antananarivo, Madagascar, [7]Institut Pasteur, Paris, France

In sub-Saharan Africa epilepsy reflects complicated delivery and sequelae of infections during childhood. However in rural countries like Madagascar the profile of epilepsy is mostly unknown. In the same time, Madagascar is one of the hotspot of cysticercosis and its burden during epilepsy is unknown. To describe this profile and relation with cysticercosis a survey was conducted on epileptics in 40 dispensaries of the Moramanga district, a rural area and in two private hospitals in Antsirabe (a small town near the capital). Patients older than 5 years were recruited after at least two unexplained seizures. For each patient recruited in dispensaries a control subject matched on age was recruited in household randomly selected in the same village. Socioeconomic and clinical data were collected. Serology for cysticercosis was conducted using glycosylated crude proteins of the cysticercus in ELISA and western blot. A total of 247 subjects were investigated (125 epileptics and 122 controls). No type of seizure was associated with positive serology for cysticercocis. Seroprevalence was the same for epileptics and controls (49.5% / 50.4%). It was higher in rural than in urban area, and for female than for male. However epileptics have higher ELISA mean OD than controls (0.461 vs 0.26), and younger epileptics have higher OD than older ones. In univariate analysis higher prevalence of cysticercosis in epileptics was associated with the number of children in the household and the use of backyard wells for water supply, but not with the presence of pigs. In multivariate analysis age, gender, urban/rural habitat and presence of pigs were retained to explain epilepsy but not seropositivity for cysticercosis. For seropositivity only the absence of toilets and the number of children in the household were retained. Over all poor access to sanitation and the number of children in the household were the major parameters associated with cysticercosis, undoubtfully in relation with taeniasis. However in this area, serology for cysticercosis is not relevant for diagnostic.

455

MONOCLONAL ANTIBODIES FOR DETECTION OF PARASITE ANTIGENS IN BODY FLUIDS FROM PATIENTS WITH NEUROCYSTICERCOSIS

Adriana Paredes[1], Patricia Saenz[1], Miguel W. Marzal[1], Andrea M. Rivera[1], Miguel A. Orrego[1], Carla M. Cangalaya[1], Cristina Guerra-Giraldez[1], Silvia Rodriguez[2], Siddhartha Mahanty[3], Hector H. García[2], Theodore E. Nash[3]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Instituto Nacional de Ciencias Neurologicas, Lima, Peru, [3]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States

Neurocysticercosis (NCC), an infection of the brain by *Taenia solium* cysts, represents the most common cause of adult-onset epilepsy in developing countries. Serologic diagnosis has historically been based on detection of antibodies to *T. solium* in serum, but antigen (Ag) detection assays using T. saginata cross reactive monoclonal antibodies (MAbs) have been applied to serum and body fluids more recently to identify individuals with live parasites. We generated and characterized 22 murine monoclonal antibodies against *T. solium* cysts in BALB/c mice using whole cyst (WA), vesicular fluid and excretion/secretion Ags of *T. solium* cysts as immunogens. Using these MAbs in an enzyme-linked immunosorbent assay (ELISA) format, we developed an Ag capture assay for antigen detection in body fluids. Only one of 20 MAbs tested demonstrated weak cross-reactivity to *Echinococcus granulosus* Ags. Localization of the target Ags on the parasite was performed by immunofluorescence and immunohistochemistry on histological sections of muscle cysts from naturally infected pigs showing reactivity to different anatomical locations on the parasite: 1 MAb was reactive to the neck and fluid spaces, 4 to the neck alone, 9 to the cyst wall and neck, and 6 to the cyst wall, neck and fluid space. Pooled samples of serum and urine from patients with NCC and negative controls were evaluated by the Ag capture ELISA, using the MAbs as a capture antibody and a polyclonal rabbit anti-*T. solium* WA antibody for detection. ELISA results show that 7 MAbs could detect antigens in serum and 3 in urine samples. Reactivity of these MAbs expressed as normalized ratios of optical densities (OD positive control/OD negative control) show that 3 MAbs had ratios >20 and 4 between 5-20. These results suggest that these monoclonal antibodies have potential utility for the diagnosis and follow up of treatment in NCC patients. Use of MAb for detection of different parasite Ag in the same patients may provide insight into the kinetics of antigen release in relation to parasite damage during treatment in NCC.

456

GENOTYPING OF *TAENIA SOLIUM*

Joselyne Olga Talledo Rodríguez[1], Holger Mayta Malpartida[1], Robert H. Gilman[2]

[1]Laboratory Infectious Diseases, Lima, Peru, [2]John Hopkins University, Baltimore, MD, United States

Human taeniasis/cysticercosis due to *Taenia solium* remains a serious public health problem particularly in developing countries. Genetic variability among *T. solium* is limited because the nature of the adult tapeworm. The objective of the present study was to develop a genetic tool to genotype tapeworm isolates. The internal transcribed spacers (ITS1 and ITS2) including the 5.8S gene of the ribosomal genes were amplified by PCR. The amplified products were then subjected to restriction enzyme analysis (REA). Restriction products were further analyzed by gel electrophoresis, different genotypes were analyzed and compared using PAUP 4 and MEGA 4 softwares. A total of 78 tapeworm isolates were analyzed, two distinct genotypes were observed when Alu I and Msp I were used, while two others genotypes were observed when Hae III and Mbo I were employed. Analysis of ribosomal spacer sequences might be an important tool to differentiate *T. solium* isolates.

457

EVALUATION OF A MONOCLONAL ANTIBODY TO DETECT OF URINE ANTIGENS FOR THE DIAGNOSIS OF NEUROCYSTICERCOSIS

Holger Mayta[1], Monica Pajuelo[1], Cynthia Anticona[1], Malena Correa[1], Adriana Paredes[2], Patricia Saenz[2], Cristina Guerra-Giraldez[2], Siddhartha Mahanty[3], Theodore E. Nash[3], Silvia Rodriguez[4], Hector H. Garcia[2], Robert H. Gilman[5], Richard A. Oberhelman[1]

[1]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Universidad Peruana Cayetano Heredia, Departamento de Ciencias Celulares y Moleculares, Lima, Peru, [3]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [4]Instituto Nacional de Ciencias Neurológicas, Cysticercosis Unit, Lima, Peru, [5]Johns Hopkins School of Public Health, Department of International Health, Baltimore, MD, United States

Neurocysticercosis (NCC), a zoonotic disease caused by the larval stage of the parasite *Taenia solium*, is considered the main cause of acquired epilepsy around the world. Computed tomography (CT) and Magnetic resonance imaging (MRI) are the standard neuroimaging techniques for the diagnosis of NCC. Diagnosis of NCC is supported by immunological testing, through antibody detection by Enzyme-linked immunoelectrotransfer blot (EITB) assay, and recently by antigen detection using an enzyme-linked immunosorbent assay (ELISA). Detection of antigens either in serum, cerebrospinal fluid or urine may be an indicator of active infection. In this study an ELISA was developed using both monoclonal (for detection) and rabbit polyclonal antibodies (for capture) raised against a crude *T. solium* cyst extract, and evaluated for its capacity to detect antigens in urine samples of patients with NCC. Urine samples from 108 patients with diverse types of neurocysticercosis (n=61) and from individuals negative for NCC by CT scan and EITB (n=47) were processed by Ag-ELISA. Fifty three of 61 NCC positive samples were Ag-ELISA positive. All patients with subarachnoid NCC were Ag-ELISA positive. Among 8 individuals with NCC who tested negative by the Ag-ELISA, 3 patients presented only viable cysts with no inflammation (2-3 cysts). All NCC negative patients gave a negative result by the Ag-ELISA test. Overall the Ag-ELISA test demonstrated 87% sensitivity and 100% specificity. Additional modification of antibodies such as conjugation of MAbs to biotin might further improve sensitivity of the test.

458

NOVEL MRI STAGING SCORE AND MONOCLONAL ANTIBODY BASED ANTIGEN DETECTION ASSAY IN SUBARACHNOID NEUROCYSTICERCOSIS: MONITORING RECURRENCE AND RESOLUTION OF DISEASE

David Goodman-Meza[1], Robert S. Sacchi[1], Patricia P. Wilkins[2], Andrew Anthony[3], John Noh[2], Pierre Dorny[4], Carlos Salama[5], Siddhartha Mahanty[6], Christina M. Coyle[3]

[1]Jacobi Medical Center, Bronx, NY, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Albert Einstein College of Medicine, Bronx, NY, United States, [4]Institute of Tropical Medicine, Antwerp, Belgium, [5]Mount Sinai School of Medicine, Manhattan, NY, United States, [6]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States

Increasingly physicians are faced with patients presenting with neurocysticercosis involving the subarachnoid space (SANCC). Treatment needs to be prolonged with close follow up, but endpoints are not well defined. A neuroimaging staging system and a monoclonal antibody based antigen detection assay were evaluated. A chart review of SANCC patients since 2000 was undertaken. An MRI staging score based on number of cysts in a location, cyst size and enhancement was used. Antigen levels were recorded at end of treatment. Twenty-three patients were identified. Median age was 35 (range 18-69), 73.9% males. Median time from immigration to presentation was 7 years (0-34). Patients presented with seizure (30.4%), hydrocephalus (26.1%), vascular complications (21.7%) and headache (21.7%). Exclusive involvement in the Sylvian fissures was present in 4, basilar cisterns in 7 and in both regions in 10. Two patients had only enhancement. Concurrent spinal disease in 5. Median MRI score was 15 (2-30). Median treatment length was 56 weeks (4-2000 weeks). During treatment the following events occurred: Vascular (17.4%), hydrocephalus (17.4%), clinical deterioration and increased inflammation on MRI (both, 13.0%). Those that resolved on MRI (52.2%) were more likely to have a negative antigen level at the end of treatment. Of the 8 who had a negative antigen 87.5% resolved, whereas only 28.8% resolved of the 7 with a positive antigen (p=0.041). Also, of the 7 patients with a positive antigen at the end of treatment 5 recurred (71.4%), whereas those with a negative antigen none recurred (p=0.007). Median baseline MRI score was lower among those resolved (p=0.006). It was no different among those whose disease recurred (p=0.784) or those who suffered events during treatment (p=0.396). In this study, three-quarters of patients had clinical events during treatment, and resolution occurred in about half the cases. MRI score and antigen level correlated with disease resolution. Antigen level was associated with disease recurrence. The two scores used in combination seem promising for use in treatment decisions.

459

PRESENTATION AND MEDICAL TREATMENT OF SPINAL NEUROCYSTICERCOSIS

Ramiro Rancier[1], Robert S. Sacchi[1], Manuela Calvo[2], David Goodman-Meza[1], Christina M. Coyle[2]

[1]Jacobi Medical Center, Bronx, NY, United States, [2]Albert Einstein College of Medicine, Bronx, NY, United States

Spinal cord involvement is noted in about 1-3% of cases of neurocysticercosis (NCC) patients. Leptomeningeal spinal NCC, evident in about 80% of spinal cases, usually presents as an extension of the subarachnoid disease of the brain (SANCC). The cysts are thought to migrate downward from the basilar cisterns eventually become fixed at one level and can be accompanied by inflammation resulting in arachnoiditis and dural thickening. Surgical treatment is most commonly reported in the literature. This is a large series of medically managed spinal NCC cases. We performed a retrospective review of spinal NCC seen in Jacobi Medical Center's Tropical Medicine Center between the years 2000 and 2014. Eight cases were identified. The median age was 35.5 (range 28-65), 7 males and 7 were born in Latin America and median time from immigration was 8 years (1-18). Six patients had concurrent SANCC of the brain, 1 had a 4th ventricular cyst and 1 was a primary spinal case. Location of lesions was: cervical (62.5%), thoracic (37.5%), lumbar (75%). Four had cysts and all had arachnoiditis and hydrocephalus at presentation. Symptoms in descending order of frequency were: headache (75%), seizures (50%), radicular pain (37.5%), extremity weakness (37.5%), and back pain (25%). The median time for treatment with albendazole and steroids was 58 weeks (12-157). Aseptic meningitis was seen in 62.5% of the patients. At the end of treatment only 1 resolved completely, the other 7 had never clumping and of these 5 had enhancement. One patient continued to have cysts and he was actively being treated. Only half improved their clinically. Spinal NCC is commonly associated with SANCC and requires prolonged medical therapy. In this series the clinical presentation was due to both brain and spinal involvement. Patients presenting with aseptic meningitis with hydrocephalus from an endemic region should be evaluated for both spinal and SANCC. Radiographic improvement was seen, but residual nerve clumping may account for the persistent clinical symptoms.

460

BIOMARKER-BASED ASSOCIATION MODELS FOR PREDICTING COGNITION DEFICITS IN CASSAVA CYANOGENIC POISONING

Guy M. Bumoko[1], Marie-Thérèse A. Sombo[1], Daniel L. Okitundu[1], Dieu-donné N. Mumba[2], Théodore K. Kazadi[1], Jean-Jacques T. Muyembe[2], Micheal J. Boivin[3], Desiré Tshala-Katumbay[4], Desiré Tshala-Katumbay[5]

[1]University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [2]University of Kinshasa, Institut National de Recherches Biomédicales, Congo-Kinshasa, Kinshasa, Democratic Republic of the Congo, [3]Department of Psychiatry and Neurology/Ophthalmology, Michigan State University, East Lansing, MI, United States, [4]University of Kinshasa, Department of Neurology and Center for Research on Occupational and Environmental Toxicology, Oregon Health & Science University, Portland, OR, United States, [5]University of Kinshasa, Department of Neurology and Center for Research on Occupational and Environmental Toxicology, Oregon Health & Science University, Portland, OR, United States

While risk factors for konzo are known, determinants of cognitive impairment in konzo-affected children remain unknown. We anchored cognitive performance (KABC-II scores) to serum levels of free-thyroxine (free-T4), thyroid-stimulating hormone (TSH), albumin, and motor proficiency (BOT-2 scores) in 40 children including 21 with konzo (median age: 9 years) and 19 without konzo (median age: 8 years). A multiple regression model was used to determine variables associated with changes in KABC-II scores. The results were: Age (: - 0.818, 95%CI: - 1.48, - 0.152) (p=0.018), gender (: - 5.72; 95% CI: - 9.87, -1.57 for females) (p=0.009), BOT-2 score (: 0.390; 95% CI: 0.113, 0.667) (p=0.008), and free-T4 (: 1.88; 95% CI: 0.009, 3.74) (p=0.049) explained 61.1% of variation in KABC-II scores. Subclinical hypothyroidism was not associated with poor cognition. A crude association was found between serum albumin and KABC-II scores (: 1.26; 95% CI: 0.136, 2.39) (p=0.029). On spot urinary thiocyanate reached 688 µmol/l in children without konzo and 1032 µmol/L in those with konzo**.** In conclusion, **f**emale gender and low serum albumin are risk factors common to cognitive and proportionally associated motor deficits in children exposed to cassava cyanogens. The two types of deficits may share common mechanisms.

461

EASE OF USE AND SAFETY OF A CONICAL CUP BLOOD TRANSFER DEVICE FOR USE WITH RAPID DIAGNOSTIC TESTS FOR HUMAN AFRICAN TRYPANOSOMIASIS (HAT)

Magoma Mwancha-Kwasa[1], Daniel Kyabayinze[2], Iveth J. González[3], Bbale Ndawula[2], Christine Kanyago[2], Sylvain Bieler[3], Joseph Ngung'u[3], Charles Wamboga[4], Enock Matovu[5], Sandra Incardona[3], David Bell[3]

[1]Ministry of Health, Nairobi, Kenya, [2]Foundation for Innovative New Diagnostics, Kampala, Uganda, [3]Foundation for Innovative New Diagnostics, Geneva, Switzerland, [4]MInistry of Health, Kampala, Uganda, [5]Makerere University, Kampala, Uganda

Rapid diagnostic tests (RDTs) are increasingly being used for screening and diagnosis of infectious diseases in remote settings. Appropriate devices to safely transfer fixed amounts of blood from finger pricks to RDTs pose a significant challenge. Recently, a 5-µL inverted cup was evaluated and found to be the most acceptable to health workers (HWs) in terms of safety, ease of use and accuracy of volume transferred when used with malaria RDTs. The success of this device and its subsequent uptake by various manufacturers led to the development of a 23-µL conical cup for RDTs requiring higher volumes of blood. This study compares this conical cup with a plastic pipette when used for screening for Human African Trypanosomiasis (HAT) infection with the SD BIOLINE HAT RDT in Yumbe and Arua districts in Northwestern Uganda. After a half-day of training, conical cups and plastic pipettes were given to 49 HWs who used them for testing of suspect patients who voluntarily accepted to participate in the study. Each HW used the blood transfer devices with at least 10 patients in the field. Questionnaires and focused group discussions were used to gather information on ease of use, blood safety and acceptability. Preliminary results show that HWs generally preferred the conical cup over the plastic pipette. A higher rate of successful blood transfers and fewer occurrences of blood safety issues were observed when using the conical cup. Results of this study not only demonstrate the ease of use of a new blood transfer device for RDTs for HAT and other diseases such as HIV, but also raise awareness about the need for further improvements in BTDs for the use of RDTs on hands of health workers in remote areas. Additional and more detailed results will be presented during the meeting. Final results will be available by the end of April.

462

THE EFFECT OF MAGNESIUM SULPHATE ON AUTONOMIC DYSREGULATION IN ENTEROVIRUS 71 RELATED HAND FOOT AND MOUTH DISEASE IN VIETNAMESE CHILDREN

Qui T. Phan[1], Bridget Wills[2], Trieu T. Huynh[1], Giang T. Pham[1], Bich T. Nguyen[1], Hung B. Nguyen[1], Chau V. Nguyen[1]

[1]Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, [2]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam

Enterovirus 71 (EV71) associated hand foot and mouth disease (HFMD) has emerged across much of Asia as a serious infection of young children during the last decade. HFMD predominantly affects children under 5 years of age, with a clinical spectrum varying from a relatively mild self-limited febrile illness with a rash, to fatal cardiopulmonary collapse. An outbreak of HFMD in 2011 in southern Vietnam resulted in over 110,000 reported cases and 116 deaths, and epidemiological studies from neighboring China report over 7 million HFMD cases between 2008 to 2012, with 2,457 deaths. Autonomic nervous system (ANS) dysregulation is considered as the main indicator of progression to severe disease, initially manifesting with hypertension, and later progressing to cardiopulmonary failure and death in a small proportion of cases. Although lacking a formal evidence base, current guidelines in many Asian countries suggest milrinone as the first choice for management of ANS related hypertension in patients with severe HFMD. Based on clinical experience and evidence from a randomized controlled trial magnesium sulphate (MgSO4) has become the mainstay of treatment for ANS dysregulation in patients with tetanus in many centres. We describe a case series involving 10 EV71 confirmed HFMD cases with ANS dysregulation managed at HTD over a 4 month period in early 2012. Magnesium sulphate was added when hypertension remained poorly controlled despite high dose milrinone (up to 0.75 ug/kg/minute), and in all cases the blood pressure reduced within 30-60 minutes and remained stable subsequently on a continuous magnesium infusion for 48-72 hours. No patient required hemofiltration, although 2 of 10 cases were ventilated because of respiratory distress. Brain MRI was performed later in 4 of these cases and in the 2 children with neurological sequelae abnormalities were found, involving the medulla in both cases, and with extensive atrophic changes in one child. These promising initial results are currently being investigated in a formal randomized controlled trial of magnesium sulphate versus placebo in Vietnamese children with severe HFMD and signs of ANS dysregulation.

463

ATTRITION AND ITS ASSOCIATED FACTORS AMONG PRE-ART CLIENTS REGISTERED IN CARE AND TREATMENT CENTERS IN MOROGORO, TANZANIA 2010 -2011

Herilinda J. Temba

Morogoro Regional Hospital, Dar es salaam, United Republic of Tanzania

Despite significant success in scaling up care and treatment programmes in Tanzania, a majority of people living with HIV (PLHIV) do not access them. Successfully enrolled PLHIV in care and treatment clinics (CTC) are lost at every step along the continuum of care. This study aims at determining factors associated with attrition among Pre-ART adults in CTC.We

conducted a clinic based retrospective cohort study that involves review of data from Pre-ART adult clients (≥15years) register and client treatment card number 2 (CTC2 Card) at three CTCs in Morogoro from July, 2010 to July 2011. Pre ART clients who were not in care at their original sites at 1 year of follow up were traced through home based care volunteers and phone calls. Relative risk was then calculated using Epi info statistical soft ware.A total of 351 CTC clients were enrolled between July 2010 and July 2011. Most enrolled clients were ART eligible 161(45.9%) of whom 92(57.1%) were initiated on ART. Despite being ART eligible 69 (42.9%) of enrolled clients were not Initiated on ART. Mortality among those not initiated ART was 47 (17.6%) of whom 30 (63.8%) were ART eligible. Majority 259 (66.7%) of enrolled clients had no ART start Status, of the 259 clients status of 25(9.6%) could not be determined until the end of the study. 67(25.9%) were confirmed not to be in care at one year of follow up due to different reasons such as death, opt out and some were reached and promised to return to care. 67(25.95) were pre ART and still attending at their original clinics whereby 80 (30.9) were in care with other providers. Being enrolled in care at an advanced disease stage, paying for transport to go to the clinic and stigma were risk factors for Pre ART attrition.Attrition due to mortality is high among Pre ART clients who are enrolled in CTC while already in advanced disease stage. Attrition from clinic is higher than attrition from care. Self stigma and advanced disease stage were pre ART attrition risk factors. Status disclosure and sensitization to reduce stigma as well as Strengthening of CTC to improve linkage and referrals between CTC and other clinics as well as prioritizing ART initiation among clients who are ART eligible is recommended.

464

COMMON CLINICAL COMPLICATIONS OF HUMAN MONKEYPOX INFECTION AT THE GENERAL HOSPITAL OF KOLE, IN DEMOCRATIC REPUBLIC OF CONGO

Placide K. Mbala[1], James W. Martin[2], John W. Huggins[2], Jean Jacques Muyembe[1], Cesar K. Mutambay[1], Bryony Soltis[2], Anne W. Rimoin[3], Fernando B. Guerena[2], Lawrence Korman[2], Phillip Pitman[2]

[1]Institut National de Recherche Biomedicale, Kinshasa, Democratic Republic of the Congo, [2]U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD, United States, [3]University of California Los Angeles Fielding School of Public Health, Los Angeles, CA, United States

Monkeypox (MPX) virus is an orthopoxvirus that is endemic in central and West Africa, causing a smallpox-like disease ranging from subclinical to severe cases. With the cessation of smallpox vaccination in 1982, rural populations in central Africa were increasingly susceptible to MPX infection due to waning herd immunity to poxviruses. We have observed an increase in severe clinical manifestations of MPX in both children and adults, leading to more frequent clinical complications. Most of these complications are moderate requiring nurse care and prolongation of the hospitalization, however, severe complications can be fatal (death, miscarriage) or lead to grave sequels (blindness), albeit rarely. To describe frequent and severe clinical complications observed in human subjects with MPX infection at the Kole General Hospital, one of the most remote Ministry of Health Hospital in the rain forest of the Sankuru District, in Democratic Republic of Congo (DRC) where the majority of the population relies on bush meat as a principal source of protein. From 2007 to 2011, we conducted an observational study of 229 cases of human MPX infection in order to describe its clinical and biological characteristic as it occurs in the DRC. Clinical signs, symptoms, and laboratory results were recorded during their hospitalization. Clinical complications observed during this study were identified and recorded. Miscarriage was the most common complication in infected pregnant women (3/4) with evidence of fetal contamination, followed by secondary dermatitis on infected skin lesions (30/229), death (4/229), abscess of cervical lymph node (6/229), keratitis (3/229), staphyloma occurring approximately 20 months later after the onset of keratitis, and caseation of eye lesions (1/229) in confluent lesions spreading in the sclera. Clinical complications in human MPX infection are common in DRC, which is likely attributable to the decline of cross-protective immunity after cessation of smallpox vaccination and the subsequent increase in the susceptible population in endemic regions. Infected pregnant women were the most likely to have complications resulting in death.. Certain complications can be treated with nursing care or minor surgery, whereas others require intensive care or plastic surgery which are not often available in remote areas where MPX most often occurs, thus leading to grave sequelae.

465

INTRAVENOUS ARTUSENATE VS. QUININE IN THE TREATMENT OF SEVERE MALARIA IN LIBERIAN CHILDREN

Benetta Andrews[1], Kanagasabai Udhayashankar[1], Eric Adu[1], Patricia McQuilkin[2]

[1]JFK Memorial Hospital, Monrovia, Liberia, [2]University of Massachusetts Medical School, Worcester, MA, United States

Liberia is a West African Country whose health care system is recovering from years of civil war. Liberia is endemic for malaria and recent reports estimate the malaria prevalence to be as high as 32% in children under 5 years of age. Recent data collected at JFK Medical Center, the national referral hospital, shows that 42% of pediatric admissions are for the treatment of severe malaria. The goal of this study was to compare the outcomes of children admitted with severe malaria and treated with either quinine or artusenate, to establish the optimal treatment regimen in this setting. We studied children age 5 and under who were admitted to JFKMC during the time period from May 2013 through October 2013 with a diagnosis of severe malaria. 92 children were treated with artusenate (2.4 mg/kg/dose IV q 12 x 4 doses) and 73 children were treated with quinine (15mg/kg/dose IV q12 x 3 doses). We measured time to symptom resolution, length of hospital stay and clearance of parasitemia for each treatment arm. Of the 165 study participants, the average age was 27 months; 43% were female, 57% were male, and 48% used bednets regularly. 39% had at least one prior episode of malaria. Children treated with artusenate cleared their symptoms sooner (2.08 days) and had shorter hospitalizations (3.56 days) than their counterparts treated with quinine (cleared symptoms in 2.32 days and hospitalized an average of 3.67 days). A larger number of children treated with artusenate had persistent parasitemia on hospital day 3 (6.5%) versus those children treated with quinine (1.3%). These results align well with prior reports of the superiority of parenteral artusenate over quinine for the treatment of severe malaria in children in Sub Saharan Africa. Future studies include following children post hospital discharge to examine clearance of parasites after completion of outpatient regimens, and screening for drug resistance mutations in this population.

466

IMPLEMENTATION AND EVALUATION OF A RAPID ASSESSMENT CLINIC FOR FEBRILE RETURNED TRAVELERS IN AMBULATORY TROPICAL MEDICINE

Farah Jazuli, Andrea V. Page, Andrea K. Boggild

University of Toronto, Toronto, ON, Canada

Fever in the returned traveler is a medical emergency warranting prompt medical attention to exclude potentially life-threatening illnesses such as malaria. However, prolonged evaluation in the Emergency Room (ER) may not be required for all patients. As a quality improvement initiative, we designed and implemented an algorithm for rapid assessment of febrile travelers (RAFT) in an ambulatory tropical medicine clinic. Criteria for referral to the RAFT clinic include: presentation to the ER, history of and/or documented fever, and travel to the tropics or sub-tropics within the past year. Exclusion criteria include a diagnosis of *Plasmodium falciparum* malaria and fulfillment of other admission criteria such as unstable vital signs or significant laboratory derangements. We performed a time series analysis pre- and post-implementation of the clinic, with primary outcome of time to definitive tropical medicine consultation. Secondary outcome measures include number of ER visits averted for repeat malaria testing

and number of ER visits averted for definitive management of an infectious illness. Interim analysis 2-weeks post-implementation indicate a mean time to RAFT clinic assessment of 1.29 days (range 0_2 days) compared to 5.44 days (range 0_26 days) prior to implementation (p=0.15). A total of 7 patients were referred to the RAFT clinic over 2 weeks, thus averting 7 repeat ER visits for follow-up malaria screening. Of 7 RAFT patients, 4 (57%) had an infectious illness that required specific therapy, thus, 4 "call-backs" to the ER over 2 weeks were averted. Extrapolated over 1 year, our interim results suggest that implementation of a RAFT clinic can avert 182 repeat ER visits for follow-up malaria screening, and 104 ER "call-backs" for management of a positive infectious work-up. In addition to provision of more timely care to ambulatory febrile returned travelers, we have demonstrated at our interim analysis that implementation of a RAFT clinic in an urban tertiary care setting can reduce ER visits by 5.5 per week.

467

COST EFFECTIVENESS OF A POINT OF CARE TEST FOR SEPSIS AMONG PATIENTS WITH FEBRILE ILLNESS IN LOW RESOURCE SETTINGS

Erin Penno, Sarah Baird, John Crump

University of Otago, Dunedin, New Zealand

Bacterial sepsis is an important cause of mortality in low- and middle-income countries, yet distinguishing patients with sepsis from others remains a challenge. Currently management decisions are based on clinical assessment using algorithms such as Integrated Management of Adolescent and Adult Illness (IMAI). Efforts to develop and evaluate point-of-care (POC) diagnostic tests for sepsis to guide decisions on the use of antimicrobials are underway. To establish the minimum performance characteristics of such a test, we varied the characteristics of a hypothetical POC test for sepsis required for it to be cost effective. We applied a decision tree model to a population of febrile patients presenting at the district hospital level in a low-resource setting. We compared existing clinical assessment algorithms against the POC test. Patient survival was the outcome of interest. Costs and performance characteristics for the POC test were benchmarked against existing malaria rapid diagnostic tests. Prevalence of bacterial sepsis among febrile patients and outcome data were informed by the literature. We used a case fatality probability of 20% for appropriately treated sepsis and of 50% for inappropriately treated sepsis. A 13.4% prevalence of sepsis among febrile patients seen at the district hospital was assumed. Based on a clinical assessment for sepsis with the established sensitivity of 83% and specificity of 62%, we found a POC test for sepsis with a specificity of 94% and a sensitivity of 83% was cost effective, resulting in parity or equivalence in survival but costing US$2.05 less per live saved than clinical assessment. A POC test with sensitivity and specificity of 100%, equivalent to the best malaria rapid diagnostic tests, yielded an incremental cost effectiveness ratio that showed strong dominance, that is being both cheaper and more effective. Our results establish performance targets for POC tests for sepsis in low-resource areas.

468

HIGH INCIDENCE OF SNAKE BITES IN THE UPPER EAST REGION OF GHANA: A CLARION CALL FOR IMPROVEMENT IN SNAKE ENVENOMING OUTCOMES

Emmanuel A. Blay[1], Emmanuel Adongo[2], Daniel A. Boakye[1], Kwadwo A. Koram[1], Irene Ayi[1]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Ghana Health Service, Bongo District Hospital, Bongo, Ghana

Snake bites and snake envenoming are a common emergency problem in many rural health centres in sub-Saharan Africa. Although this phenomenon is not peculiar to sub-Saharan countries due to the presence of poisonous snakes on every continent and in almost every country, the incidence of snake bites is higher in the inter-tropical world than the temperate regions. Records on snake bites are minimal and scanty and the statistics may be misleading. This study sought to estimate the incidence of snake bites and mortality rates in a savannah farming area in Ghana. A review of records on snake bites in the Regional Hospital, a referral facility of the Upper East Region of Ghana for the year 2013. The Region has a population of approximately, 1,084,621. A total of 146 persons (male: 68/146 (46.6%); female: 78/146 (53.4%)) with snake bites were referred to the hospital out of a total of 94,707 OPD cases for year 2013. The overall snake bites incidence in the region for the year was 13.4 per 100,000 persons. A mortality rate of 7.5% (11/146) was reported (54.5% (6/11) females; 45.5% (5/11) males). 83.2% (129/146) of the snake bites were recorded in the peak farming season of March-August, which is also the major raining season. This study shows a high incidence of snake bites and relatively high mortality. This calls for efficient health care delivery for snake bite victims to improve snake envenoming outcomes.

469

TRAVEL MEDICINE SPECIALIZATION REDUCES MEDICAL ERRORS: CHLOROQUINE PRESCRIPTIONS SIGNAL A BROADER PROBLEM

Edward J. Park, Patrick W. Hickey

Uniformed Services University of Health Sciences, Bethesda, MD, United States

There has yet to be a systematic analysis of antimalarial prescription errors in the United States. Utilizing the U.S. Military Health System's (MHS) outpatient medical records, all chloroquine (CQ) prescriptions from military facilities to the pediatric patients during the period 2006 to 2010 were reviewed. Adherence to destination specific prescribing guidelines from the CDC and MHS was assessed. Records from 547 encounters were reviewed, yielding 440 with destination information. Results were stratified to compare travel medicine clinics vs. primary care clinics. Travel medicine clinics in the MHS are operated by pediatric/adult infectious disease, preventive medicine and allergy & immunology specialties, all others were considered non-travel medicine clinics. Errors were categorized as either contraindicated (CI) for areas with CQ resistance or not indicated (NI) for areas without a recommendation for chemoprophylaxis. Travel Clinics provided 42% (n=185) of patient care. No CI errors were made at travel clinics. Non-travel clinics made CI errors in 6.3% (n=16) (p=0.001) of prescriptions. The overall rate of CI errors was 3.6%. Overall error rates (CI+ NI) for travel clinics was 6.5% (n=12) and non-travel clinics was 11.4% (n=29) (p=0.08) for a weighted average of 9.3% based on proportions of total chloroquine prescriptions. Travel Medicine specialty clinics have significantly lower rates of prescribing errors that place patients at risk for malaria. CQ-NI risks are common across clinic types and offer little to no benefit to the patient and exposes them to increased risk for adverse drug effects. These results suggest other aspects of care may also show disparities in quality of care. Strategies to improve travel medicine care, particularly among non-specialists are needed.

470

HIGH PREVALENCE OF HYPOGLYCEMIA AMONG PEDIATRIC PATIENTS ADMITTED TO A RURAL MOZAMBICAN HOSPITAL AND RISK FACTORS FOR DEATH

Lola Madrid, Sónia Amós Macuvule, Miguel Lanaspa, Elisa López-Varela, Tacilta Nhampossa, **Quique Bassat**

Centro de Investigação em Saúde da Manhiça (CISM), Manhiça, Mozambique

Hypoglycaemia is a common and life-threatening problem in African children, and is associated with a wide variety of diseases. We describe the prevalence and incidence of hypoglycaemia among admitted African children, establishing risk factors for a poor outcome. We retrospectively reviewed 13 years of systematic clinical data collected through a morbidity surveillance system ongoing in a rural Mozambican hospital. Data from all paediatric admissions were analysed, and independent risk factors assessed for 1) Hypoglycaemia and 2) death in children with

hypoglycaemia. Additionally, we used a glucose sensor to continuously monitor interstitial glucose levels (every 5 minutes) during the first 72 hours of admission for a subset of malaria patients. From January 2001 to December 2013, 45,593 children <15 years had a glycaemia determination performed on admission. 1477 children (3.2%) presented hypoglycaemia (glycaemia<3mmol/L), of which about 2/3 (971) with levels < 2.5mmol/L. Independent risk factors for hypoglycaemia on admission included being male (OR 1.17; p=0.033); having respiratory distress (OR 1.30; p=0.001), anorexia (OR 2.60; p<0.001), a positive blood culture (OR 1.69; p<0.001), malaria (OR 1.41; p<0.001), being a newborn (OR 2.50; p<0.001) or being severely malnourished (WAZ<3SD; OR 1.28; p=0.006). Hypoglycaemic children were significantly more likely to die than normoglycaemic ones (OR 6.8; p<0.001), with an associated CFR of 19.4% (245/1266). Independent risk factors for death among hypoglycaemic children included having respiratory distress (OR 2.75; p=0.031), anorexia (OR 3.55; p=0.015) and being severely malnourished (OR 5.07; p=0.002). We also aim to present data on continuous monitoring of hypoglycaemia (first 72 hours) for 80 malaria patients, which suggests that up to a third of admitted children have at least one hypoglycaemia episode detected through continuous monitoring. Hypoglycaemia remains a frequent and hazardous condition for African children, not only on admission but also throughout hospitalization, with many episodes remaining undetected. Symptoms, signs or conditions found to be associated with an increased risk of hypoglycaemia should trigger the verification of glycaemia, and the implementation of life-saving corrective measures.

471

TRAVEL RELATED VACCINATION COVERAGE RATES IN A COHORT OF DEPARTMENT OF DEFENSE BENEFICIARIES SEEKING PRE-TRAVEL MEDICAL CONSULTATION

Tahaniyat Lalani[1], Mark Johnson[2], Edward Grant[3], David Tribble[3], Jason Maguire[1], Jamie Fraser[3], Anuradha Ganesan[3], Timothy Burgess[4], Ryan Maves[2], Heather C. Yun[5], Philip Coyne[4], Karl Kronmann[6]

[1]IDCRP, Portsmouth, VA, United States, [2]Naval Medical Center San Diego, San Diego, CA, United States, [3]IDCRP, Bethesda, MD, United States, [4]Walter Reed National Military Medical Center, Bethesda, MD, United States, [5]United States Air Force - SAUSHEC Infectious Disease, San Antonio, TX, United States, [6]Naval Medical Center Portsmouth, Portsmouth, VA, United States

Vaccine-preventable diseases are significant threats to international travelers. We evaluated the rates of travel-related vaccinations in Department of Defense beneficiaries seeking care at military travel clinics who enrolled in the TravMil cohort. 1323 patients were enrolled at 3 military travel clinics; 321 (24%) were active duty personnel, and 1002 (76%) were retirees or dependents. The most common destinations for travel were South/Central America and the Caribbean (31%) followed by Southeast/North Asia (28%) and Africa (28%). The median time between pre-travel consultation and departure was 26 days. Active duty personnel had higher immunization rates prior to travel when compared to retired military and dependents (hepatitis A: 97% vs 66%; hepatitis B: 80% vs 45%; Td/Tdap: 97% vs 74%; influenza: 90% vs 67% (p<0.05 for all)). The typhoid vaccine was the most common vaccine prescribed; 462 of 478 travelers to high-risk regions in Asia (97%) had current vaccines or were newly vaccinated. 26% (89/343) of at-risk travelers to destinations with endemic Japanese encephalitis (JE) did not receive vaccination, most often due to insufficient time to complete the series in 53% (47/89) of cases. Yellow fever vaccination was not prescribed in 14% (68/482) of patients traveling to endemic areas either due to low risk of exposure or presence of relative contraindications. 97% (156/161) of travelers to meningococcus-endemic regions of Africa received appropriate meningococcal vaccinations. The rabies vaccine was prescribed for 72 (5%) participants, 20 of whom were traveling for > 28 days. No vaccine-preventable diseases were identified in participants during post-travel follow-up visits. Travelers should be encouraged to schedule pre-travel visits at least 6 weeks prior to departure to allow for sufficient time to complete vaccination series such as JE or rabies. The pre-travel visit is a good opportunity for providing routine vaccines to retirees and dependents.

472

POTENCY OF POLYVALENT ANTI-SNAKE VENOM SERUM AGAINST VIPERS AND ELAPIDS VENOMS IN GHANA

Emmanuel A. Blay, Irene Ayi, Mark Ofosuhene, Alexander K. Nyarko, Kwadwo A. Koram, Kwabena M. Bosompem

Noguchi Memorial Institute for Medical Research, Accra, Ghana

Parenteral preparations "Inoserp® Pan-Africa" (from Veteria Labs, Mexico for Inosan Biopharma, Spain) for activity against venoms of some snakes common in Ghana was evaluated for safety, potency and sterility. *In vitro* demonstration of antibody/toxin reactivity using immunological methods and determination of effective neutralization doses, as well as, *in vivo* demonstration of biological activity by neutralization of snake venom toxins in laboratory mice (ICR strain) were carried out. Results of the experiments revealed that samples of the product submitted for testing are sterile and contains no contaminating microbes. The product contains antibodies that are capable of binding toxins in the venom of the representative Elapidae and Viperidae snake species tested namely *Naja nigricollis* (Spitting cobra), *Bitis arietans* (Puff adder) and *Echis ocellatus* (Saw-scaled viper). The product was thus found to contain antibodies that could neutralize both Elapidae and Viperidae venoms *in vivo* and thus may therefore be used clinically as post-exposure prophylaxis in snake bite victims.

473

COST EFFECTIVENESS OF SURVEILLANCE FOR BLOODSTREAM INFECTIONS FOR SEPSIS MANAGEMENT IN LOW RESOURCE SETTINGS

Sarah Baird, Erin Penno, John Crump

University of Otago, Dunedin, New Zealand

Bacterial sepsis is a leading cause of mortality among febrile patients in low- and middle-income countries, but blood culture services are not widely available. Consequently, empiric antimicrobial management of suspected bloodstream infection is based on generic guidelines that are rarely informed by local data on etiology and patterns of antimicrobial resistance. To evaluate the cost-effectiveness of surveillance for bloodstream infections to inform empiric management of suspected sepsis in low-resource areas, we compared costs and outcomes of generic antimicrobial management with management informed by local data on etiology and patterns of antimicrobial resistance. We applied a decision tree model to a hypothetical population of febrile patients presenting at the district hospital level in Africa. Focusing on patient survival as the outcome of interest, we used a probability of death of 20.0% for appropriately treated sepsis and 50.0% for inappropriately treated sepsis. Laboratory and treatment cost data were obtained from Tanzania and costs of antimicrobials were derived from World Health Organization data. Costs were inflated to 2011 U.S dollars. Based on the literature, a 13.4% prevalence of sepsis among febrile patients was assumed. Using susceptibility patterns from bloodstream infection studies in Africa obtained by systematic review, we estimated that 44.0% of organisms were covered by a regimen tailored to local etiology and patterns of antimicrobial resistance of bloodstream infections whereas 12.0% of organisms were covered by a generic regimen. We found that the evidence-based regimen saved an additional 1,067 lives per 100,000 persons with fever at a cost of US$43.14 per life saved. Based on World Health Organization recommendations that interventions below a threshold of US$5,705 per Disability-Adjusted Life Year avoided should be pursued, our findings indicate that routine surveillance for bloodstream infections is likely a cost-effective service in the African context.

474

EVALUATION OF CHILDREN ADMITTED IN KOROGWE DISTRICT HOSPITAL, TANZANIA FROM 2005 TO 2012 WITH INTENTION TO TREAT FOR MALARIA

John P. Lusingu[1], Martha M. Lemnge[1], Thor G. Theander[2]

[1]National Institute for Medical Research, Tanga, United Republic of Tanzania, [2]Centre for Medical Parasitology, Copenhagen, Denmark

Malaria has been documented over years to be the leading cause of hospital admissions in Tanzania. It has recently been reported that malaria is declining but fever episodes are still the common feature of admission. The study aimed at monitoring children admitted in Korogwe District Hospital (KDH) with intention to treat for malaria. Children below five years admitted with a primary diagnosis of malaria from outpatient department from January 2005 to December 2012 were enrolled into this study after obtaining informed consent and collecting clinical features and performing laboratory investigations which included blood smear for malaria parasites, levels of haemoglobin, lactate and glucose. Treatment was based on the initial assessment at the admission desk and changed accordingly after obtaining laboratory results. A total of 3,260 children were admitted into KDH with an intention to treat for malaria between 2005 and 2012. Out of these, 1,243 (38%) had a confirmed diagnosis of malaria; and 395 (12.12%) had severe malaria. There was a trend of declining malaria admission across years from 2005 to 2012. Febrile episodes remained high across years providing evidence that there are multiple causes of febrile episodes that are non-malaria. A total of 2623 (80.46%) admitted children had fever and 189 (5.8%) had respiratory distress syndrome, 79 (2.5%) were hypoglycaemic and 524 (45.9%) had high lactic acidosis. Moderate and severe anaemia were 42% (95% CI: 40.9% - 44.4%) and 13.9% (95% CI: 12.7% - 15.2%), respectively. The risk of death was associated with day of admission, odds ratio on day of admission being 275 (9% CI: 601 - 1243, p <0.001), glycaemic levels, 18 (95% CI: 6 - 54, p <0.001) for hypoglycaemia, 9 (95% CI: 2 - 31, p<0.001) for hyperglycaemia, 2.4 (95% CI: 1.2-5, p=0.014) for lactic acidosis and respiratory distress, 4.7 (95% CI: 1.8 - 12.2, p=0.001). However, anaemia and malaria parasitaemia were not associated with the risk of death, odds ratio being 2.4 (95% CI: 0.28 - 19.66, p = 0.43) for anaemia and 3.2 (95% CI: 0.5 - 20.0, p = 0.213), respectively. In conclusion, this study has provided evidence that there has been a progressive decline in malaria morbidity for children who are admitted in the paediatric ward. The management of malaria and severe anaemia in this study conformed to the highest standard of care that can be found in a District Hospital in a resource constrained society.

475

EFFECTS OF DOXYCYCLINE ADJUVANT THERAPY ON PRIMARY KNEE OSTEOARTHRITIC PATIENTS IN IRAQ EVALUATED USING THE WOMAC INDEX

Sami Salman[1], Arwa M. Ahmed[2]

[1]Rheumatology Unit, College of Medicine, Baghdad University, Baghdad, Iraq, [2]Baghdad Teaching Hospital, Baghdad, Iraq

Osteoarthritis (OA) is a common chronic joint disease that involves degeneration of articular cartilage and its risk factors are many, including gender, obesity, low vitamin D and increased age. There are limited options to treat or prevent OA in Iraqi subjects, especially those in lower socioeconomic categories. Pre-clinical data suggested that doxycycline might act as disease-modifying agent for the treatment of osteoarthritis with the potential of slowing cartilage degeneration. To examine the short-term effects of doxycycline on knee OA we designed a double blind, placebo controlled study to determine the effects of daily doxycycline 100 mg BID on OA. One hundred forty patients were enrolled and equal numbers were randomly assigned to either treatment or placebo groups. Seventy patients received an oral doxycycline capsule 100 mg twice daily and controls received a starch containing capsule twice daily. The efficacy outcome measured was the change in the WOMAC (Western Ontario and McMaster Universities) index of knee OA over the course of three monthly visits. Our results demonstrated that there were no significant differences between the drug and placebo groups in pain score at the baseline visit (p=0.63) or at the first visit one month later (p=0.29). However, at the second visit, there was a significant reduction in pain score in the drug group compared to placebo group (p=0.028). At three months, the difference between treatment and placebo groups became more significant (p=0.013). Joint stiffness and physical function scores measured at each visit showed no significant differences between study groups. Mean WOMAC scores were reduced at each visit compared to baseline values in doxycycline group compared to the placebo group, and the reduction in mean WOMAC score by the third monthly visit was significantly much greater in the treatment group (8.37±2.1) than in controls (3.83±3.1, p<0.001). In conclusion, oral twice-daily dosage of 100 mg doxycycline demonstrated significant symptomatic benefit in patients with primary knee OA in terms of pain reduction and total WOMAC score.

476

RESULTS OF BASELINE OPHTHALMOLOGICAL EXAMINATIONS CONDUCTED IN *ONCHOCERCA VOLVULUS* INFECTED PARTICIPANTS FROM ONCHOCERCIASIS MESO- AND HYPERENDEMIC AREAS IN NORTH-EAST DRC AND GHANA IN A STUDY OF THE EFFECTS OF A SINGLE DOSE OF 8 MG MOXIDECTIN VS. IVERMECTIN

Amos Nyathirombo[1], Eric Kanza[2], Ebenezer Addy[3], Didier Bakajika[4], Nicholas O. Opoku[5], Kambale Kasonia[6], Germain Mambandu[7], Michel Vaillant[8], Christine Halleux[9], Piero L. Olliaro[9], **Annette C. Kuesel**[9]

[1]Centre de Recherche en Maladies Tropicales de l'Ituri, C.R.M.T-ITURI, Hôpital Général de Référence de Rethy, Rethy, Democratic Republic of the Congo, [2]Centre de Recherche Clinique de Butembo, Université Catholique du Graben, World Health Organization, Butembo, Democratic Republic of the Congo, [3]Onchocerciasis Chemotherapy Research Centre, Hohoe, World Health Organization, Hohoe, Ghana, [4]Centre de Recherche en Maladies Tropicales de l'Ituri, C.R.M.T-ITURI, Hôpital Général de Référence de Rethy, World Health Organization, Rethy, Democratic Republic of the Congo, [5]Onchocerciasis Chemotherapy Reserch Centre, Hohoe, Ghana, [6]Centre de Recherche Clinique de Butembo, Université Catholique du Graben, Butembo, Democratic Republic of the Congo, [7]Centre de Recherche en Maladies Tropicales de l'Ituri, C.R.M.T-ITURI, Hôpital Général de Référence de Rethy, Rethy, Province Orientale, Democratic Republic of the Congo, [8]CCMS CRP-SANTE, Luxembourg, Luxembourg, [9]World Health Organization, Geneva, Switzerland

A study comparing the safety and efficacy of a single dose of 8 mg moxidectin vs. a single standard dose of ivermectin (150 µg/kg) treated 762 males and 411 females, including 79 children of 12-17 years, with ≥10 microfilaria/mg skin in Nord-Ituri (DRC, N=460), Nord-Kivu (DRC, N=472) and Nkwanta North district (Ghana, N=241). Ophthalmological pre-treatment examinations included: detailed history, visual acuity, anterior segment examination with up to x25 slit lamp, dilated fundus examination by direct and indirect ophthalmoscopy, color vision, intraocular pressure, visual field with FDT perimetry, ocular mobility, pupillary reflex, external ocular structures, and number of dead and life microfilaria in the anterior chamber and the cornea. Medical histories were to be reported into the data base if, in the investigator's judgement, they were relevant for assessing post-treatment effects. They were coded according to the Medical Dictionary for Regulatory Activities. All ocular examination results were entered into the data base and their severity graded according to the Onchocerciasis Chemotherapy Research Center Common Toxicity Criteria [grade 0 (normal) - grade 4] or for events not included in these criteria, by method specific grading systems, NCI CTC version 2 or a generic grading system. Substantial variations between the three sites were observed in the percentages of participants with grade 3 or 4 abnormalities in visual acuity and visual field (range 6-16% and 21-40%, respectively). Different types of abnormalities of the anterior and posterior segments were diagnosed in 0-11% and 0-15% of participants,

respectively. Ocular symptoms were reported for 37-75% of participants. The most frequent symptoms (beyond those related to vision itself) reported by the participants or detected at the baseline examination were eye pruritus (6.6-40.2%), blurred vision (6.1-30.3%), corneal disorders (0-8.0%), eye pain (2.5-6.6%), conjunctivitis (0-1.9%).

477

DELAY IN HANSEN'S DISEASE DIAGNOSIS IN THE UNITED STATES

Stephen Waterman[1], Laura Blum[2], Alfonso Rodriguez-Lainz[1], Richard Truman[3], Leisha Nolen[4], David Blaney[4]

[1]Centers for Disease Control and Prevention, San Diego, CA, United States, [2]University of California Berkeley, CA, United States, [3]HRSA, Baton Rouge, LA, United States, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States

The burden of Hansen's disease (HD) (leprosy) has declined worldwide but it remains endemic in many less-developed countries. In developed countries, HD is rare, mostly associated with immigrants from endemic areas. In the United States, 72% of 213 HD cases reported in 2009 were foreign born. Untreated HD can progress to a severely debilitating disease, involving functional loss of extremities and disability. Risk of impairment increases with longer delay between onset of symptoms and disease diagnosis. We identified risk factors associated with longer interval between HD symptom onset and diagnosis in the United States. HD cases registered with the National Hansen's Disease Program and diagnosed during 2000-2010 (n=1,241) were included in the analysis. Cox proportional hazards models were used to assess median times between symptom onset and diagnosis. Foreign-born persons whose symptoms began before their arrival in the United States had a longer delay in diagnosis than U.S-born and foreign-born cases with onset after arrival (3.3 years vs. 1 year, $p<0.05$). U.S-born patients residing in states with an HD specialized ambulatory care clinic experienced a shorter delay to diagnosis, compared with those with no clinic nearby (0.9 vs. 2.3 years) ($p<0.05$). Proximity to a HD clinic did not affect delay to diagnosis among the foreign born. Among the U.S-born delay in diagnosis was significantly ($p<0.05$) longer for those whose symptoms began when they were 30-44 years old (2.3 years) compared to other age groups. For the foreign-born the longest delay (3 years) was for those with onset with onset at ≤15 years old (3 years). Better understanding of the reasons for delayed diagnosis is needed, particularly for younger foreign-born patients and for those with symptom onset before U.S arrival. The role of enhanced HD screening and education during immigration-related medical examination needs to be assessed. For the U.S-born, improved access to HD clinics should be explored. Increased HD awareness and outreach to physicians are also needed, especially in states without HD clinics and in areas with large foreign-born populations.

478

PHYSIOLOGICAL ROLE AND CHEMOTHERAPEUTIC POTENTIAL OF THE ECDYSONE RECEPTOR HOMOLOGUE OF THE HUMAN PARASITE *BRUGIA MALAYI*

Amruta Mhashilkar, Canhui Liu, Thomas Unnasch

University of South Florida, Tampa, FL, United States

A homologue of the ecdysone receptor (Ecr), a master regulator of development in insects has previously been identified and shown to be responsive to 20-OH ecdysone in transfected *Brugia malayi*. As the EcR is not found in vertebrate animals, it and the regulatory pathways it controls represent an attractive potential chemotherapeutic target. To further delineate the physiological role that the EcR plays in filarial parasites, adult female parasites were treated with 20-OH ecdysone in culture and microfilarial output and embryograms monitored in treated and control parasites. Females treated with 20-OH ecdysone produced significantly more microfilaria that control worms, implicating the EcR in regulation of microfilarial development. RNAseq of the transcripts of adult females treated with 20- OH ecdysone was also conducted to observe changes in gene expression. RNAseq identified 67 genes whose expression was genes significantly up-regulated in the treated parasites compared to untreated controls. A mammalian two hybrid system was used develop a high throughput assay to identify agonists and antagonists of the filarial EcR. This assay was based upon a mammalian two hybrid system involving BmEcR Gal4 and RXR VP16 fusion constructs and a GAL4 Gaussia luciferase reporter. The fusion constructs heterodimerize and when bound to the cognate ligand, activates transcription of the luciferase reporter. On induction with 20-OH ecdysone, transactivation of the luciferase gene was seen in triply transfected mammalian cells, with a signal to noise ratio of roughly 6 and Z' of the assay is 0.7. This assay will be employed to screen natural product libraries and a collection of ecdysone analogs in the near future.

479

INVESTIGATING *ONCHOCERCA VOLVULUS - WOLBACHIA* BACTERIAL ENDOSYMBIONTS COPY NUMBER VARIATIONS AFTER IVERMECTIN TREATMENT

Samuel Armoo, Stephen R. Doyle, Warwick N. Grant

Genetics Department, La Trobe University, Bundoora, Melbourne, Australia

Onchocerca volvulus - causative agent of onchocerciasis - harbour *Wolbachia* bacterial endosymbionts (wOv), whose activities are vital for worm survival. For instance, doxycycline treatment caused parasite mortality due to wOv death. In addition, co-evolution wOv and worm genomes, and lateral gene transfers have been reported. Despite this survival benefit, "un-interrupted" worm autophagy mechanisms regulate wOv copy numbers to achieve a homeostatic balance. Here, we investigate whether interruptions in worm biology, such as ivermectin (IVM) treatment, can lead to changes in wOv copy number. We used a relative real time quantitative PCR assay to measure wOv copy number variations (CNV) between IVM treated and naïve worms. The *Wolbachia* surface protein gene (wsp) for wOv and the glutathione reductase gene (gr) for worm are single copy, therefore gene copy numbers were equivalent to genome copies. In all, 143 adult parasites (34 naïve and 109 treated), sampled from Ghana, were assayed. Treated parasites had significantly reduced variance of wsp/gr ratio compared to naïve ones ($P = 0.001$). Also, treated parasites had a lower median wsp/gr ratio (4.25, S.E. = 2.31) than naïve (10.36, S.E = 10.11), though difference not statistically significant ($P = 0.531$). These results may be indicative of IVM treatment influencing the worm wOv copy numbers.

480

RAPID, SENSITIVE DETECTION OF FILARIAL DNA WITH MINIMAL EQUIPMENT

Weam Ismail Zaky, Francesca Tomaino, Nils Pilotte, Steven A. Williams

Smith College, Northampton, MA, United States

Currently, molecular xenomonitoring efforts for lymphatic filariasis (LF) rely on the PCR or RT-PCR-based detection of *Brugia malayi, Brugia timori* and *Wuchereria bancrofti.* Typically, extraction of DNA from infected mosquitoes is performed using the column-based DNeasy Blood and Tissue Kit from Qiagen. However, this extraction is both time consuming and expensive, and the diagnostic testing which follows requires expensive thermal cyclers or Real-Time PCR instruments. Such expenses make these molecular tests impractical for laboratories within many endemic areas. Accordingly, in such locations, there exists a substantial need for an inexpensive, equipment-minimizing diagnostic option. In this work, we evaluate a crude, NaOH extraction method for the isolation of template DNA that minimizes the presence of inhibitors, in conjunction with an isothermal DNA amplification method that requires minimal laboratory equipment. This simple extraction reduces the total cost of the DNA isolation by 50%. We show that for pools of up to 25 mosquitoes, spiked with one infected *B. malayi* or *W. bancrofti* L3 worm, detection

by real-time PCR, and tHDA isothermal amplification are equally sensitive using both NaOH extraction, and Qiagen-based extraction methods. Furthermore, as a portable and simple diagnostic assay, tHDA isothermal amplification can be coupled with HybriDdetect® test strips, enabling detection of amplified *B. malayi* DNA with equal sensitivity to gel electrophoresis product visualization. By eliminating the need for expensive equipment without compromising sensitivity, this assay and product detection combination provides a diagnostic alternative for endemic locations lacking the means to perform the molecular assays currently employed for xenomonitoring purposes.

481

APPLICATION OF RECOMBINANT HUMAN ANTIBODY FOR VALIDATION AND QUALITY CONTROL OF SEROLOGICAL ASSAYS IN SUPPORT OF ONCHOCERCIASIS ELIMINATION PROGRAMS

Allison Golden[1], Eric Stevens[1], Lindsay Yokobe[1], Roger Peck[1], Melissa Valdez[1], Dunia Faulx[1], Potochoziou K. Karabou[2], Meba Banla[3], Kangni Adade[2], Richard Gbandi Gantin[3], Peter Soboslay[4], Vitaliano Cama[5], Thomas R. Unnasch[6], Thomas B. Nutman[7], Gonzalo J. Domingo[1], Tala de los Santos[1]

[1]PATH, Seattle, WA, United States, [2]National Onchocerciasis Control Programme, Lome, Togo, [3]Onchocerciasis Research Laboratories, Sokode, Togo, [4]University of Tubingen, Tubingen, Germany, [5]Centers for Disease Control and Prevention, Atlanta, GA, United States, [6]University of South Florida, Tampa, FL, United States, [7]National Institute of Health, Bethesda, MD, United States

Monitoring progress toward onchocerciasis or river blindness elimination has largely relied on the detection of microfilariae in skin snips of infected patients, a technique that presents challenges: acceptability, timing with mass drug administration (MDA) interventions, and sensitivity when the disease burden is low. The detection of antibodies to *Onchocerca volvulus* (Ov)-specific antigens provides an alternative and proven approach that requires only capillary blood from a finger stick and where the sensitivity of antibody detection is not dependent on timing with recent MDA. One challenge with serological assays is the availability of a reliable and consistent source of positive control. To address this issue, a recombinant, monoclonal human IgG4 antibody was developed to the antigen Ov16. With anti-Ov16 IgG4 ELISA-based serological assays already widely used to confirm elimination in the Americas and parts of Africa, the recombinant Ov16-specific human IgG4 antibody could be used for standard curves on ELISA-based assays and as a quality control reagent for an Ov16 rapid diagnostic test (RDT). The performance of two ELISA tests for anti-Ov16 human IgG4 was assessed when using the recombinant IgG4 antibody, plasma samples, and dried blood spots. The data demonstrates that use of a horseradish peroxidase-based (HRP) ELISA results in a lower limit of detection than the currently used alkaline-phosphatase-based ELISA format. A dried-down formulation of the recombinant antibody was developed and used under field conditions without refrigeration as a quality control for Ov16 RDTs. Stability was demonstrated for over 15 weeks, unrefrigerated. The recombinant human anti-Ov16 IgG4 antibody will be useful for inter-laboratory validation of ELISA assays and as a quality control reagent for RDTs at different points of the supply chain from manufacturer to field use.

482

EXPRESSION PATTERNS OF NICOTINIC ACETYLCHOLINE RECEPTORS (NACHRS) IN *BRUGIA MALAYI* FILARIAL WORMS

Benwen Li, Amy H. Rush, Gary J. Weil

Washington University School of Medicine, St. Louis, MO, United States

Nematode nicotinic acetylcholine receptors (nAChRs) are the targets for a number of anthelmintics including levamisole, pyrantel, morantel and oxantel. The levamisole AChR type is composed of five subunits, Cel-unc-29, Cel-unc-38, Cel-unc-63, Cel-lev-1 and Cel-lev-8 in C. elegans. Nine nAChRs are present in the *Brugia malayi* genome including orthologues of Cel-unc-29, Cel-unc-38, and Cel-unc-63. We studied expression these genes by qRT-PCR in *B. malayi* adult female, male, and microfilariae. Six of eight nAChRs genes studied were differentially expressed. Four were more highly expressed in males, 1 in females, and 1 in microfilariae. We performed *in situ* hybridization with cRNA probes to localize expression of five *B. malayi* nAChRs genes in adult worms. Most of them had similar expression patterns with signals in developing embryos, spermatogonia, the uterine wall, vas deferens, and lateral chords. For example, Bm1_35890, an orthologue of cel-unc-29 encoding the subunit of levamisole-sensitive receptors that had equal expression in male and female worms by qRT-PCR, was strongly expressed in both male and female worms. In females, strong expression signals were detected in the ovary, developing embryos and lateral hypodermal chords, with moderate expression in the uterus wall adjacent to stretched microfilariae. Expression signals in males were strong in spermatogonia and in the wall of vas deferens. Expression patterns for the novel gene Bm1_48815 (with no orthologue in other nematodes and equal expression in males and females) were similar to those of Bm1_35890. Anti-peptide antibody to Bm1_48815 bound to the same tissues that were labeled by *in situ* hybridization, but the antibody also bound stronngly to body muscle in both male and female worms. Increasing evidence suggests nicotinic receptors regulate developmental events in the nervous system and neuronal AChRs may play important developmental roles as the receptors are expressed early during embryogenesis in vertebrates. The expression patterns of these genes suggest that they are involved in reproduction, and this may explain the effect of drugs that target nAChR on reproduction in filarial worms.

483

HIGH CONTENT IMAGING: MORE THAN A PRETTY PICTURE

Kelly L. Johnston, Rachel Clare, Darren A. Cook, Andrew Cassidy, Vera T. Unwin, Louise Ford, Stephen Ward, Mark J. Taylor

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

The A·WOL consortium aims to find a novel macrofilaricidal drug to treat the debilitating diseases lymphatic filariasis and onchocerciasis, through targeting the *Wolbachia* bacteria that reside within these parasitic nematodes. Typically, screening chemical libraries directly against parasitic nematodes is cumbersome, low throughput and relies on animal reservoirs. By focusing on the endosymbiont, we have been able to utilise an insect cell-based screening approach that originally relied upon a quantitative polymerase chain reaction readout, but now employs a High Content Imaging readout running on the Perkin Elmer Operetta® platform. This assay uses texture analysis of cells stained with SYTO®11 (fluorescent DNA stain) as a direct measure of bacterial load and allows the consortium to screen up to 10x 384 well plates per day; a radical increase in throughput from the qPCR screen. Further to its use as a screening tool, the Operetta® is also being used for more fundamental biological investigations such as experiments surrounding the infection dynamics within host cells and, as part of a separate project aiming to create a novel cell-based screen for discovery of a macrofilaricide, the development of a filarial nematode cell line. The nature of the anti-*Wolbachia* screening approach will be presented in addition to preliminary data from other High Content Imaging investigations surrounding *Wolbachia,* host cells and cell line development.

484

A•WOL MACROFILARICIDAL DRUG DISCOVERY AND DEVELOPMENT - OPTIMIZATION OF ANTI-*WOLBACHIA* EFFICACY

Louise Ford[1], Gemma L. Nixon[1], Kelly L. Johnston[1], Joseph D. Turner[1], Neil G. Berry[2], Paul M. O'Neill[2], Stephen A. Ward[1], Mark J. Taylor[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]University of Liverpool, Liverpool, United Kingdom

There is an urgent need to develop a novel treatment for filariasis, and targeting *Wolbachia* provides safe macrofilaricidal activity with superior therapeutic outcomes compared to standard anti-filarial treatments. The Anti-*Wolbachia* (A·WOL) Consortium has developed both *in vitro* and *in vivo* assays, to screen chemical libraries for anti-*Wolbachia* activity. The outputs from the A·WOL program are now being pursued as part of A·WOL II Macrofilaricide Drug Discovery & Development programs. Screening of >10000 compounds from the BioFocus library and chemoinformatic analysis have generated six independent lead series chemotypes with the potential to enter a medicinal chemistry "hit-to-lead" and lead optimization program. A·WOL Drug Discovery is now progressing these lead series through a rigorous lead optimisation and candidate selection process, using iterative cycles of medicinal chemistry and biological testing in order to deliver at least one novel pre-clinical candidate and a chemically distinct back-up, aligned with our Target Product Profiles for an anti-*Wolbachia* macrofilaricide. In addition, ongoing screening of large diversity-based libraries (150-500k compounds) aims to provide additional, chemically diverse hits, with one-order improvement in absolute potency or significant shortening of treatment time, in order to expand the structural diversity of anti-*Wolbachia* chemotypes. A·WOL Drug Development is optimising regimens of anti-*Wolbachia* monotherapy and combination treatment of registered anti-*Wolbachia* and anti-filarial drugs *in vivo* using an adult *Brugia malayi* mouse model. This efficacy testing is driven by a rational PK/PD modelling approach which supports dosage regimens, in order to identify the best treatment regimens to test in field trials.

485

THE DEPLETION OF *WOLBACHIA* FROM *BRUGIA MALAYI* MICROFILARIAE AND SUBSEQUENT EFFECT ON THE DEVELOPMENT OF INFECTIVE LARVAE IN *AEDES AEGYPTI*

Darren A. Cook, Gemma Molyneux, Andrew Steven, Ana F. Guimaraes, Kelly L. Johnston, Hayley E. Tyrer, Mark J. Taylor

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

The filarial nematode *Brugia malayi* is a human pathogen that harbours the bacterial endosymbiont, *Wolbachia.* Tetracycline treatment causes the depletion of *Wolbachia* leading to infertility and early death in the adult worm. If tetracycline treatment is ongoing at the time of infection, development from the L3 stage to adult is inhibited. The depletion of *Wolbachia* in microfilariae (Mf) has been shown to affect development to L3 infective larvae in the intermediate host *Litomosoides sigmodontis.* To assess the effect of tetracycline treatment on *B. malayi* microfilariae and development to the L3 infective stage within the mosquito, *Aedes aegypti,* infected gerbils (*Meriones unguiculatus*) were treated for 2, 4 and 6 weeks. At each time-point, Mf were extracted and fed to mosquitoes and the subsequent development to L3 larvae was assessed versus controls. To further understand the relationship between *Wolbachia* and L3 development, live Mf were directly visualised within the mosquito midgut and also proteomic analysis was conducted of secretory/excretory products from treated and control Mf. The results show the significant effect of *Wolbachia* depletion on the ability of Mf to progress to L3 stage and treated Mf are unable to escape the mosquito midgut. The retardation of L3 development by tetracycline may have an important additional blocking effect on the transmission of filarial parasites and understanding the mechanism by which this occurs may offer further insight into the important role *Wolbachia* play in each of the life-cycle stages of *B. malayi*.

486

MICRORNAS IN PARASITIC NEMATODES - DEFINING A FUNCTION

Eileen Devaney, Victoria Gillan, Kirsty Maitland, Henry Gu, Alan Winter, William Weir, Collette Britton

University of Glasgow, Glasgow, United Kingdom

We previously identified a large number of microRNAs in the filarial nematode *Brugia* species and in *Haemonchus contortus*, a sheep nematode related to human hookworms. Recent experiments have sought to define the function of two microRNAs, *Bpa-mir-5364* and *Hco-mir-5352*. The Brugia miRNA was selected for study on the basis of an intriguing expression pattern, being ~x12-fold increased between vector derived L3 and L3 recovered at 24 h post-infection. This miRNA is a novel member of the *let-7* family. Using target prediction programs combined with a comparative genomics approach we sought to identify predicted mRNA targets, and selected three of these for further analysis. We established a dual luciferase assay in transfected HEK cells to examine the relationship between *Bpa-mir-5364* and the 3'UTR of the predicted targets and used anti-sense oligonucleotides to attempt to inhibit *Bpa-mir-5364 in vitro*. For *H. contortus*, we focused on a single miRNA that is parasite-specific and only found in nematode parasites that live in the gastrointestinal tract (irrespective of nematode clade). *Hco-mir-5352* is one of a cluster of four microRNAs that is conserved in a number of gastrointestinal nematodes. We could detect *Hco-mir-5352* in adult worm excretory-secretory products and in tissues from infected sheep, indicating that it may be released by the parasite *in vivo* and thus could interact with a host mRNA. A predicted target of *Hco-mir-5352* is mammalian CD69 and we have used the dual luciferase assay in an attempt to confirm this relationship. An improved understanding of the function of parasite microRNAs casts novel light upon the intricacies of the host-parasite relationship.

487

DIRECT VEGF-SPECIFIC ANTI-ANGIOGENIC ACTIVITIES OF THE ANTI-*WOLBACHIA* DRUGS, DOXYCYCLINE AND MINOCYCLINE, IN AN *IN VITRO* MICROVASCULAR BLOOD AND LYMPHATIC ENDOTHELIAL CELL CULTURE SYSTEM

Hayley E. Tyrer, Amber Fanthome, Ana Guimaraes, Rachel Clare, Mark Taylor, Joseph D. Turner

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Lymphatic filarial infection (LF) is the cause of elephantiasis and hydrocoele lymphoedemas (LE). LE is initiated as a result of episodic inflammatory damage and remodelling of parasitized lymphatics. Heightened inflammatory responses in LE patients are associated with pro-angiogenic/lymphangiogenic molecules in circulation, such as vascular endothelial growth factors (VEGFs), which suggests inflammatory angiogenesis is relevant in the pathophysiology of LF. Current mass drug administration treatments for LF have mainly microfilaricidal activities and do not help alleviate the suffering of current LE patients. As such, there is an unmet need to identify new therapeutics to help reduce LF morbidity. Encouragingly, in two recent trials, 6-week doxycycline therapy was identified to improve LE grade after 12-24 months. The anti-morbidity mode of action (MoA) of doxycycline has not been defined. LE grade is improved both in doxycycline treated patients with and without active LF infection, suggesting a separate MoA to that of targeting the filarial inflammatory endosymbiont, *Wolbachia.* We developed an *in-vitro* blood and lymphatic endothelial cell culture system to assess the VEGF-specific anti-angiogenic activities of the anti-*Wolbachia* compounds, doxycycline and minocycline. Utilising a 96 well/200 µL based Operetta™ (Perkin Elmer) fluorescent bio-imaging system we have stimulated the proliferation of

human adult dermal microvascular blood endothelial cells (HMVECd) and equivalent lymphatic endothelial cells (HMVECdLy), using VEGFs targeting VEGF receptors 1-3. Titrations of both doxycycline and minocycline toward physiological plasma levels have demonstrated a dose-dependent reduction in both VEGF-specific HMVECd and HMVECdLy proliferation. This data supports a direct anti-angiogenic MoA of second-generation tetracyclines in use for the treatment of LF, which may target pathological inflammatory VEGF processes. The bio-imaging platform has been validated for onward screening to identify new candidate compounds with VEGF-specific anti-angiogenic activities.

488

ONCHOCERCIASIS: TRANSCRIPTOMIC AND PROTEOMIC APPROACHES FOR IDENTIFYING BIOMARKERS ASSOCIATED WITH THE PRESENCE OF VIABLE ADULT FEMALE PARASITES FOR POST-CONTROL SURVEILLANCE

Sasisekhar Bennuru[1], Nancy Holroyd[2], Alan Tracey[2], James Cotton[2], Matthew B. Rogers[3], Jose M. Ribeiro[1], Elodie Ghedin[4], David Abraham[5], Matthew Berriman[2], Sara Lustigman[6], Thomas Nutman[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Wellcome Trust Sanger Institute, Cambridge, United Kingdom, [3]University of Pittsburgh, Pittsburgh, PA, United States, [4]New York University, New York, NY, United States, [5]Jefferson Medical College, Philadelphia, PA, United States, [6]New York Blood Center, New York, NY, United States

Control of onchocerciasis or 'river blindness' has had considerable success in Africa. As efforts shift from control to disease elimination better tools are needed to identify viable adult females (OvAF) for the "end game" needs. The goal of this project is to identify host- and/or OvAF-specific biomarkers for use in rapid field-friendly tests during post-control surveillance programs. By taking advantage of the recently sequenced genome and assembly of Onchocerca volvulus (~97.4Mb) organized by optical mapping into chromosomes, we have overlaid RNAseq analyses of the important mammalian stages of O. volvulus (Ov) L3, adult males, adult females and microfilariae. Of the predicted 12994 genes, a total of 12416 transcripts were identified from all stages, with over 9600 adult female transcripts. Analysis of transcripts across all the stages resulted in the identification of 75 select gene products that were abundant in OvAF and absent or minimally expressed in other stages. In parallel, shotgun proteomics of the somatic extracts from each of the Ov mammalian stages (including excretory-secretory proteins of OvAF) are in progress using UPLC MS/MS (Thermo Easy nLC 1000 UPLC coupled to Thermo Q-exactive quadrupole-Orbitrap mass spectrometer). Of even more interest is the proteomic analysis of O. volvulus infected human sera in which hundreds of Ov-specific proteins have been identified, of which four are among the female-specific proteins found during the transcriptomic analysis. Recombinant antigens and monospecific polyclonal antibodies are being made to test the validity of these targets as biomarkers for use in post-control surveillance assay systems.

489

THE MUTUALISTIC SYMBIOSIS OF *WOLBACHIA* AND THE FILARIAL NEMATODE *BRUGIA MALAYI* - UNRAVELLING THE PROTEOME AND TRANSCRIPTOME

Gemma R. Molyneux, Christina Bronowski, Simon Wagstaff, Mark J. Taylor

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

The parasitic nematode *Brugia malayi* is a causative agent of lymphatic filariasis, a disfiguring disease affecting over 120 million people worldwide. *B. malayi* exists in a mutualistic symbiotic relationship with the α-proteobacterium *Wolbachia*. We have applied a global proteomic profiling approach to investigate the molecular basis of this symbiosis. Adult female *B. malayi* in the mammalian host *Meriones unguiculatus* were sampled at multiple time-points post-antibiotic depletion. Deep proteome mining combined with high-resolution mass spectrometry was used for comprehensive proteome profiling of *Wolbachia*/worm at these selected time-points. *Wolbachia*/worm ratios were also monitored by qPCR. Using a combination of extensive peptide pre-fractionation and established proteomic workflows we observed improved proteome coverage by an increase in peptide/protein identification. Such proteomic approaches coupled with reversed phase liquid chromatography and analysis by high-resolution mass spectrometry provides a powerful tool for global proteome profiling. Proteins of interest from this initial global proteomic 'screen' have been further investigated using a targeted proteomics approach. Following basic analysis through established bioinformatics pipelines, transcriptomic, proteomic, and published datasets will be integrated in a systems biology approach with the objective of understanding the molecular basis of the mutualistic *Wolbachia*/*B. malayi* symbiosis.

490

IDENTIFICATION OF CHEMOSENSORY RESPONSES IN THE FILARIAL WORM, *BRUGIA MALAYI*

Lisa Fraser, Michael Kimber, Lyric Bartholomay

Iowa State University, Ames, IA, United States

Lymphatic filariasis (LF) is a disease caused by mosquito-borne filarial nematodes including *Wuchereria bancrofti* and *Brugia malayi*. Although over 120 million people suffer from this disfiguring disease, chemotherapeutic options for LF are limited to three drugs: diethylcarbamazine citrate, albendazole and ivermectin. The threat of drug resistance combined with the inefficacy of these drugs against adult parasites highlights the need for new anthelmintic drugs. Chemosensation is an essential behavior used by multi-cellular organisms to interact with the environment. In the free-living nematode, *Caenorhabditis elegans*, chemosensation plays a crucial role in development, avoiding noxious conditions and finding food and mates. In parasitic nematodes, chemosensation is thought to play a critical role in host-seeking and host-invasion behaviors making genes involved in this system attractive targets for drug or vaccine development. However, little is known about the chemosensory system in filarial parasites of animals. In this study, we sought to elucidate chemosensory-induced behavior in *B. malayi* during the infectious L3 stage of the parasite. Scanning electron microscopy revealed that amphids, the major chemosensory organs of nematodes, are present in both juvenile and adult stages of *B. malayi*. In addition, orthologues of several genes known to be involved in chemosensory behavior in *C. elegans* were identified in *B. malayi* based on sequence homology. Finally, we identified over 10 chemical compounds that were either attractive or repellent to *B. malayi* L3 stage parasites using chemotaxis assays. This research is the first study to demonstrate that *B. malayi* has a responsive chemosensory pathway. In addition, the results obtained indicate that the chemosensory response in *B. malayi* plays an important role in host-seeking and host-invasion behavior, and therefore is a prime candidate for chemotherapeutic intervention.

491

GENOMICS OF *WUCHERERIA BANCROFTI* FROM FOUR ENDEMIC REGIONS OF INFECTIVITY: HAITI, KENYA, MALI AND PAPUA NEW GUINEA

Peter A. Zimmerman[1], Christopher L. King[1], Patrick J. Lammie[2], Yaya Coulibaly[3], Abdallah Diallo[3], Thomas B. Nutman[4], James W. Kazura[1], Peter M. Siba[5], **Scott T. Small**[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]NIAID-Mali ICER, Bamako, Mali, [4]National Institutes of Health, Bethesda, MD, United States, [5]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea

Lymphatic filariasis (LF) is a major threat to human health in the tropics, leading to the disfiguring and debilitating conditions hydroceles and

elephantiasis. There are approximately 1.2 billion people living in LF endemic countries where 120 million people currently suffer from the disease. The parasites that cause LF are separated into two genera, *Brugia* and *Wuchereria*, with *W. bancrofti* (Wb) responsible for ~90% of LF cases. Until recently, Wb has been neglected in genomic studies due to complications in sample collection and preparation, such as lack of adult stage samples. Our previous work has shown it is possible to concentrate Wb microfilaria from an infected blood sample resulting in high quality genomic sequence. Here we expand our previous studies in Papua New Guinea to three more endemic areas of Wb infectivity: Mali, Kenya, and Haiti. We report on thousands of unique single nucleotide polymorphisms (SNPs) and identify genes that are highly variable among localities. We utilize discovered SNPs to *i)* construct an assay to identify the geographic source of new Wb outbreaks, *ii)* explore the historical context of admixture between endemic areas, retracing the possible routes of Wb migration, and *iii)* root the gene trees of Wb to identify the species origin. Our results provide a new context for studying Wb infection by identifying endemic areas of high genetic diversity that may hinder elimination.

492

CHARACTERIZATION OF PUTATIVE *BRUGIA MALAYI* ACETYLCHOLINE-GATED CHLORIDE CHANNELS

Elizabeth M. Asque, Mostafa Zamanian, Lyric Bartholomay, Michael Kimber

Iowa State University, Ames, IA, United States

The neglected tropical disease, lymphatic filariasis (LF), affects over a million people and an even greater number of individuals are at risk for this disease in endemic tropical regions. The filarial nematodes, *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*, can cause severe lymphedema leading to gross disfigurement in the infected human host. Mass Drug Administration (MDA) programs deliver chemotherapeutics diethylcarbamazine, ivermectin, and albendazole, annually to endemic areas to manage and treat this disease. Despite success with MDA, growing concerns of resistance combined with the inefficacy of these drugs against the adult stages of the parasites emphasizes the need for novel and broadly effective antifilarial chemotherapeutics. Cholinergic neuro transmission is a proven source of effective anti-nematodal drug targets. Although much is known about Na+ conducting nicotinic acetylcholine receptors, the family of acetylcholine-gated chloride channels (ACC) is poorly understood. We used reverse genetics to investigate *Brugia malayi* acetylcholine chloride channels (BACCs), which are a novel family of ACCs. Transcriptomics, homology based searches, and 5' and 3' RACE-PCR identified 8 putative BACCs. qRT-PCR results revealed that three BACCs, BACC-1, BLGC-46, and BLGC-47, are expressed in both juvenile and adult stages. Results from *in situ* hybridization demonstrate that BACCs are distinctly localized. We are further probing the potential function of BACC-1, BLGC-46, and BLGC-47 by using RNA interference and microfluidics assays. Our results show that BACCs have similar characteristics to other ACCs thus these genes are prime candidates for further investigation as novel chemotherapeutic targets.

493

CASE REPORT: FOUR-YEAR-OLD FEMALE FROM INDIA WITH NIGHTTIME COUGH, PULMONARY INFILTRATES AND AN ABSOLUTE EOSINOPHIL COUNT OF 53,000

David P. Del Bello[1], Anusha Viswanathan[1], Gina Allegretti[1], Paul Cantey[2], Michael Tosi[1]

[1]Mount Sinai Medical Center, New York City, NY, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States

A four-year-old female with a prior history of failure-to-thrive, who recently emigrated from a lymphatic filariasis endemic area in Northern India, was admitted to the pediatric floor of Mount Sinai Medical Center with low-grade fevers and night-time cough for over four weeks, diffuse bilateral centrilobular ground glass opacities on chest CT and an absolute eosinophil count in excess of 53,000. Blood smear examination was negative for parasites. Filariasis Bm14 ELISA (IgG4) performed at the Centers for Disease Control was positive at 100 units per microliter (upper limit of normal 12.4 units per microliter). Clinical presentation was most consistent with Tropical Pulmonary Eosinophilia secondary to *Wuchereria bancrofti*. Serology was also strongly positive for *Strongyloides* and *Toxocara,* and was weakly positive for *Trichinella*. She was treated with two days of oral ivermectin 200 micrograms/kilogram per day for the *Strongyloides*, then 21 days of oral diethylcarbamazine 6 milligrams/kilogram divided three times a day for the suspected *W. bancrofti* and finally 14 days of albendazole 15 milligrams/kilogram per day for the *Toxocara*. Seven weeks after completing therapy, the patient clinically improved and her absolute eosinophil count was reduced to 3300. Tropical Pulmonary Eosinophilia is a rare diagnosis in children in the United States. Blood smears for *W. bancrofti* are negative in the setting of Tropical Pulmonary Eosinophilia, so history, identification of epidemiologic risk, and antibody testing are the keys to appropriate diagnosis. The case also demonstrates the importance of checking for other parasites in patients from an area co-endemic for multiple parasites who have been identified to already have a particular parasitic disease, as polyparasitism is not uncommon.

494

A CASE REPORT: SUBCONJUNCTIVA EYE WORM

Aye T. Lin

Presbyterian Intercommunity Hospital, Whittier, CA, United States

We report a case of ocular *Loa loa* (10/9/2013) in a 46 year old Cameroonian Male who reported a history of a motile foreign body sensation in right eye for five years. Examination of patient's right eye showed live Loa Loa worm in subconjunctiva space. The worm was extracted by making a small incision in the conjunctiva with a scalpel and removing the worm with small forcep. Patient was treated with Albendazole after the worm removal.

495

HIGH THROUGHPUT SCREENING FOR ANTI-*WOLBACHIA* DRUGS TO TREAT ONCHOCERCIASIS AND LYMPHATIC FILARIASIS

Rachel Clare[1], Roger Clark[2], Kelly L. Johnston[1], Darren A. Cook[1], Louise Ford[1], Eileen McCall[2], David C. Murray[2], Kirsty Rich[2], Stephen Ward[1], Mark J. Taylor[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]AstraZeneca, Cheshire, United Kingdom

The Anti-*Wolbachia* consortium (A•WOL) at Liverpool School of Tropical Medicine (LSTM) has partnered with the Global High Throughput Screening (HTS) Centre at AstraZeneca in the first open access HTS project for the World Intellectual Property Organization's (WIPO) Re:Search program against Neglected Tropical Diseases. The A•WOL consortium aims to identify novel macrofilaricidal drugs targeting the essential bacterial symbiont (*Wolbachia*) of the filarial nematodes causing onchocerciasis and lymphatic filariasis. The project aimed to scale-up the throughput of the A•WOL cell-based screening assay using AstraZeneca's leading automation, screening technologies and expertise. The A•WOL screen at LSTM uses a DNA fluorescent (SYTO®11 stained), *Wolbachia* infected C6/36 *Aedes* albopictus cell line imaging assay (running on the Perkin Elmer Operetta® platform) to screen up to 10x 384 well plates per day. The development of a higher throughput screen at AstraZeneca has dramatically evolved the assay protocol, through the optimisation of the cell culture, assay analysis, washing methodology and plate reading technologies. The process is now fully automated from cell and compound addition through to data analysis. The validation of this assay will allow screening of AstraZeneca's chemical library of compounds in

a single screening activity, dramatically increasing our throughput. Hits identified will then be progressed through the A•WOL drug discovery and development programme for new macrofilaricides.

496

ECONOMIC COSTS AND BENEFITS OF MORBIDITY MANAGEMENT AND DISABILITY PREVENTION FOR LYMPHATIC FILARIASIS

Eileen Stillwaggon[1], Larry Sawers[2], LeAnne Fox[3], David Addiss[4]

[1]Gettysburg College, Gettysburg, PA, United States, [2]American University, Washington, DC, United States, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Children Without Worms, Decatur, GA, United States

Lymphatic filariasis (LF) is endemic in 73 countries, with 120 million people infected, of whom 40 million suffer serious disability, including lymphedema of the legs (15 million), arms, and breasts; hydrocele in 25 million men; and acute attacks of fever and disabling pain that last several days, termed adenolymphangitis (ADL), which contribute to worsening lymphedema and substantial productivity loss. The Global Programme to Eliminate Lymphatic Filariasis (GPELF) has two goals: interrupting LF transmission by 2020 and caring for people already infected through morbidity management and disability prevention (MMDP). While 53 countries have ongoing mass drug administration, only 27 had begun MMDP by 2013, in part, due to the perceived high cost and low return on investment for MMDP. To address this concern, we estimate costs and benefits of MMDP for the global population affected and at risk over the lifetime for affected cohorts. MMDP changes the distribution of disability and productivity over time. Actual program costs are based on 3 delivery modes: community-based care (CBC), referral to primary care clinics, and specialty clinics. We calculate societal costs of untreated morbidity due to acute attacks, chronic lymphedema and hydrocele, consequent lost wages, and other costs. In each country with cost comparisons, research to date suggests societal cost of untreated LF far exceeds cost of MMDP. In India, patients enrolled in CBC morbidity management averaged 29 fewer lost work days per year. In Haiti, ADL was reduced substantially after simple training in self-care. A program in Togo, after 3 years of MMDP, reported stabilization of lymphedema stage and slight decrease in ADL. In Ghana, patients reported substantial improvement in work capacity after hydrocele surgery. We will combine results from these and other countries and adjust costs and benefits to each context, to estimate savings in direct costs, productivity loss, and marginalization that provide economic rationale in addition to the ethical mandate for MMDP, the second pillar of GPELF.

497

LOA LOA IN A CONGOLESE REFUGEE WOMAN IN MINNESOTA

Hope Pogemiller[1], Ann Settgast[2]

[1]University of Minnesota, Minneapolis, MN, United States, [2]Health Partners Center for International Health, St. Paul, MN, United States

An estimated 13 million people are living with the neglected tropical disease Loiasis. The *Chrysops* deerfly vector can be found in rainforest canopies of Central and West Africa. Loa loa microfilaria migrate into subcutaneous tissue, maturing into adult worms, mating, and producing more microfilaria for more than a decade in a human host. This could cause a refugee to experience intermittent symptoms for many years after resettlement to the US. Calabar nodules (pruritic, non-tender skin swellings) and white worms moving across the visual field should lead to the search for *Loa loa*. A 49 year old Congolese refugee presented with 2 days of a "needly" sensation in her right eye after 6 days of left eye pain with periorbital swelling. She had temple pain and intense pruritis in her palms and soles. She reported numerous episodes of migratory pruritic swellings on her left forearm and foot and a sensation of threads moving across her vision in the previous 2 years. While living near Kinshasa 9 years earlier, she was treated with an herbal poultice that allowed tiny white worms to be removed from her eyes. Her husband has been treated for onchocerciasis in the past. An NIH onchocerciasis antibody test was negative, and a slit-lamp exam found no evidence of coinfection. Her filarial serologies were consistent with a past or acute filarial infection. She was treated with 3 weeks of DEC, causing only mild nausea. Her eosinophilia decreased from an AEC of 800 to 200 after treatment. There is a 50% chance that this course will have treated her filarial infection definitively. This woman suffered from Loiasis, an illness that can plague patients with intermittent symptoms for over a decade. Although short-term travelers are rarely infected, the current influx of Congolese refugees to the US heightens the importance of Loiasis awareness. As onchocerciasis can be endemic in the same regions, coinfection must be considered when selecting treatment for Loiasis to avoid the severe Mazzotti inflammatory reaction that occurs when given DEC while infected with onchocerciasis.

498

ORAL FLUBENDAZOLE: A POTENTIALLY USEFUL MACROFILARICIDE

Charles D. Mackenzie[1], Timothy G. Geary[2], Sabine Specht[3], Maeghan O'Neill[2], Achim Hoerauf[3], Kennan Marsh[4], Ivan Scandale[5], Steven Silber[6]

[1]Michigan State University, East Lansing, MI, United States, [2]McGill University, Quebec, QC, Canada, [3]Medical University, Bonn, Germany, [4]AbbVie, North Chicago, IL, United States, [5]DNDi, Geneva, Switzerland, [6]JRD, Raritan, NJ, United States

The search for a macrofilaricide that can enhance the goal of elimination of filarial infections, and the diseases they cause, is a current and relevant goal. The most attractive approach to macrofilaricide discovery is to re-purpose agents already in use in humans. High amongst these potential candidates is flubendazole, a drug that was shown in a screening survey in the early 1980s to be effective against *Onchocerca volvulus* in humans but was difficult to administer due to the properties of the formulation used. The need to reformulate this agent to overcome the issue of insolubility and make it suitable for use as an oral administration was a goal regarded as important for field program use of this anhelminthic in filariasis. We will discuss here results obtained with a new formulation of flubendazole that achieves, after a single oral doses of 2-6 mg/kg, a plasma concentration of between 1-3µg/ml with a peak plasma level at 3-5 hr which decreases to control levels by 12-20hr in rodents. Using the *Litomosoides sigmodontis* model in gerbils, we have achieved >90% killing of adult worms when the animals were assessed 9 weeks after treatment with 6 mg/kg of the oral formulation of flubendazole given daily for 5 days. *In vitro* incubation of *Brugia sp.* with pharmacologically-relevant concentrations of flubendazole caused alterations in the internal organs of the adult worms. Histological observations in both filarial species exposed to flubendazole *in vitro* and *in vivo* showed that several structures in the worms were affected by the drug; the early developing forms in the uterus were the most affected component, and hypodermal cells were also significantly damaged when worms were incubated at concentrations of flubendazole equivalent to the plasma levels obtained after oral dosing. The beneficial effect of treatment with oral flubendazole on host tissue pathology induced by the presence of the parasite was marked when comparing untreated animals to flubendazole-treated animals 9 weeks after dosing. The degree of tissue and organ changes were scored subjectively, and the histopathology occurring examined and scored histologically. The very significant reduction in pathology in animals treated with 6 mg/kg flubendazole suggests that the death of adult worms induced by this drug is not likely to invoke severe adverse events in the host. Our study shows that oral flubendazole can safely kill the majority of adult filariae in this experimental model.

499

ONCHOCERCA LUPI: AN EMERGING ZOONOSIS IN NORTHERN EUROPE AND THE UNITED STATES?

Matthew Belenchia[1], David Adams[2]

[1]AASU, Savannah, GA, United States, [2]AASU/Medical College of Georgia, Savannah, GA, United States

The geographical distribution of cases caused by *Onchocerca lupi,* a common parasitic worm in wolves, has widened during the last decade. Moving from Southern European countries such as Greece and Turkey, an increasing number of cases have begun to emerge in Northern Europe and the Americas. Common arthropod vectors for *O. lupi* include blackflies (e.g., *Simulium yahense* and midges (e.g., *Culicoides* spp.). *O. lupi* commonly causes ocular problems such as conjunctivitis, photophobia, and excessive lacrimation. In recent years, however, clinicians have identified cases that have involved extra-ocular sites such as the spinal canal. As of 2014, more than sixty cases of *O. lupi*, ocular and otherwise, have been identified throughout Europe and the United States. This presentation will examine not only the global epidemiology of *Onchocerca lupi* but potential surveillance measures for this emerging zoonosis.

500

OPTIMIZATION OF THE *ACANTHACHEILONEMA VITEAE* LIFE CYCLE FOR INTENSIVE PRODUCTION

Steven Schaar[1], **Leah Mann**[1], Zachary Williams[1], Katy Anderson[2], Jordan Trentadue[1], Shelly Michalski[1]

[1]University of Wisconsin Oshkosh, Oshkosh, WI, United States, [2]Purdue University, West Lafayette, IN, United States

The filarial nematode *Acanthacheilonema viteae* is of great interest to the filariasis community because it lacks the endosymbiotic *Wolbachia* bacterium found in several filarial parasites of humans. The NIH/NIAID Filariasis Research Reagent Resource Center (FR3) began distributing A. viteae to its users in 2011. The FR3 strain of A. viteae and of its tick vector *Ornithodoros tartakowskyi* were originally acquired from TRS Laboratories and the life cycle is currently being optimized for intensive production to meet increasing demand. Variables targeted in this optimization include 1) the microfilaremia of gerbils used for infecting ticks, 2) the life stage and sex of ticks used for infections, 3) the number of subcutaneous injections used to infect hamsters with third-stage infective larvae (L3), and 4) the medium used for subcutaneous infection of gerbils with L3. By feeding only adult stage ticks on gerbils selected for high microfilaremias we increased our yields from 13 L3/tick (n=497) to 126 L3/tick (n=107 ticks). Similarly, the average recovery of adult A. viteae from hamsters has risen from 51 ± 45 (n=13) to 90 ± 48 (n=22), partially due to the finding that worms isolated in Hanks' Balanced Salt Solution (HBSS) produce patent infections whereas those isolated in RPMI 1640 do not. Methods to infect ticks with *A. viteae* via artificial membrane feeding and inoculation are currently being developed, and a recent trial resulted in recovery of 208 L3s/tick inoculated by enema with microfilaremic gerbil blood (n=4 ticks). The increased recovery of *A. viteae* from host animals has allowed the FR3 to meet higher demand from the filariasis research community for all life stages of the worm.

501

ACTIVATING AUTOPHAGY AS A NOVEL ANTI-*WOLBACHIA* MODE OF ACTION FOR MACROFILARICIDAL DRUG DISCOVERY

Amber Fanthome, Denis Voronin, Mark J. Taylor

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

River blindness (*Onchocerca volvulus*) and elephantiasis (*Wuchereria bancrofti* and *Brugia malayi*) affect over 150 million people in more than 80 countries, with a further 1 billion at risk of infection. Each of these nematode parasites has evolved a mutualistic association with the bacterial endosymbiont *Wolbachia*. Depletion of *Wolbachia* with the antibiotic doxycycline arrests development, fertility and viability and delivers potent macrofilaricidal efficacy in clinical trials. In order to identify alternative anti-*Wolbachia* drugs with a more rapid activity we have exploited the host nematodes immune regulation of *Wolbachia* populations through autophagy to discover drugs with a wolbachiacidal mode of action. We have screened libraries of 100 autophagy inducing drugs and compounds against *B. malayi*. Selected 'hits' are then screened against transgenic *C. elegans* and human embryonic kidney (HEK) cells expressing a fluorescent autophagy marker, ATG8 to identify drugs, which are selectively more potent against nematode versus human autophagy activation. Hits ranked by relative nematode potency are progressed through the A·WOL drug discovery and development screening pipeline to identify pre-clinical lead candidates and optimized combinations of anti-*Wolbachia* drugs to reduce treatment timeframes.

502

PHARMACOKINETIC/PHARMACODYNAMIC MODELLING OF ANTI- *WOLBACHIA* CHEMOTHERAPEUTIC AGENTS IN A LYMPHATIC FILARIASIS MURINE INFECTION MODEL

Raman Sharma, Ghaith Aljayoussi, Ana de Castro Guimaraes, Jill Davies, Lousie Ford, Alison Shone, Joseph Turner, David Waterhouse, Stephen A. Ward, Mark Taylor

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

An estimated 120 million people are infected by lymphatic filariasis throughout the tropics leading to a profound public health and socio-economic burden in severely affected communities. *Wolbachia* is an essential endosymbiont of the filarial nematodes *Wuchereria bancrofti, Brugia malayi* the causative agents of lymphatic filariasis. Doxycycline is currently the gold standard for the targeting of *Wolbachia* in lymphatic filariasis chemotherapy. However, the current drug regimen is a 100-200 mg/day doxycycline dose given for 4 to 6 weeks to patients. The A·WOL consortium plan to reduce the current treatment time to 7 days or less to improve drug regimen adherence and to reduce drug resistance and costs of treatment. To achieve a rapid 7-day or less kill rate of *Wolbachia*, a number of drug combinations will be employed. These include different tetracyclines (Doxycycline and minocycline) rifamycins (Rifampicin or Rifapentine), Moxifloxacin as well as anti-helminthic drugs. The complexity of multiple drug combinations necessitates a rational approach in the identification and choice of the best treatments in *in-vivo* models and translating the animal treatments in the lab into clinical trials on the field. We have done series of PK-PD models and simulations using parameter and non-parameteric population PK-PD modelling software programs to further dissect and quantify the dynamics of anti-bacterial activity of these drugs in the treatment of Lymphatic Filariasis and Onchocerciasis. As an example here, we identified the PK parameters of doxycycline, minocycline and rifampicin in *in-vivo* PK studies in the SCID *Brugia malayi* model and used the PK data along with pharmacodynamic output to interpret the PK-PD relationships in light of the effect of each drug upon *Wolbachia* viability in parasites. The data display the power of PK-PD modelling in quantifying the PK-PD relationships of different drugs whilst giving insight to predictions of drug dynamics and interaction with *Wolbachia* viability.

503

DEVELOPMENT OF A SMALL ANIMAL MODEL OF ONCHOCERCIASIS FOR THE SCREENING OF MACROFILARICIDAL DRUGS

Alice Halliday[1], Haelly Mejane Metuge[2], Winston Patrick Chounna Ndongmo[2], Jonas Arnauld Kengne-Ouafo[2], Tayong Dizzle Bita Kwenti[2], Ana Guimaraes[1], Hayley Tyrer[1], Peter Enyong[2], Mark Taylor[1], Samuel Wanji[2], Joseph Turner[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Research Foundation for Tropical Diseases and the Environment, Buea, Cameroon

Onchocerciasis affects >37 million people with approximately 800,000 suffering visual impairment (river blindness). Development of macrofilaricides for onchocerciasis is hampered by the lack of a facile animal model. Our laboratories and others have demonstrated protracted survival of non-murine filariae in immune-compromised mice. Therefore we tested whether cattle-sourced *Onchocerca* could persist in Severe-Combined Immuno Deficient (SCID) mice. *O. ochengi* nodules were harvested from the skins of infected cattle while infective L3 were produced from blackflies fed on cattle in Cameroon. Motile male *O. ochengi* were isolated following culture of disrupted onchocercomas, prior to surgical implantation into the peritoneal cavity of SCID mice. Viable macrofilariae were recovered in 100% of recipients assessed between 1-5 weeks post implant (mean survival=49.22% ±5.08, n=22). Therefore, the implant model was assessed for suitability to test for efficacy of direct anti-nematodicidal drugs using the standard macrofilaricide, flubendazole suspension (FBZ). FBZ or vehicle control (VC) was administered parenterally (10mg/kg sc, +5 days) into groups of SCID recipients (n=7-8) of 15 male *O. ochengi*. FBZ induced an almost total macrofilaricidal response assessed 4-5 weeks after implant (mean survival FBZ=1.67%±1.09 vs VC=43.81% ±11.44, p=0.0089). *O. ochengi* L3 larvae were also inoculated into SCID mice. However, only 1 viable larva could be recovered after 7-14 days following sc or ip injection of between 40-100 L3 (n=26). In conclusion, we have developed a small animal model of onchocerciasis using adult *O. ochengi* parasites that is sufficiently robust to commence screening of macrofilaricidal candidates. We are currently validating this model for additional screening of anti-*Wolbachia* candidates. Further, we have commenced development of a murine onchocercoma xenograft model to evaluate whether male and female macrofilariae and released microfilariae can survive in SCID recipients for periods necessary to further validate efficacy of macrofilaricidal drugs.

504

AN INTERACTIVE WEB BASED COMPUTER SIMULATION FOR THE PREDICTION OF MASS DRUG ADMINISTRATION OUTCOMES IN ONCHOCERCIASIS CONTROL AND TREATMENT

Ghaith Aljayyoussi, Louise Ford, Raman Sharma, Joseph Turner, Mark Taylor, Stephen Ward

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Here we show for the first time a user friendly, interactive, internet based computer simulation that will help in the prediction of mass drug administration outcomes in onchocerciasis treatment and control. The model is being developed within the A·WOL consortium, to predict the impact of anti- *Wolbachia* drugs currently being advanced. The simulation program focuses on how different treatments, such as microfilaricidal, macrofilaricidal or combinations, would reflect upon the overall outcome of treatment regimes in a specific endemic area. We utilise dynamic models using to form an interactive platform where the user can experiment with different scenarios with complete control over parameters such as degree of endemicity, drug modes of action, drug pharmacokinetic profiles, vector biting rates and the application of vector control and drug resistance. The model also puts into consideration population variations in response to the drug which aids in predicting worst case scenarios and developing alternative plans. It can also be used to simulate the impact of applying combination therapies, drugs with different pharmacokinetics parameters and different potencies and modes of action. As an example, we show a case study where the model predicts the treatment outcomes in endemic areas when using ivermectin alone or a combination of ivermectin and doxycycline. The modelling shows that the use of a combination of ivermectin and doxycycline treatment vastly increases the chances of elimination success. The use of a macrofilaricidal drug such as doxycycline shows a much higher potential in achieving overall elimination than microfilaricidal drugs (such as ivermectin) alone which agrees with previous computer models

505

LASER DIFFRACTION ASSAY FOR LOW RESOURCE QUANTIFICATION OF MICROFILARIAL BURDEN

Philip J. Budge[1], David W. Wright[2]

[1]Washington University School of Medicine, St. Louis, MO, United States, [2]Vanderbilt University, Nashville, TN, United States

Efforts to eliminate lymphatic filariasis and to control onchocerciasis by mass drug administration (MDA) in West and Central Africa have been hampered by the risk of severe adverse reactions among persons harboring high-level loiasis infections (>8,000 microfilariae per mL of blood). A test-and-NOT-treat strategy has been proposed for excluding persons with high-level loiasis from MDA. This will require low-resource, real-time, point-of-care diagnostics that can be used at the time of MDA to identify persons with high-level loiasis and exclude them from treatment. Using *Caenorhabditis elegans* L1 larvae as surrogates for microfilariae, we have developed a low-resource laser diffraction assay for microfilarial quantification. The prototype apparatus is composed of a low-power red laser (similar to a laser pointer) directed through a sample chamber (cuvette, flow cell, or capillary tube) towards a photodiode connected to a laptop-powered oscilloscope. Preliminary studies show the assay is capable of quantifying worm concentrations between 500 and 50,000 L1 larvae/mL. Assay sensitivity is dependent on depth of the sample chamber: a 1 cm chamber depth gives maximum sensitivity while a 0.05 cm depth gives lower sensitivity but greater precision. Cellular blood components interfere with the diffraction assay, and low-resource methods to separate larvae from blood components prior to quantification are being developed. These preliminary data suggest that a portable, battery-powered, low cost laser-diffraction device may be capable of rapid, point-of-care quantification of microfilarial burden in the setting of MDA.

506

FIELD EVALUATION OF STANDARD DIAGNOSTICS' ONCHOCERCIASIS IGG4 RAPID DIAGNOSTIC TEST PROTOTYPES

Dunia Faulx[1], Allison Golden[1], Melissa Valdez[1], Roger Peck[1], Eric Stevens[1], Lindsay Yokobe[1], Pooja Bansil[1], Potochoziou K. Karabou[2], Meba Banla[3], Kangni Adade[2], Richard Gbandi Gantin[3], Peter Soboslay[4], Thomas B. Nutman[5], Gonzalo J. Domingo[1], Tala de los Santos[1]

[1]PATH, Seattle, WA, United States, [2]National Onchocerciasis Control Programme, Lome, Togo, [3]Onchocerciasis Research Laboratories, Sokode, Togo, [4]University of Tubingen, Tubingen, Germany, [5]National Institute of Health, Bethesda, MD, United States

The progress that has been made toward the elimination of onchocerciasis, or river blindness, in the Americas and Africa has been the culmination of decades of commendable dedication to controlling this disease. Over the past few years, the use of annual or semi-annual community-based ivermectin distribution through mass drug administration (MDA) programs has moved the goal from control to elimination. As elimination goals are being reached, appropriate diagnostic tools are needed to identify exposure to Onchocerca volvulus (Ov) for purposes of identifying possible recrudescence. A rapid diagnostic test (RDT) that detects IgG4 antibodies

specific to the antigen Ov16 has been developed and will be manufactured by Standard Diagnostics, Inc. Prior to commercial availability, the test has been evaluated in the PATH laboratory and in rural settings in Togo with 1,500 participants during routine Ov epidemiological surveillance activities. In the laboratory setting, our data indicate that the RDT has a sensitivity of 89% and a specificity of 99%, when compared to an Ov16-specific ELISA. The read window, an important indicator of test result stability, is highly consistent over a 24-hour time period. We will also present the performance of the RDT relative to the Ov16 ELISA and sensitivity as compared to skin-snip derived microfilaria status when tested in real-world settings in Togo that include factors such as exposure to high temperature and humidity. Finally, we describe possible facilitators and barriers for integration of the test into routine surveillance practices. Our data verify the performance of the test in field settings prior to a commercial launch of the Ov16-based RDT and informs how it should be used in current onchocerciasis control and elimination efforts.

507

SURVIVOR COPYCAT BEHAVIORS AND AUTOCHTHONOUS HELMINTHIASES IN THE U.S.

James H. Diaz

Louisiana State University Health Sciences Center, New Orleans, LA, United States

Popular *Survivor* TV programs glamorize rugged outdoorsmen surviving wilderness adventures by seeking natural sustenance. To identify risk factors for autochthonous helminthiases resulting from *Survivor* copycat behaviors, Internet searches identified 54 cases of helminthiases transmitted by the raw consumption of animals recommended as safe, natural foods by *Survivor* series programming during the period, 1984-2013. Cases were defined by positive microscopic, serologic, or molecular diagnostics. Continuous variables were analyzed for significant differences by unpaired t-tests; categorical variables were analyzed by chi squares. Statistical significance was indicated by p-values ≤0.05. 38 autochthonous cases of neuroangiostrongyloidiasis (NAS) with eosinophilic meningoencephalitis and 16 cases of autochthonous paragonimiasis (PG) with hemorrhagic pneumonitis were reported following consumption of raw intermediate hosts infected by causative parasites, the rat lungworm (*Angiostrongylus cantonensis*) and the American lung fluke (*Paragonimus kellicotti*) respectively. The mean age of NAS cases was 21.5 years; most cases were in males (P = 0.039) from Hawaii (P = 0.039), 37% of whom reported consuming raw snails or frogs or eating unwashed greens harboring infective larval snails (P = 0.003). The mean age of the PG patients was 27.3 years; most cases (93%) were reported in males (P < 0.0001) from Missouri, most of whom (67%) had consumed raw crayfish (P < 0.0001), often while intoxicated (47%) on camping, paddling, or floating trips within the Mississippi River Drainage Basin (73%, P = 0.028). There was one death in a 71-year-old male with PG. The most significant risk factors for autochthonous helminthiases from *Survivor* copycat behaviors included male gender; and consumption of raw, wild animals in parasite-endemic regions, especially while intoxicated outdoors. Recommended preventive-behavior interventions included proper food preparation of self-harvested wild animals, wilderness survival training, and alcohol avoidance during outdoor recreation.

508

EPIDEMIOLOGY OF SOIL TRANSMITTED HELMINTHS AND *PLASMODIUM FALCIPARUM* AMONG SCHOOL CHILDREN IN BUMULA DISTRICT, WESTERN KENYA

Stella Kepha[1], Birgit Nikolay[2], Rachel L. Pullan[2], Charles S. Mwandawiro[3], Sammy M. Njenga[3], Fred Nuwaha[1], Simon J. Brooker[2]

[1]Makerere University, Kampala, Uganda, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Kenya Medical Research Institute, Nairobi, Kenya

Soil-transmitted helminthiasis and malaria continue to be important health problems among Kenyan school children, many of whom are at risk of coinfection. It has been suggested that the immune response evoked by helminth infections may modify immune responses to plasmodia species and consequently alter risk of infection; however previous studies have been inconclusive. As part of baseline activities of a current trial investigating deworming impact on risk of clinical malaria among school children in Bumula District in western Kenya (ClinicalTrials.gov: NCT01658774), a cross-sectional survey was conducted to investigate the prevalence, intensity and geographic distribution of soil-transmitted helminth (STH) and *Plasmodium falciparum* infections among 4,842 school aged children in purposively selected 22 primary schools. Single stool samples were collected and examined in duplicate for STH (hookworm, *Ascaris lumbridoides* and *Trichuris trichiura*) using the Kato Katz method. A finger-prick blood sample was taken and examined for *P. falciparum* by expert microscopy. Overall, 23.4% of the children were infected with *P. falciparum* and 26.5% with one or more STH species, with hookworm (16.4%) and *A. lumbricoides* (14.3%). The prevalence of STH and *P. falciparum* infections varied significantly by age group and sex and by geographical location. After adjusting for age, sex and clustering within schools, the risk of *P. falciparum* infection was higher among children with any STH infection (odds ratios= 1.2, 95% confidence intervals [CI] (1-1.4), p=0.03), although there was no significant association between *P. falciparum* and individual STH species. Interestingly however, *P. falciparum* infection was positively associated with medium to high intensity *A. lumbricoides* infections (odds ratios= 1.5, 95% CI (1.1-2.1), p=0.006). Coinfection with *P. falciparum* and STH is common among school children in western Kenya, supporting the need for integrated helminth and malaria control. There is also a need to investigate the consequences of deworming on the risk of malaria.

509

PHYTOCHEMICAL, EFFICACY AND SAFETY PROFILES OF *EMBELIA SCHIMPERI* VATKE

Mesfin Tefera[1], Dawit Abebe[1], Asfaw Debella[1], Walelingn Mekonennen[1], Ibrahim Naser[2], Enadale Seifu[3], Asrat Dawit[4], Berhanu Tesfaye[1], Samuel W/Kidan[1]

[1]Ethiopian Public Health Institute, Addis Ababa, Ethiopia, [2]National Taiwan University of Science and Technology, Taipei, Taiwan, [3]Addis Ababa University, School of Pharmacy, Addis Ababa, Ethiopia, [4]Addis Ababa University, Medical Faculty, Addis Ababa, Ethiopia

Embelia schimperi fruits are among the most common traditionally used agents to teat parasitic / helminthes infections in Ethiopia. Despite the plants widespread use, scientific evidence on constituents responsible for the biological activity, efficacy and safety is not well documented. A 96 well micro titer pale assay technique was used to evaluate the *in vitro* anthelmintic properties of crude hydro alcoholic extract and solvent fractions of *E. schimperi* fruits against hookworm and strongyloides spp. The *in vivo* anthelmintic activity of the extract was determined in albino mice experimentally infested with *Hymenolopis nana* worms. The percent killing activity of the crude extract was from 92-100% when tested at a concentration of range of 50-400mg/ml against hook worm and *Strongylodes* spp. The percent motility inhibition against hookworm larvae was from 66.6-86.6% when fractions were tested at a concentration of

50mg/ml. 100% adult parasite clearance was observed from the mice intestine when the crude extract was administered at a single oral dose of 1kg/kg. Phytochemical screening indicated the presence of steroids, polyphenols, quinone and alkaloids. Preliminary sub chronic toxicity study was also conducted to make gross observation on measurements of change in body weight, hematological, biochemical parameters and histopathology of kidney and liver of albino mice. The toxicity study indicated that the crude extract of *E. schimperi* fruit has wide safety margin in all tested parameters and the plant may be relatively safe as an oral anthelmintic medication if further investigations is carried out. This study also indicates the presence of active principles in *E. schimperi* fruits with anthelmintic activity that may support the usage of preparations from the plant by local people to treat parasite helminthes infection. In this study average worm expulsion tine and possible mechanism of action of the extract is also suggested. However, further research is required to study long term toxicity, effect on beneficial micro biota, mechanism of action, identification of marker compounds and effectiveness of the plat *in vivo* / human model/ and clinical trails before it can be recommended for human use.

510

THE EFFICACY OF ALBENDAZOLE AND LEVAMISOLE DRUG COMBINATION IN INDIVIDUALS WITH REDUCED EFFICACY FOR SINGLE-DOSE ALBENDAZOLE TREATMENT AGAINST HOOKWORM INFECTIONS

Buhari B. Hamidu[1], Edward J. Tettevi[2], John A. Larbi[1], Mike Y. Osei-Atweneboana[2]

[1]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]Council for Scientific and Industrial Research - Water Research Institute, Accra, Ghana

The Soil-Transmitted Helminth (STH) infections control programme has been on-going in Ghana since 2000 with mass drug administration (MDA) of albendazole (ALB) to school children. However, our research revealed, 100% cure rate with albendazole-levamisole (ALB-LEV) combination in hookworm population responding sub-optimally to ALB treatment in four endemic communities in the Northern region of Ghana. This was an eighteen months longitudinal study. A total of 421 school children between the ages of 2-17 years were randomly selected from four endemic communities in Kpandae district of the Northern region of Ghana. One hundred and three (103) patients positive for hookworm were categorised into four groups: Control, ALB only, LEV only and LEV - ALB drug combinations with 26 patients in each group. Coprological assessment for parasites was based on the Kato-Katz technique. Parasites recorded were hookworm (*Ancylostoma doudenale* and *Necator americanus*), *Trichuris trichiura, Hymenolepis nana*, and *Taenia* sp. in the communities selected. Overall, the highest cure rate after 21 days of treatment was recorded for LEV-ALB treatment (100%) followed by LEV alone (92.31%) and ALB alone (88.46) whiles the Control was zero. Also, faecal egg count reduction rate was 100% (LEV-ALB), 96.15% (LEV alone), 91.84 (ALB alone) and 9% for Control.Cumulative re-infection rates were 12, 32 and 48% and 23, 50 and 69% respectively for LEV-ALB, LEV alone and ALB alone at 6 months and one year after treatments. Hookworm still remains a significant public health burden in Africa. The result shows a significant efficacy for LEV - ALB combination in hookworm population with reduced ALB treatment (100% FEC) after 21days treatment and therefore can be used to augment ALB for clearing possible resistant parasites of hookworm infections. All parasitic samples are currently being analysed to identify polymorphism related to benzimidazole resistance in hookworms.

511

MOLECULAR DIAGNOSIS OF SOIL-TRANSMITTED HELMINTH INFECTIONS USING A NOVEL ISOTHERMAL AMPLIFICATION METHOD

Nour Rashwan, Catherine Bourguinat, Roger Prichard

McGill University, Montreal, QC, Canada

Ascariasis, Trichuriasis, and Hookworms are the three main soil-transmitted helminth infections, causing human morbidity in tropical areas of the world. Diagnosis of human soil-transmitted helminths (STHs) relies on microscopy to identify eggs in the feces. The precise detection of STHs by microscopy requires training and is labor intensive and can lead to false negative results. As a result of the limitations of microscopic methods, it was of interest to develop new methods to identify different STH species. Accurate diagnostic is not only essential for epidemiological studies but is vital for monitoring the possible emergence of drug resistance and for the development of new anthelmintics since the efficacy of different drugs shows variation among the species. For a variety of infections, studies have shown that molecular diagnostic tools can be more reliable and sensitive. Therefore, development of a simple, rapid and sensitive molecular tool for STH species diagnosis is desirable. We developed novel diagnostic assays based on the Smart Amplification method (SmartAmp) to detect STH infections under isothermal condition. This isothermal method uses asymmetric primer design to prevent non specific amplification. Control plasmids were employed to develop and optimize the assays. The assays were applied to analyze fecal samples using species-specific primer sets. Real-time PCR monitoring of the amplification was achieved within 20-40 min with complete suppression of the background amplification. SMartAmp assays were developed for diagnosis of STH infections and the reliability of the method was validated using the conventional PCR method.

512

IDENTIFICATION AND CHARACTERIZATION OF THE IMMUNOMODULATORY MOLECULE(S) IN EXCRETORY-SECRETORY (ES) PRODUCTS DERIVED FROM THE GASTROINTESTINAL NEMATODE *HELIGMOSOMOIDES POLYGYRUS*

Rajesh M. Valanparambil[1], Mifong Tam[1], Yovany Moreno[2], Armando Jardim[2], Timothy G. Geary[2], Mary M. Stevenson[1]

[1]The Research Institute of McGill University Health Centre, Montreal, QC, Canada, [2]Institute of Parasitology, McGill University, Montreal, QC, Canada

Heligmosomoides polygyrus (Hp) is a murine gastrointestinal (GI) nematode used as a model of helminth parasites that infect humans and livestock. We previously showed that Hp-derived excretory-secretory products (HES) potently suppress effector $CD4^+$ Th cell responses to an unrelated antigen by modulating the antigen presenting function of dendritic cells (DC) to induce an anti-inflammatory network involving regulatory T cells. Interrogation of mass spectrometry (MS) data from 1D-SDS PAGE and LC-MS/MS analysis of HES with transcriptomic information indicated that HES contains over 200 proteins, including several with known immunomodulatory effects. To better define the immunomodulatory molecules(s) in HES, we used biochemical approaches and assessed the suppressive activity of the separated fractions by measuring IL-12p70 secretion by bone marrow-derived DC (BMDC) pre-treated with HES fractions prior to stimulation with the TLR9 ligand CpG-ODN. First, we performed size exclusion chromatography to separate HES proteins based on their molecular weight and identified 12 fractions that suppressed DC secretion of IL-12p70 in response to CpG-ODN. The suppressive fractions were pooled and subjected to further separation by anion-exchange chromatography followed by testing on CpG-ODN-stimulated BMDC. We identified 3 fractions that suppressed IL-12p70 secretion. Analysis of these fractions by LC-MS/MS identified 21 candidate proteins. To further narrow down the immunomodulatory candidate(s), HES was resolved by cation-exchange chromatography. Only one fraction

was identified to have suppressive activity in the BMDC assay; LC-MS/MS analysis is underway to identify the proteins in this fraction. Our goal is to clone and purify the candidate immunosuppressive protein(s) and to test the ability of recombinant protein to modulate DC polarization of effector $CD4^+$ T cell responses *in vitro* and *in vivo*. Together, our findings provide novel information on biochemical and immunomodulatory aspects of GI nematode-derived ES products.

513

DEXAMETHASONE DOWNREGULATED THE EXPRESSION OF MATRIX METALLOPROTEINASE 2, 9 AND TH2 CYTOKINES IN MICE WITH EOSINOPHILIC MENINGITIS CAUSED BY *ANGIOSTRONGYLUS CANTONENSIS* INFECTION

Hung-Chin Tsai, Yu-Shin Chen, I-Tzu Chen, Pei-Yun Chou, Susan Shin-Jung Lee, Yao-Shen Chen

Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan

Angiostrongylus cantonensis, also known as the rat lungworm, is the major cause of eosinophilic meningitis in the Pacific Islands and Southeast Asia. Rats serve as the definitive host of the nematode. Humans are infected incidentally and lead to eosinophilic meningitis. Previous BALB/c animal study had demonstrated increased matrix metalloproteinase 9 (MMP-9) expressions and blood brain barrier damage in mice infected with *A. cantonensis*. Steroids have been shown to be one of the effective treatment options for eosinophilic meningitis caused by *A. cantonensis* infection. However, the mechanism of how steroids can influence on eosinophilic meningitis are still unclear. We hypothesize that the beneficial effect of steroid on eosinophilic meningitis are mediated by decrease MMP and cytokines expressions. In a BALB/c mice model, mice were orally infected with 40 *A. cantonensis* L3 via an orogastric tube and were sacrificed every week for 3 consecutive weeks after infection until the end of the study. Dexamethasone was injected via the intra-peritoneal routine from 7th days of infection until the end of the study. Evans blue method was used to measure the blood brain barrier changes and the serum/CSF and brain homogenates expression of MMP-2, 9 and cytokines were analyzed by gelatin zymography, western blot, ELISA and reverse transcriptase polymerase chain reaction (RT-PCR) respectively. There were an increased MMP-2 and MMP-9 expressions in CSF and brain homogenates by western blot and gelatin zymography following 2-3 weeks of infection. Dexamethasone administration could down-regulate the expression of MMPs. These changes were parallel to the blood brain barrier disruption as evidence by the Evans blue extravasation following infections. Furthermore, the brain homogenates cytokines , such as IL-5, IL-10 and INF-γwere also elevated following 2-3 weeks of infection. Dexamethasone could decrease the expression of IL-5, IL-6, IL-10 and TNF-αby using ELISA. The brain homogenates mRNA expressions of IL-5, IL-6, INF-γ and TNF-α were decreased gradually by RT-PCR. All of these findings suggested that the Th2 cytokines play an important role in mice with eosinophilic meningitis caused by *A. cantonensis* infection and provided the evidences supporting steroid effects on *A. cantonensis* infection by inhibiting MMP-2, 9 and Th2 inflammatory cytokines expressions.

514

THE IMPACT OF COMMUNITY DEWORMING AGAINST SOIL TRANSMITTED HELMINTHIASES ON PREVALENCE, WORM BURDEN AND MORBIDITY IN A HIGH PREVALENCE AREA OF ARGENTINEAN GRAN CHACO

Adriana Echazu[1], Paola Vargas[1], Marisa Juarez[1], Pamela Cajal[1], Ruben Cimino[1], Diego Cruz[2], Roxana Ruiz[2], Viviana Heredia[3], Gladys Paredes[3], Luis M. Arias[4], Alejandro J. Krolewiecki[5]

[1]Instituto de Investigaciones en Enfermedades Tropicales - Universidad Nacional de Salta, Salta, Argentina, [2]Fundación Mundo Sano, Buenos Aires, Argentina, [3]Hospital J.D. Perón, Tartagal, Salta, Argentina, [4]Ministerio de Salud Pública de la Provincia de Salta, Salta, Argentina, [5]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

Soil-transmitted helminthiases (STH) are the most prevalent infections of human-kind, relevant to public health due its morbidity and role in poverty perpetuation. Tartagal, Gran-Chaco, Argentina is a high prevalence area where no preventive anthelminthic chemotherapy was recently applied. This study, conducted in Tartagal since March 2012, investigates the results of community-based mass drug administration (MDA) and lasting until 2015. Population: ~3600 individuals. Surveillance of a statistically representative random group, with fresh stool sample analysis, complete blood count and detection of IgG against S.stercoralis with NIE-ELISA. Treatment intervention: single dose albendazole + ivermectin. Survey: n=157 stool; n=149 blood for baseline and n=103 stool; n=152 blood 6 months later. Baseline prevalences for any STH at baseline and follow-up were 55 and 16% respectively; and for hookworm (HKW) 50 and 13% respectively ($p<0.05$). HKWs main species was A.duodenale (85%). Baseline S.stercoralis prevalence was 13% and at follow-up 8% ($p=0.22$). T.trichiura and A.lumbricoides were rarely found. Worm burden revealed 3 heavy HKW infections at baseline and none at follow up. At baseline 56% of the individuals were anemic, mean Hgb value 11 gr/dL (SD=1.6; minimum 4.9) which improved to 10% anemic (all young women), mean Hgb value 13 gr/dL (SD=1.5; minimum 9) at follow up ($p<0.05$). HKW infection was significantly associated with anemia ($p=0.001$), risk ratio (RR)=1.54 (CI 95% 1.03-2.30) at baseline, however at follow up it was no longer significant ($p=0.58$). 75% had eosinophilia at baseline and 43% after MDA ($p<0.05$). No significant difference in S.stercoralis seroprevalence was found between baseline (51%) and follow up (44%) ($p=0.25$). MDA coverage: 80%. One round of community-based MDA was effective in reducing the prevalences of STH infection, anemia and eosinophilia and in decreasing anemia severity and HKW infection burden. Individuals who remained anemic after MDA were women of reproductive age, with other probable causes of anemia.

515

PLANT NATURAL PRODUCTS SHOW *EX VIVO* ACTIVITY AGAINST ADULT HOOKWORM

Blaise Dondji[1], Kaitlin L. Koppinger[1], Averyl Shindruk[1], Brianda Cardenas-Vegas[1], Jocelyn McCornack[1], Susmita Unni[1], Gil Belofsky[2], William Ray[2], Eric Winterstein[2]

[1]Laboratory of Cellular Immunology and Parasitology, Department of Biological Sciences, Central Washington University, Ellensburg, WA, United States, [2]Department of Chemistry, Central Washington University, Ellensburg, WA, United States

Hookworms, blood feeding intestinal parasites, remain a major health burden with approximately a billion people infected in tropical and subtropical regions of the world. In these geographical areas, they are a major contributor to iron-deficiency anemia, weight loss, stunted growth and malnutrition. Control strategies have relied on mass treatment with benzimidazole drugs. However, recent reports have described the resistance of hookworms and other soil-transmitted nematodes to these drugs. Considering that in several endemic areas, local populations use plant products to treat several ailments including parasitic diseases, we

tested compounds from plants for their anthelminthic activity against the adult stage of the hookworm, *Ancylostoma ceylanicum*. The present study screened extracts from five plant species and chromatographically-enriched fractions of the most active one. These plants were collected from the western United States. Extracts from two of the plants namely *Dalea ornata* and *Oemlaria cerasiformis* showed anthelminthic activity (mortality and/or reduced motility) of their crude extracts and enriched fractions against *A. ceylanicum*. Associated worm mortality rates ranged from 25% at 24 hours to 100% at 120 hours, after incubating worms with the test compounds. Three concentrations of the compounds were tested (100, 50, 10 mg/mL). Our study revealed a dose-dependent activity where the lowest concentration (10 mg/mL) achieved 100% mortality 120 hours post exposure while the same activity level was obtained at 48 hours with 100 mg/mL. Our data show that plants represent a relatively untapped source of potentially effective anthelminthic molecules. Studies are underway with the aim of purifying and testing active components of the extracts *ex vivo* and *in vivo*. The anthelminthic activity of these compounds in the animal model of the disease will be evaluated using clinical, parasitological and immunological parameters such as weight gain, anemia, egg output, worm burden, immune cell proliferation indices, and immune cell population types and sizes by flow cytometry.

516

CYTOKINE EXPRESSION PROFILES AND IMMUNITY TO MEASLES VACCINATION IN KENYAN CHILDREN WITH HELMINTH INFECTION

Joshua R. Lacsina[1], Jacqueline M. Naulikha[2], Patricia Pavlinac[1], Doreen Rwigi[2], Alfred Odiwuor[2], Frankline M. Onchiri[1], Loice W. Mbogo[2], Benson O. Singa[2], Judd L. Walson[1]

[1]University of Washington, Seattle, WA, United States, [2]Kenya Medical Research Institute, Nairobi, Kenya

Helminths are among the most prevalent infections in resource-limited countries, with an estimated one billion school-age children worldwide living in areas at high risk for helminth transmission. Children are particularly susceptible to high-burden infections, which can result in derangements in host cytokine expression. These derangements, in turn, lead to both immunosuppression and pathologic inflammation in the host. The disruption of cytokine signaling by helminths has been proposed to impair the ability of children to mount protective immune responses to vaccination. We are currently enrolling children in a cross-sectional study to test the hypothesis that helminth infection is associated with a modified Th2 cytokine response, which is permissive for chronic helminth infection and reflected by an elevated IgG4/IgE ratio. Furthermore, we will explicitly test the prediction that helminth infection is associated with impaired vaccine-induced immune responses, specifically for measles vaccination. This study is nested within a large pediatric surveillance cohort at three clinical sites in Western Kenya. Plasma and stool samples are collected at the time of enrollment from eligible children aged 1 through 5 years with documented measles vaccination. Shortly after collection, stool samples are tested by Kato-Katz and formol-ether concentration methods to diagnose helminth infection and quantify parasite burden. Plasma from children with and without helminth infection will be analyzed by ELISA to compare serum levels of Th1 and Th2 cytokines, as well as titers of IgE, IgG4, and anti-measles antibodies. Preliminary enrollment demonstrates a helminth infection prevalence of approximately 25% in our Western Kenya cohort. Improving our understanding of helminth-mediated immune dysfunction will facilitate the rational design of both anti-helminth vaccines and novel adjuvants to enhance vaccine efficacy in helminth-endemic areas.

517

TOXOCARIASIS IN NORTH AMERICA: A SYSTEMATIC REVIEW

Rachel M. Lee[1], Laura B. Moore[2], Maria Elena Bottazzi[3], Peter J. Hotez[3]

[1]Baylor College of Medicine, Houston, TX, United States, [2]James A. Baker Institute of Public Policy, Rice University, Houston, TX, United States, [3]National School of Tropical Medicine, Baylor College of Medicine, Houston, TX, United States

Toxocariasis is an important neglected tropical disease that can manifest as visceral or ocular larva migrans, or covert toxocariasis. All three forms pose a significant public health problem in areas of high prevalence. To determine the burden of toxocariasis in North America we conducted a systematic review of the literature following PRISMA guidelines. We found 18 articles with original prevalence, incidence, or case data for toxocariasis. Of these articles, 5 reported data for Canada, 8 reported data for the United States, and 5 reported data for Mexico. One article reported only prevalence data, no articles reported incidence data, 5 articles reported only affected cases, and 12 articles reported both prevalence data and number of affected cases. 63% of articles with seroprevalence estimates for the United States tested blood samples collected during the 1988-1994 National Health and Nutrition Examination Survey. The most commonly cited risk factors for toxocariasis included male sex, pet ownership, particularly if the animals are allowed to live outdoors and eat other animals or otherwise consume unconventional pet food, African American race, age less than 18, low level of education, poverty, foreign birth, living in the southern United States, and playing at parks or in sandboxes where dogs and cats have defecated. Of the studies that reported prevalence data by sex 73% found a statistically significant greater prevalence in males compared to females, while 27% of studies found no significant differences between genders. No studies found females to have significantly higher prevalence of Toxocara infection than males. Further research is needed to determine the true current burden of toxocariasis in North America, however the prevalence estimates gathered in this review suggest that the burden of disease is significant.

518

THE ROLE OF SOIL-TRANSMITTED HELMINTH INTENSITY AND CO-INFECTION IN THE SPATIOTEMPORAL VARIATION OF CHILDHOOD ANEMIA: RESULTS OF A 5-YEAR MDA PROGRAM IN BURUNDI

Mohamad Assoum[1], Giuseppina Ortu[2], Maria Gloria Basanez[2], Archie Clements[3], Ricardo Soares Magalhaes[1]

[1]The University of Queensland, Herston, Australia, [2]Imperial College London, Norfolk Place, United Kingdom, [3]Australian National University, Canberra, Australia

We investigated the role of *Ascaris lumbricoides, Trichuris trichiura* and hookworm infection and coinfections in the anaemia burden in school age children as a result of a 5-year mass drug administration (MDA) program in Burundi. The aim of the study was to investigate for the first time the impact of the MDA program on the spatiotemporal variation in anaemia burden in the country. Longitudinal parasitological data was collected from 40,553 children from 2007 to 2011 in 31 schools in Burundi. These data included faecal egg counts for *A. lumbricoides, T. trichiura* and hookworm, coinfections with these parasites and blood iron levels, age, sex, weight and height. Locational data included the GPS coordinates of the schools, annual mean land surface temperature, NDVI, precipitation and the distance to perennial water bodies. Spatiotemporal Bayesian geostatistical models of anaemia were built adjusting for age, sex, helminth intensity and co-infections, malnutrition and physical environment factors associated with the geographical location of the school. Our results indicate that the geographical distribution of the prevalence of anaemia did not change significantly during the 5-years. Our results indicate an association between the spatiotemporal variation in anaemia burden in children in Burundi and STH intensity and co-infections and environmental

conditions. Our study demonstrates the important role of malnutrition in anaemia burden over the five year period. The findings of this study suggest that MDA has played an important role in the control of anaemia in school age-children in Burundi. The results also suggest the need to integrate nutritional interventions to the current MDA programme to achieve the desired level of anaemia reduction in Burundi. Our maps can be useful to guide future MDA campaigns to achieve this reduction.

519

DOMESTIC ANIMALS MECHANICALLY TRANSMIT *NECATOR AMERICANUS* HOOKWORM TO HUMANS

Ryan H. Boyko[1], Jeffrey P. Townsend[1], Debbie Humphries[1], David P. Durham[1], Dickson Osabutey[2], Yvonne A. Ashong[2], Lisa M. Harrison[3], Joseph Otchere[2], Michael Wilson[2], Michael Cappello[3], Alison P. Galvani[1]

[1]School of Public Health, New Haven, CT, United States, [2]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [3]Ghana-Yale Partnership for Global Health, New Haven, CT, United States

Necator americanus hookworm infections cause serious impairment in millions of children across the tropics. While humans are the only definitive host of *Necator americanus*, epidemiological evidence from South America, Africa, and Asia suggest that risk for hookworm infection is increased by contact with dogs and pigs. Building on experiments showing that *N. americanus* eggs can survive passage in the pig digestive tract, we demonstrate that pigs and dogs in twelve rural communities in central Ghana harbor and excrete viable human hookworm eggs in their stool. Using logistic and ordinal regressions, we show that pig ownership and the proportion of households owning dogs in one's village significantly predicted hookworm prevalence and infection intensity. In these communities, 67% of dog feces and 55% of pig feces contain hookworm eggs. Using PCR, we confirmed a majority of collected animal stools contained *N. americanus*. Further, infective third stage hookworm larvae developed from all of the samples (n=41), demonstrating egg viability after natural passage through an animal's digestive tract. The presence of high rates of antibodies to *Toxocara canis* in their serum suggests humans in these communities are frequently exposed to helminth parasites through contact with dogs. We introduce a new hookworm transmission model that indicates that animal-mediated transmission of hookworm eggs may play an important role in human disease. Hookworm control strategies embracing the 'One Health' concept by interrupting hookworm transmission from animals should be developed.

520

A PARADIGM FOR STUDYING ANTHELMINTIC COMBINATIONS *IN VIVO*

Melanie M. Miller[1], **Yan Hu**[2], Thanh-Thanh H. Nguyen[2], Christina Gee[1], James D. Lui[1], Raffi V. Aroian[2]

[1]University of California San Diego, La Jolla, CA, United States, [2]University of Massachusetts Medical School, Worcester, MA, United States

Soil-transmitted helminth (STH) nematode parasites (hookworms, *Ascaris* and *Trichuris*) are key contributors to morbidity and poverty worldwide. Few anthelmintics are available for treatment, and only anthelmintic, albendazole, is commonly used in mass drug administrations, even though there are more than 1 billion people are infected. New anthelmintics and treatment strategies are greatly needed, in particular as albendazole resistance is inevitable given its current method of usage and as commonly occurs with veterinary use of this drug. Our group has identified Cry5B made by *Bacillus thuringiensis* as a promising new anthelmintic for treating STH parasites. Apart from developing Cry5B as a much needed new class of anthelmintic, we are also interested in preserving the potency of Cry5B and other anthelmintics as much as possible—i.e., preventing resistance. To achieve this aim, combination therapies with anthelmintics is an excellent approach. The challenge with anthelmintic combinations is defining a good combination and at what ratio drugs can be productively combined. Although we have published combination studies using the nematode *Caenorhabditis elegans*, it was not clear how these translate *in vivo* in infected mammals. Surprisingly, little work has been done to define the characteristics of anthelmintic combinations *in vivo*. By using the hookworm (*Ancylostoma ceylanicum*) infections in hamster, we establish a new and powerful *in vivo* paradigm for studying anthelmintics combinations—defining not only how well two drugs combine but also providing some optimization of the ratio for combinations. Here, we will update you our latest results of the anthelmintic combinations with Cry5B and other anthelmintics, e.g., tribendimidine. We show that, like in C. elegans, these two drugs can be combined to give powerful effects *in vivo*. Work on other *in vivo* combinations is also proceeding. Our paradigm here provides a powerful means on how to examine and demonstrate the anthelmintic combination therapies.

521

THE ROLE OF SOIL-TRANSMITTED HELMINTH INFECTIONS IN THE GEOGRAPHIC RISK OF FUNCTIONAL ILLITERACY OF SCHOOL-AGED CHILDREN IN THE PHILIPPINES: SPATIAL VARIATION AND NUMBERS AT RISK

Kei Owada[1], Maria Sonia Salamat[2], Lydia Leonardo[2], Archie Clements[3], Laith Yakob[1], Colleen Lau[1], Ricardo Soares Magalhaes[1]

[1]University of Queensland, Herston, Australia, [2]University of the Philippines, Manila, Philippines, [3]Australian National University, Canberra, Australia

We aimed to understand the contribution that soil-transmitted helminth (STH) infections make to functional literacy status of school-aged children in the Philippines and investigated, for the first time, the geographical distribution of prevalence of functional literacy indicators adjusting for STH infections. STH infection data were collected from 29,919 individuals during the most recent National Schistosomiasis Survey conducted in 2005 to 2007 in 177 locations throughout the Philippines. Data were also provided by the National Statistics Office on three functional literacy indicators (i.e. ability to compute, read and comprehend) of 19,673 school-aged children in the Philippines. Our study was conducted in two phases: first, Bayesian geostatistical models were developed to predict the complete geographical distribution of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm co-infection and intensity of infection across the Filipino population. Second, Bayesian geostatistical models were built for each of the functional literacy indicators, adjusting for the location and the predicted co-infection and intensity of *A. lumbricoides*, *T. trichiura* and *hookworm* generated during the first phase. Our results indicate that *A. lumbricoides* and *T .trichiura* co-infection is an important contributor to the spatial variation of functional illiteracy of school-aged children. We identified significant spatial heterogeneity in functional literacy indicators between regions of the Philippines and remarkable spatial variation in functional literacy indicators within different regions of the Philippines accounted by STH co-infection and intensity of infection. Our results demonstrate the important role of STH co-infection and intensity of infection in the geographical variation of prevalence of functional illiteracy in the Philippines. The findings of this study can be useful to guide the Integrated Helminth Control Program to improve the health and functional literacy in the Philippines by reducing STH infection levels in school-aged children.

522

SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHS IN SCHOOL-AGED CHILDREN: EPIDEMIOLOGICAL PROFILE IN KINSHASA AND BAS-CONGO PROVINCES OF THE DEMOCRATIC REPUBLIC OF CONGO

Sylvie Linsuke[1], Sabin Sabin[1], Kirezi Kanobana[2], Faustin Mukunda[3], Raquel Inocêncio da Luz[4], Marleen Boelaert[2], Jean-Pierre Van Geertruyden[4], Katja Polman[2], Pascal Lutumba[1]

[1]Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo, [2]Institute of Tropical Medicine, Antwerp, Belgium, [3]Programme National de Lutte contre la Bilharziose et Parasitoses Intestinales, Kinshasa, Democratic Republic of the Congo, [4]University of Antwerp, Antwerp, Belgium

The lack of epidemiological data on schistosomiasis (SCH) and soil-transmitted helminthes (STH) in the Democratic Republic of Congo (DRC) hampers effective disease control, although these diseases have a significant impact on population health. In 2009-2010 we conducted a random survey in school-aged children (3rd grade) in 11 health areas of the provinces Kinshasa and Bas-Congo. We collected socio-demographic data and examined stool and urine samples of each child. A total of 2399 children (1559 children from Kinshasa and 840 from Bas-Congo) were included. The overall prevalence of SCH was 13.5 %; CI95%: 12.1-14.8. The highest prevalence of SCH was found in Bas-Congo province (32.1; CI95%: 29-35.3). A total of 61.3 % (CI95%: 59.4-63.3) school-aged children were infected STH with a predominance of A. lumbricoïdes. This prevalence was higher in Kinshasa (64%; CI95%: 61.6-66.4) compared to Bas-Congo province (56.3%; CI95%: 53.4-60). The data generated in this study provide baseline data for the formulation of control strategies on SCH and STH infections in Kinshasa and Bas-Congo. More work is needed in other provinces.

523

DETERMINANTS OF INFANT-FEEDING CHOICES OF HIV-POSITIVE WOMEN ATTENDING PREVENTION OF MOTHER-TO-CHILD TRANSMISSION CLINICS IN OYO STATE, SOUTHWESTERN NIGERIA, 2013

Aishat B. Usman[1], Olufunmilayo Fawole[1], Magbagbeola David Dairo[2], Saheed Gidado[1], Patrick Nguku[1]

[1]Nigeria Field Epidemiology and Laboratory Training Programme, Abuja, Nigeria, [2]Department of Epidemiology and Medical Statistics, University of Ibadan, Ibadan, Nigeria

The Nigerian National HIV Guidelines recommends avoidance of all breastfeeding when replacement feeding is acceptable, feasible, affordable, sustainable and safe.This study determined factors influencing the infant-feeding choices of HIV-positive women enrolled in Prevention of Mother-to-Child Transmission (PMTCT) of HIV clinics in Oyo State, Nigeria. A cross-sectional survey of 450 HIV-positive women who had received infant-feeding counselling prior to delivery using a two-stage sampling technique. A semi-structured questionnaire was administered to obtain data on socio-demographics, infant feeding choice and factors influencing these choices. We defined mixed feeding as addition of other food or water to breast milk in the first six months of life. Four Focus Group Discussions (FGD) were conducted. Data was analyzed using Epi-info software version7.Detailed content analyses of the FGDs were done. The mean age of the mothers is 31 ± 3.5years. Exclusive breastfeeding (EBF), exclusive replacement feeding (ERF) and mixed feeding (MF) were 62.0%, 25.0%, and 13.0% respectively. Determinants of ERF were mode of delivery (AOR 2.3, 95%CI 0.8-4.3) and the desire to reduce the risk of transmission (AOR 5.4, 95%CI 2.8-6.4). For EBF, household income (AOR 3.6, 95%CI 1.8-5.4) and health workers influence (AOR 2.5, 95%CI 1.2-3.8), while for MF, non-disclosure of HIV status to spouse (AOR= 4.3,95%CI 1.5-12.8), Neighbours' advice ((AOR 1.8,95%CI 1.5-4.7) and infant illnesses (AOR= 2.9, 95%CI2.3-7.8) were the predictors. FGDs revealed pressure from family members as the major determinant of mixed feeding practice. In conclusion, pressure from family/neighbours to practice mixed feeding should be discouraged. Incorporation of family members into programs promoting safer infant feeding options in mothers living with HIV/AIDS and male involvement is imperative. Keywords: infant food, HIV, Women, Nigeria

524

EVALUATION OF THE IMPACT OF HIV INFECTION ON THE DIGESTIVE FLORA OF CHILDREN

Celine N. Nkenfou[1], William B. Abange[2], Nelly N. Kamgaing[3], Hortense K. Gonsu[3]

[1]"Chantal Biya" International Reference Centre for Research on HIV/AIDS Prevention and Management, Yaounde, Cameroon, [2]Catholic University of Central Africa, Yaounde, Cameroon, [3]Faculty of Medecine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon

The recurrent bacterial infections and affections observed in HIV positive infants could be explained by the imbalance in their digestive flora. The study aims at evaluating the impact of HIV infection on the digestive flora of infants aged 3-24 months, since there have been no studies addressing this issue in Cameroon. A cross-sectional and case-control study was carried out in two hospitals in Cameroon. Stool sample was collected from each of the proxy-consented HIV positive and HIV negative children. These stools were cultured using aerobic, strict anaerobic, 10% CO_2 and microaerophilic conditions. Identification of the bacteria species were done by biochemical characterization. Out of the 80 children enrolled for the study, 33 (41.25%) were HIV positive children and 47 (58.75%) were HIV negative children. 15 different types of bacteria species were isolated from HIV positive infants with high presence of *Lactobacillus* spp. (96.97%) *Streptococcus* spp. (84.85%) and *Bifidobacterium* spp. (81.81%). Opportunistic bacteria like *Shigella* spp. (24.24%), *Staphylococcus aureus* (15.15), *Klebsiella* spp. (12.12%), *Acinetobacter* spp. (3.03%), *Pseudomonas* spp. (3.03%) and *Proteus* spp. (3.03%) were also identified. Statistically, *Clostridium* spp. (p=0.009), *Shigella* spp. (p=0.002), *Enterococcus* spp. (p=0.000) *Staphylococcus aureus* (p=006) and *Streptococcus* spp. (0.015) were significantly more present in HIV positive infants than in HIV negative infants. Bacteria species like *Proteus* spp. *Pseudomonas* spp. *Acinetobacter* spp. *Staphylococcus aureus* were isolated only in HIV positive infants and absent in HIV negative children giving a frequency rate of 24.2%. HIV positive infants at stage 3 and 4 harbored more opportunistic bacteria. We remarked the imbalance in bacteria flora of HIV infected infant's harbouring quantitatively more isolated bacteria than in HIV non-infected children. Systematic stool culture would significantly benefit for the follow-up of HIV infected children to reduce the risk of recurrent bacteria infection.

525

A THEORETICAL PERSPECTIVE OF THE LIVED EXPERIENCES OF HIV INFECTED MOTHERS TOWARDS ADMINISTERING DAILY COTRIMOXAZOLE PROPHYLAXIS TO THEIR HIV EXPOSED BUT NOT INFECTED CHILDREN IN MALAWI

Nyanyiwe M. Mbeye[1], Feiko ter Kuile[2], Kamija Phiri[1]

[1]Malawi College of Medicine, Blantyre, Malawi, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Although new HIV infections among adults in sub Saharan Africa have declined by 34% since 2001, the epidemic continues to disproportionately affect this region bearing 70% of all new infections in 2013. However, the sustained scale up of interventions for prevention of mother to child transmission of HIV has led to fewer newly HIV infections in children. The World Health Organization recommends that all HIV exposed infants be started on cotrimoxazole prophylaxis (CPT) until breastfeeding is stopped and HIV infection is excluded to provide adequate prevention against early opportunistic infections. Adherence may be challenging when CPT is given to very small infants who need to take it for long periods of time. Previous studies have only focused on adherence to PMTCT services hence it is

important to build a grounded theory of the social and cultural influences of CPT adherence in this group. Three focus group discussions and seventeen In depth Interviews were conducted with HIV infected mothers administering daily CPT to their HIV exposed but uninfected infants in a semi urban district in Malawi. A thorough literature search supplemented the concepts that emerged from the data. Findings We propose a fusion of the ecological theory of perception and the Health Belief Model to explain adherence to CPT in this study. The women themselves, their families, the communities in which they live and the health care system relate and influence each other through social interaction which play a role in influencing actions either positively or negatively. Motivators such as a supportive family help someone to take a positive action and factors such as shortage of drugs at the health facility act as deterrents. Interestingly, the data in this study demonstrates that despite negative influences that might arise at any level, the determination of the individual to take a health related action and the realisation that the recommended action will prevent any negative outcomes motivated the mothers to believe that they could successfully do something to prevent any negative health outcomes on their children. In conclusion, these findings could be used to design individualised interventions on HIV disease counselling as well as importance of treatment adherence in infants. The most currently used theory of Behavioural Learning lacks this capacity as it ignores effects such as past behaviour, habits and lack of acceptance of one's diagnosis.

526

KNOWLEDGE, ATTITUDE AND PRACTICES OF SEXUALLY TRANSMITTED INFECTIONS AMONG WOMEN OF REPRODUCTIVE AGE LIVING IN KATANGA SLUM KAMPALA, UGANDA

Faith Nawagi

Makerere University College of Health Sciences, Department of Nursing, Kampala, Uganda

Sexually transmitted infections (STIs) still stand as one of the commonest health problems affecting women of reproductive age especially in slums. These have further been idenified as cofactors of HIV transmission, multiple complications such as, abortion, infertility and ectopic pregnancy among others.The burden of STIs continues to remain high in Uganda at 69%. In order to design appropriate preventive measures ,there is need to establish the profile of knowledge, attitudes and practices of sexually transmitted infections among susceptible populations such as women of reproducitive age living in slums like Katanga in Kampala Uganda.This was a descriptive crosssectional study with 339 participants.A consecutive sampling method was done. Using a standardized questionnaire, woman in Katanga slum who met the eligibility criteria were interviewed and data collected from them. Data was coded, cleaned, transcribed and double entered using EPI-data 3.1 and analyzed using SPSS 17.0. Coded data was summarized using frequencies for categorical data and medians for continuous data.In this study76.7% knew what STIs are with 41.9% giving Syphilis, Candida, HIV and gonorrhoea as examples. Most of the participants (99.1%) were between the age of 25-49 years with the majority (31%) being between 25-29years. The commonest symptoms known to the participants were genital itching (59.2%) and genital rash (14.5%).Only 2.9% did not know about the predisposing factors for STIs, however most mentioned multiple patners (63.7%) and unprotected sex (50.7%).Only 51% could identify STIs by the signs and symptoms with 71.9% knowing that they are treatable and curable.40% of the participants lacked knowledge on the effects and complications of STIs on their health and body. Although knowledge on methods of prevention was high (92.3%), it was not followed by appropriate behavioural patterns since 18.8% were found positive for STIs using the symdromic approach and 82% mentioned having suffered from STIs in the past 06 months more than once.The level of knowledge about STIs and their prevention is not matched by sexual behavioural patterns. Many women in Katanga slum still dont practice the approppriate preventive measures for STIs . There is a need to lay strategy on how the preventive measures which are well known by this vulnerable population can actually be effectively adopted and practiced.

527

CROSS SECTIONAL ASSESSMENT ON THE PREVALENCE OF HIV AMONG DOTS CLIENTS IN ADDIS ABABA ADMINISTRATIVE REGION 2011-2012

Zegeye H. Tessema, A. Mengesha

Federal Ministry of Health, Addis Ababa, Ethiopia

TB and HIV are the major public health problems in Addis Ababa, considering this fact; Health institutional based cross-sectional study was undertaken in Addis Ababa administrative region from September 2011 to February 2012. The objective of the study was to determine HIV sero prevalence among registered tuberculosis patients in Addis Ababa public DOTs clinics. The HIV antibody was determined using a single ELSA technique. A total of 417 tuberculosis patients in 26 Dots (intensive care) clinics aged 16 years and above was enrolled in the study. The over all HIV sero prevalence rate among tuberculosis patients was 32.5%. The highest rate was observed in the age group 30 to 39 years. Almost equal proportions of male 49.3% were found to be HIV sero positive compared to the females 50.7%. Being unmarried was found to be associated with HIV positive test result ($p<0.005$). Those divorced and widow/widower patients had high proportion of HIV positive. Daily labourers and patients who are living alone were found to be significantly infected with HIV ($p<0,001$). The HIV positive rate was higher among pulmonary tuberculosis patients compared to extra-pulmonary tuberculosis cases. Smear positive pulmonary patients 25% were found to be significantly associated with HIV sero positive test result compared with smear negative pulmonary tuberculosis cases15.6%. It was concluded that HIV infection is highly prevalent among smear positive TB, higher schooling, daily labourers, young age group, unmarried TB- clients, people who had history of STD, history of multiple sexual partners. Finally, additional qualitative supportive study on KAP on TB patients towards HIV/AIDS is recommended.

528

MYCOBACTERIUM TUBERCULOSIS-SPECIFIC CD8+T CELL RECALL IN CONVALESCING TB SUBJECTS WITH HIV CO-INFECTION

Violet Asiko Ongaya[1], Matthew B. Huante[2], Paul W. Mwangi[1], Philip H. H. Keiser[2], Evans I. Amukoye[1], Janice J. Endsley[2]

[1]Kenya Medical Research Institute, Nairobi, Kenya, [2]University of Texas Medical Branch, Galveston, TX, United States

Memory T cell populations recover following phase I chemotherapy for tuberculosis (TB) and augment the effectiveness of antibiotics during the continuation phase of treatment. For those with human immunodeficiency virus (HIV), the CD8+T cells may have an especially important role in host defense to Mycobacterium tuberculosis (M.tb) as CD4+T cell function and/or numbers decline. Here we performed a preliminary study to investigate the impact of HIV infection status on CD8+T cell effector function during the convalescent TB period. Peripheral blood samples from convalescent HIV+ and HIV- TB subjects were used to determine CD4+T cell count and monitor antigen-specific CD8+ T cell activation of effector function (lymphoproliferation, IFN-γ;, granulysin) in response to M.tb antigen. Our preliminary results suggest that HIV co-infection is associated with moderate suppression of the M.tb-specific memory CD8+T cell compartment in many subjects convalescent for TB. Interestingly, highly activated CD8+T cells were observed in recall experiments using peripheral blood from several HIV+ subjects that had low (<200 cells/mm3) CD4+T cell counts. Further investigation may provide important information for development of novel approaches to target M.tb-specific CD8+T cell memory to protect against TB in HIV-endemic regions.

529

PREVALENCE AND RISK FACTORS OF PULMONARY TUBERCULOSIS AMONG HIV/AIDS PATIENTS IN IHIALA, ANAMBRA STATE, NIGERIA

Ikechukwu N. Dozie, Donatus C. Nnadi, **Chinonyerem J. Njoku**, Uche M. Chukwuocha

Federal University of Technology, Owerri, Nigeria

The prevalence and risk factors of pulmonary tuberculosis among HIV/AIDS patients were determined in this study. A cross-sectional study design was adopted and hospital records from 2007 to 2011 of 375 HIV/AIDS patients attending Highly Active Antiretroviral Therapy (HAART) Centre of Our Lady of Lourdes Hospital Ihiala, Anambra, Nigeria, were reviewed. A standard proforma was used to generate data on other risk factors and diagnostic records. The prevalence rate of PTB among HIV/AIDS patients was 6.1%. The mean age, BMI and CD4 levels were 35.53 years (SD 9.37), 22.22Kg/m^2 (SD 2.23) and 429.85cells/mm^3 (SD 268.41) respectively. Pulmonary TB prevalence was highest among HIV/AIDS patients aged 30-39 years (60.9%), married patients (60.9%), patients with normal BMI (100%) and patients with CD4 levels <200cells/mm^3 (60.9%). Lower CD4 levels <200cells/mm^3 (P<0.0001), age 30-40 years (P <0.019) and marital status (being married; P<0.005), respectively, were significantly associated with the occurrence of PTB among HIV/AIDS patients in this study. Co-treatment of TB/HIV/AIDS (DOTS-HAART), lifestyle modification and education to minimize exposures to risk factors should be scaled up and encouraged.

530

THE IMPACT OF NUTRITIONAL STATUS ON FIRST LINE HAART FAILURE IN HIV-INFECTED CHILDREN IN CAMBODIA

Jaroslava Brnova[1], Katarina Dudakova[2], Nada Kulkova[3], Lenka Michalikova[1], Andrea Shahum[2], Veronika Sladeckova[2], Martin Kolibab[2], Julia Vujcikova[2], George Benca[2], **Vladimir Krcmery**[4]

[1]Faculty of Health Sciences and Social Work, Trnava, Slovakia, [2]St. Maximilian Kolbe Clinic, Phnom Penh, Cambodia, [3]Laboratory of Molecular Microbiology, Bratislava, Slovakia, [4]St. Elizabeth University, Bratislava, Slovakia

While the impact of antiretroviral treatment on growth in children is well established, the influence of prior nutritional status on the response to treatment is not well known. The objective of this study was to assess impact of nutritional status on treatment failure in HIV infected children receiving first line highly active antiretroviral therapy (1st line HAART). Nutritional status was assessed by height-for-age, weight-for-age and weight-for-height Z scores using the 2006 World Health Organization growth reference. CD4 cells counts and viral load (VL) were measured every 6 months or when CD4 drop-down has been observed, consequently minimally twice per years. Laboratory parameters and genotypic resistance tests were performed in case of clinical or immunological failure by National Paediatric Hospital laboratory affiliated to Institute Pasteur Cambodia, Phnom Penh. First line therapy was consisted of Zidovudine + Lamivudine + Nevirapine or Efavirenz in those with concomitant tuberculosis infection. In our study group of HIV infected children treated with HARRT (n=98) the median Z score for HAZ, WAZ, BAZ at baseline of HAART was -3,3 (IQR = -4,4/-2), -3,4 (IQR=-4,1/-1,4), -1,4 (IQR=-2,3/-0,4) and decreased during study period -2 (IQR=-2,7/-1,5), -2,1 (IQR=-2,4/-1,5), -0,9 (IQR=-1,8/0,2) respectively. Concerning risk factors for treatment failure, we compared 77 (79%) children on 1st line HAART with 21 (21%) children with treatment failure (treated with second line regiments). Low CD4 cell percentage (<5%) and wasting (WAZ > - 3) at the baseline were not significantly associated with treatment failure. In our study, baseline poor nutritional status was not associated with failure of 1st line HAART among HIV-infected Cambodian children. All of our children were placed in 2 orphanages with good nutrition (five times a day) on inpatient basis, which may contribute to low failure rate. We advise the development of further studies to assess the nutritional status of children with HIV/AIDS using anthropometric measurements.

531

DECREASING OCCURRENCE OF BACTERIAL SEXUALLY TRANSMITTED DISEASES AFTER INTRODUCTION OF VOLUNTARY COUNSELING AND TESTING HIV-PROGRAM IN ELDORET, KENYA IN 2009-2013

Iveta Gagova[1], Zuzana Paukovova[1], John M. Muli[1], Jirina Kafkova[1], Marian Bartkovjak[1], Daria Pechacova[1], Jaroslava Brnova[2], Nada Kulkova[3], Lenka Michalikova[2], G. Mikolasova[3], **Vladimir Krcmery**[4]

[1]Bl. Ladislaus Batthyany-Strattmann Clinic, Eldoret, Kenya, [2]Faculty of Health Sciences and Social Work, Trnava, Slovakia, [3]Laboratory of Molecular Microbiology, Bratislava, Slovakia, [4]Slovak Tropical Institute, Bratislava, Slovakia

Incidence of bacterial sexually transmitted diseases (STD) correlates with HIV as well with Hepatitis B and C and introduction within integrated program usually has impact on multiple diseases. The aim of this research is to assess the impact of community based integrated health program focused on HIV/AIDS and tuberculosis (TB) on the occurrence of bacterial STD in rural community of about 50 000 inhabitants in north Eldoret, Kenya, in area of HIV prevalence of 8-14 %. Among all outpatient department (OPD) visits during last 4 years (2009-2013) in Bl. Ladislaus Batthyany-Strattmann Clinic serving for about 50 000 people, incidence of STD and HIV in infections cases was assessed and correlated. HIV VCT program has been established in 2008 as VCT center next to the clinic with one VCT counselor and 1 trained nurse. All VCT/HIV program and all OPD visits for STD were recorded monthly. Syphilis and gonorrhea were evaluated as STD. Among 42711 OPD visits in last 4 years, STD was diagnosed in 1446 patients (3.39 %) and HIV in 462 patients (1.1 %). However, 10 years ago, when the clinic started its work, HIV prevalence in males was 8.6 % and in females 11.9 %. Dramatic decrease of HIV was correlated with sustained decrease of bacterial STD´s (syphilis, gonorrhea). While in 2009, 505 cases of bacterial STD and 110 new cases of HIV were detected, 421 STD´s were diagnosed in 2010, 176 in 2011 and 201 in 2012 and 148 in 2013 were recorded followed decrease of HIV from 110 in 2009 to 53 in 2013. Unfortunately, proportion of adults with HIV and STD was decreasing in 2009-2013, more then 3-fold, while pediatric SDT in children <5 years increased from 0 % to 1 % in 2009 , 3 % and 6% in 2010 and in 2013, respectively. Integrated HIV/STD community program led to more 3.3 - fold decrease of STD ad 2.1-fold decrease of AIDS in rural community of Eldoret after 5 years of the introduction of VCT. Moreover, increasing prevalence STD in children <5 years of age is of great concern.

532

BACTERIAL INFECTION AND MALARIA IN HIV POSITIVE CHILDREN ADMITTED TO HOSPITAL IN NORTHEAST TANZANIA; RESULTS OF A ONE-YEAR OBSERVATIONAL STUDY

Behzad Nadjm[1], Natalia R. Valero[2], George Mtove[3], Ben Amos[4], Hugh Reyburn[2]

[1]Oxford University Clinical Research Unit and The London School of Hygiene & Tropical Medicine, Hanoi, Vietnam, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]National Institute for Medical Research, Tanga, United Republic of Tanzania, [4]Teule Hospital & European Centre for Environment and Human Health, University of Exeter Medical School, Exeter, United Kingdom

Over three million children under 15 are now infected with HIV in sub-Saharan Africa, with profound health consequences. Both HIV infection, HIV treatment and malaria infection affect the incidence of invasive bacterial disease in children and the range of causative organisms, yet few studies have provided these data systematically. We present data from an observational study of paediatric admissions between June 2006 and July 2007 to a district hospital serving a rural population in northeast Tanzania. Children aged 2 months to 13 years admitted to the hospital during study hours with fever, or history of fever, were considered eligible.

Enrolled children underwent a standard clinical examination and panel of diagnostic blood tests including a research blood slide and rapid-test for malaria, blood culture and HIV serology. Children aged less than 18 months with positive serology were tested for HIV-1 RNA. CD4 T-cell counts were performed by flow cytometry on-site. Results from this study have been published, but data pertaining to the range infections in HIV positive patients are presented her for the first time. 3,704/4,334 (85.5%) of those children admitted during study hours were eligible, consented and enrolled. The data on 3,639/3704 (98.2%) were complete. HIV infection was diagnosed in 142/3,639 (3.9%). The most common clinical diagnosis in children admitted with HIV was very severe pneumonia (33/142 (23.2%)). In HIV positive children the prevalence of *Plasmodium falciparum* by blood slide was significantly lower (48/142, 33.8%) than in HIV negative (2,147/3,497, 61.4%, p<0.001), though the median parasite density in HIV positive (22,731 parasites/µl) and HIV negative children (36,758 parasites/µl) were similar (p=0.128, Wilcoxon rank-sum). HIV positive children had a higher prevalence of invasive bacterial disease (27/142, 19.0%) than HIV negative children (314/3,497, 9.0%, p<0.001). In children negative for HIV the most common bacterial isolate was non-typhi *Salmonella*, in HIV positive children *Streptococcus pneumoniae* was the most common isolate. HIV was implicated in over 1 in 8 inpatient deaths. We were unable to show such an association between HIV infection and severe malaria and malarial death in this population.

533

PUBLIC-PRIVATE DIAGNOSTICS AND REFERRAL SERVICES FOR HIV/TB CO-INFECTED PATIENTS: A SYSTEMATIC REVIEW

Mollie Hudson, Elizabeth Fair

University of California San Francisco, San Francisco, CA, United States

In 2012, there were an estimated 8.6 million new infections and 1.3 million deaths due to tuberculosis (1). Tuberculosis is the second leading cause of death due to infectious diseases worldwide (2, 3), and is the leading cause of death among people living with HIV (3). The successful integration of tuberculosis and HIV services in both the public and private sector is essential to the fight against the dual burden of TB and HIV. The WHO Stop TB department has called for a collaboration of public and private healthcare providers (often referred to as public private mix [PPM]) to maximize TB/HIV integrative services while minimizing costs. Many challenges remain for engaging all care providers into integrated HIV/TB services, particularly in regards to co-current diagnostics and referrals. HIV patients must be screened for tuberculosis, and tuberculosis patients must be screened for HIV. An efficacious public private partnership has the potential to integrate tuberculosis and HIV diagnostics and referral services to improve health outcomes and reduce patient costs. This study was undertaken to determine the frequency of public-private diagnostic and referral services for HIV/TB co-infected patients in high burden countries? Using several electronic databases and WHO indictors, we will conduct a systematic review to assess the degree of integration of various public-private partnership models for co-current HIV/TB diagnostics and referral services. We would like to evaluate the efficacy of various PPP models using published indicators of successful integration of services, and discuss the subsequent policy implications of our findings.

534

PREVALENCE OF HIV/AIDS AND TOXOPLASMOSIS IN NIGERIA

Alex D. Acholonu

Alcorn State University, Alcorn State, MS, United States

Human Immunodeficiency Virus (HIV) has been infecting people all over the world at an alarming rate and giving rise to full blown AIDS (Acquired Immune Deficiency Syndrome). Its prevalence in Nigeria is a concern. It is at present believed to infect 3.1% of a population of 150 million. Associated with HIV/AIDSs are opportunistic infections. Some of these are cryptosporidiosis, *Pneumocystis carinii* infection and Toxoplasmosis. Toxoplasmosis is one of the most serious opportunistic infections in HIV/AIDS patients that lead them to their early demise. For HIV/ AIDS patients, cerebral toxoplasmosis causes encephalitis which worsens the patient's condition. Human beings get infected by eating raw or undercooked meats containing *Toxoplasma gondii* cysts. Such meats come mainly from pigs, sheep and goats, especially the last two which Nigerians eat a lot and most commonly during festivities like Christmas celebration, birthday celebration, Muslim festivals, etc and are sold commercially. There is scarcity of information about co-infection of HIV/AIDS and Toxoplasmosis in Nigeria. A review of available literature on the prevalence of toxoplasmosis in humans in Nigeria shows that there are about 17 reports between 1960 and 2010. Of these only three show co-infection of HIV/AIDS and toxoplasmosis. Even so, the infection rate is fairly high (38.8%/ 219 and 43.4%/60 and a positive case report). There is a need in Nigeria to step-up activities involving the screening of HIV/AIDS patients for toxoplasmosis and treating those found infected as there is treatment for toxoplasmosis. This will prolong the lives of patients.

535

POVERTY, FOOD INSECURITY AND LOW BODY MASS INDEX, ARE ASSOCIATED WITH POOR QUALITY OF LIFE FOR PEOPLE LIVING WITH HIV IN RURAL HAITI

Phillip J. Summers[1], Molly F. Franke[2], Louise C. Ivers[2]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Partners In Health, Boston, MA, United States

Poverty, food insecurity, and HIV/AIDS often co-occur and have a synergistic negative effect on health. However, the impact of these factors on quality of life (QOL) has only recently begun to be explored for people living with HIV (PLWHIV) in resource-poor settings. This study investigates the relationships between poverty, food insecurity, body mass index (BMI), and QOL in a cohort of 524 HIV-positive patients in rural Haiti. Multivariate logistic regression models were fitted to identify predictors of high QOL based on a survey containing a validated poverty score, the Household Food Insecurity Access Scale, and three domains of the AIDS Clinical Trials Group Health-Related Quality of Life Scale: general health, physical functioning, and role functioning. Of 489 respondents, 246 (50%) reported severe food insecurity and 428 (88%) reported some food insecurity. Mean QOL scores were 45 (Health), 62 (Physical), and 65 (Role), out of 100. Not being severely food insecure (OR=0.453, p=0.035; OR=0.387, p=0.008), having less poverty, (OR=0.957, p<0.001; OR=0.969, p=0.005), and having a higher BMI (OR=1.1, p=0.002; OR=1.08, p=0.007) were each independently associated with higher health and physical QOL. Having access to food from a garden (OR=1.79, p=0.05; OR=2.01, p=0.016) and younger age (OR=0.978, p=0.039; OR=0.97, p=0.005) were associated with higher physical and role functioning QOL. Food insecurity is highly prevalent at 88% in this population of PLWHIV in rural Haiti. This population's mean QOL scores were also substantially lower than scores reported from other cohorts of PLWHIV in resource-poor settings. Food insecurity, low BMI, and poverty are independently associated with poor QOL in PLWHIV and must be addressed as an integral component of comprehensive HIV programs in this setting.

536

EVIDENCE OF A DISTINCT PROFILE OF METALLOPROTEINASES 2 AND 9 AND THEIR INHIBITORS IN CARDIAC REMODELING OF CHAGAS DISEASE

Rafaelle Fares Gusmao[1], Juliana A. Gomes[2], Luciana R. Garzoni[3], Mariana C. Waghabi[3], Roberto M. Saraiva[4], Nayara I. Medeiros[2], Roberta Oliveira-Prado[1], Luiz Henrique C. Sangenis[4], Mayara C. Chambela[4], Fernanda F. de Araujo[1], Andrea Teixeira-Carvalho[1], Marcos P. Damasio[2], Vanessa A. Valente[2], Karine S. Ferreira[2], Giovane R. Sousa[5], Manoel Otavio C. Rocha[5], Rodrigo Correa-Oliveira[1]

[1]Centro de Pesquisas Rene Rachou, Fiocruz-MG, Belo Horizonte, Brazil, [2]Universidade Federal de Minhas Gerais, Belo Horizonte, Brazil, [3]Instituto Oswaldo Cruz – FIOCRUZ, Rio de Janeiro, Brazil, [4]Instituto de Pesquisa Evandro Chagas, FIOCRUZ, Rio de Janeiro, Brazil, [5]Faculdade de Medicina, Programa de Pós-graduação em Ciências da Saúde: Infectologia e Medicina Tropical, UFMG, Belo Horizonte, Brazil

Dilated chronic cardiomyopathy (DCC) in Chagas disease is associated with myocardial remodeling and interstitial fibrosis, resulting in significant extracellular matrix (ECM) modifications. ECM remodeling is regulated by proteolytic enzymes such as matrix metalloproteinases (MMPs). The main objective of this study was to evaluate the involvement of MMPs 2 and 9 and their inhibitors (TIMPs) in indeterminate (IND) and cardiac (CARD) clinical forms of Chagas disease. We evaluated, for the first time, the serum levels of MMPs 2 and 9 and TIMPs 1 and 2, as well as the main cell sources of MMPs 2 and 9 in the peripheral blood from patients presenting IND or CARD clinical forms of Chagas disease. Our results showed that MMP-9 serum levels are associated with the severity of Chagas disease. The serum levels of TIMP-1 were not different between the studied groups; however the serum levels of TIMP-2 were higher in CARD group. The correlation analysis showed a possible specificity of TIMP-1 for MMP-9. The analysis of MMPs production by T lymphocytes showed that CD8+ T cells are the main source of both MMP-2 and MMP-9 molecules. Using a new 3-dimensional model of fibrosis we also observed that serum from patients with Chagas disease induced an increase in the extracellular matrix components in cardiac spheroids obtained from mice cardiomyocytes. Furthermore, MMP-2 and MMP-9 have showed different profile of correlation with matrix proteins (laminin and fibronectin) and inflammatory cytokines (TNF-alfa and IL-1beta) in patients with Chagas disease. Our results suggest that MMP-2 and MMP-9 show distinct activities in Chagas disease pathogenesis. While MMP-9 seems to be involved with the inflammation and cardiac remodeling of Chagas disease, MMP-2 does not show any correlation with inflammatory molecules. In conclusion, our data stress the involvement of MMP-9, and not of MMP-2, in heart disease and, for the first time, its participation in chagasic cardiomyopathy. These data are innovative and represent an advance in the knowledge of the mechanisms involved in the establishment/maintenance of the Chagas heart disease pathology.

537

EFFECTS OF INFECTION AND *TRYPANOSOMA CRUZI* SOLUBLE ANTIGEN EXPOSURE ON A HUMAN ASTROCYTE CELL LINE

Gabriel A. Durán-Rehbein, Juan C. Vargas-Zambrano, John M. González

Universidad de los Andes, Bogotá, Colombia

Trypanosoma cruzi can compromise the human central nervous system (CNS), especially during the acute phase of infection or in immune-suppressed hosts. Astrocytes could be essential cells in the pathogenesis of *T. cruzi*'s CNS infection. It is known that protozoans such as Toxoplasma gondii and *Leishmania* spp. secrete proteins that facilitate invasion and intracellular survival. This work evaluated the changes induced in a human astrocyte line when: a) infected with *T. cruzi* trypomastigotes, b) exposed to the live parasite separate by membrane filter, or c) exposed to a soluble antigen from trypomastigotes. The number of cells and morphology were evaluated by light microscopy on Giemsa-stained cells; HLA molecule expression and cell cycle were assessed by flow cytometry. An increase in the number of cells was observed, proportional to the amount of soluble antigen used in culture from 3.95x105 cells/ml in control cells to 7.0x105 cells/ml with 10 µg/ml, and 9.9x105 cells/ml with 100 µg/ml (p=0.0174). The percentage of cells in G2/M phase of the cell cycle was higher in cultures exposed to soluble antigen (8.43% and 9.36% at 10µg/ml and 100 µg/ml, respectively) than control cultures (6.06%, p=0.018). *T. cruzi* infection at day four post-infection increased the intensity, but not the percentage of HLA class I expression (mean fluorescence intensity 9,634 ±2,260 for infected cultures vs. 2,986 ±1,877 for control cells, p=0.0000126); and raised the percentage of HLA class II molecules in infected cultures (11.71% ± 5.63%) compared to control cultures (1.4%± 0.17% p=1x10-8). Exposure to the soluble antigen increased expression of HLA class II (7.71% ±4.04 with 10 µg/ml, 11.36% ±8.36 100 µg/ml) in comparison to cells without antigen (1.93% ±0.9, p=0.0168), and no changes in HLA class I were detected. Infection by *T. cruzi* and exposure to a parasitic soluble antigen induce cellular proliferation as well as changes in HLA molecule expression patterns of human astrocytes line, implicating astrocytes as participants in the local cellular response during CNS infection.

538

EVIDENCE OF HOMOGENEOUS DISTRIBUTION OF *LEISHMANIA* AMASTIGOTES IN ULCERS OF CUTANEOUS LEISHMANIASIS

Milagros Suárez[1], Braulio M. Valencia[1], Marlene Jara[1], Milena Alba[1], Alejandro Llanos-Cuentas[1], Andrea K. Boggild[2], Jorge Arévalo[1], Vanessa Adaui[1]

[1]Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Tropical Disease Unit, Division of Infectious Diseases, Department of Medicine, University Health Network-Toronto General Hospital, University of Toronto, Toronto, ON, Canada

Polymerase chain reaction (PCR) testing of skin lesion specimens currently provides the most sensitive method for diagnosis of cutaneous Leishmaniasis (CL), a disease highly endemic in Latin America. Previous studies that have compared the sensitivity of *Leishmania* detection either by microscopy or PCR using different sampling methods and sites on the lesion showed discrepant results. Sensitivity can be variable and dependent upon the infectious agent load and its dispersion in the lesion. We applied a quantitative real-time PCR assay targeting *Leishmania* (*Viannia*) minicircle kinetoplast DNA to quantify the parasite load in biopsy, scraping and cytology brush specimens obtained from the ulcer center, base and raised border. A total of 31 patients with parasitologically confirmed CL were enrolled: 29 males and 2 females, with median age of 34 years and median disease duration of 2 months. Parasite loads in skin samples varied between 1.1E+00 and 5.7E+06 parasites per µg of tissue DNA. Median parasite loads did not differ significantly among the three sites of the ulcer in all sampling methods, but there was a trend towards fewer parasites in the ulcer border where the diagnostic specimen is usually obtained. Compared to biopsies, a greater amount of parasites could be quantified from dermal scrapings of the border (p=0.007) and from cytology brushes of the ulcer base (p=0.02) and center (p=0.01). Parasite load measurements on biopsies versus scrapings or cytology brushes were highly correlated [Spearman's rho range 0.75-0.95, p<0.0001]. There was no significant difference in parasite load according to the infecting species (p=0.39), with L. braziliensis and L. peruviana the predominating species. Our results suggest that in recent onset CL, *Leishmania* amastigotes distribute homogenously within the ulcer; thus, samples can be easily and safely obtained from ulcer centers and bases preserving diagnostic performance. The use of scrapings and cytology brushes outperforms invasive biopsy. Further studies with larger samples and with longer disease duration are needed.

539

3D MODELS OF SAND FLY SALIVARY PROTEINS

Michal Sima, Marian Novotny, Iva Kolarova, Petr Volf

Charles University in Prague, Faculty of Science, Prague, Czech Republic

Sand flies (Diptera: Phlebotominae) are vectors of *Leishmania* (Trypanosomatidae), the causative agents of cutaneous and visceral Leishmaniasis. During the blood feeding, sand fly females inject saliva into the host skin to overcome host haemostatic mechanism. Many proteins with distinct functions were found in cDNA library of sand fly salivary glands. 3D models of salivary proteins would allow us to better understand the protein function in antihaemostatic and immune responses. Our study was focused on yellow-related proteins, the high affinity binders of the host prohemostatic and proinflammatory biogenic amines such as a vasoconstrictor serotonin. In *Lutzomyia longipalpis*, it has been shown that salivary yellow-related proteins bind serotonin into their binding pocket located in the central part of the molecule, thereby hamper its physiological function and allow blood feeding of sand fly females. We took advantage of the known 3D structure of *L. longipalpis* yellow-related protein. The predicted 3D structures showed interspecies variability in the amino acid residues inside the binding pocket. For example, the bond of serotonin is probably stronger in P. orientalis yellow-related protein PorMsp24 than in 3Q6K of *L. longipalpis* due to the substitution of phenylalanine for glutamine and the new possible hydrogen bond between serotonin and glutamine. Such substitutions may affect the binding ability of yellow-related proteins. In *L. longipalpis*, the binding ability differs also between the yellow-related protein variants within the species. Since there are several yellow-related proteins in each sand fly species, we constructed 3D models to predict also the interspecies variability in serotonin binding ability. Yellow-related proteins are also highly antigenic for hosts and are considered as candidates for transmission blocking vaccine against Leishmaniasis, which means the need to know their exact function and structure. 3D models of sand fly salivary yellow-related proteins were constructed in MODELLER and visualised in The PyMOL Molecular Graphics System.

540

SPONTANEOUS HEART DISEASE IN THE ADULT CHIMPANZEE: THE ROLE OF *TRYPANOSOMA CRUZI*

Nestor G. Astorga

St. Mary's University, San Antonio, TX, United States

Heart disease is the leading cause of death in the United States, and kills over 650,000 people annually. A high incidence of heart disease, especially idiopathic cardiomyopathy, is seen in chimpanzees and is the leading cause of death in chimpanzees at the Southwest National Primate Research Center (Seiler 2009). The high prevalence of heart disease-related mortality in captive colonies makes chimpanzees a valuable animal model for studying heart disease. The aim of this study was to retrospectively examine potential contributing factors and causes of heart disease in chimpanzees with emphasis on *Trypanosoma cruzi* (*T. cruzi*). We reviewed necropsy reports of adult chimpanzees that died with heart disease as a histopathological finding. We examined age, sex and cause of death. The overall prevalence of heart disease in chimpanzees was 67.81% (Seiler 2009). Of these, 30.12% have myocarditis and the remainder died of cardiomyopathy. We divided the animals into two groups based clinical findings and histopathological characteristics. Group1: myocarditis and Group2: cardiomyopathy. Myocarditis was indicated by lymphocytic infiltration of the myocardium. Cardiomyopathy was characterized by diffuse myocardial fibrosis with minimal inflammation. By simple PCR, we tested 78 heart tissue samples from chimpanzees in these groups for the presence of *T. cruzi* DNA. Of the chimpanzees that died of heart disease, only 19% tested positive for the presence of *T. cruzi*. Of all of the animals tested, the myocarditis group was 23% positive for *T. cruzi* while 18% of the cardiomyopathy group tested positive. Combined, the percentage that tested positive was 19% (15/78). In some tissues (2/35), *T. cruzi* microorganisms were observed. The cause of heart disease in these animals is still under investigation. Based on these results, *T. cruzi* is one of the causes of heart disease in the chimpanzees at SNPRC, however it is not the main cause.

541

INFECTION BY *LEISHMANIA BRAZILIENSIS* IS ALTERED IN U937 DERIVED MACROPHAGES EXPRESSING EXOGENOUS GFP

Clemencia Ovalle-Bracho[1], Diana Londoño-Barbosa[1], Carlos Franco-Muñoz[1], Carlos Clavijo-Ramirez[2]

[1]Centro Dermatologico Federico Lleras Acosta, Bogotá, Colombia, [2]Universidad Nacional de Colombia, Bogotá, Colombia

Gene knock-down mediated by RNA interference is increasingly used during the study of host-parasite interaction. Often exogenous gene expression such as GFP is used as a reporter during functional assays. The aim of this work was to evaluate exogenous gfp as a positive control in order to verify RNAi mediated gene silencing, in the context of U937 derived macrophages infected by *Leishmania* braziliensis. Parental cell line U937 was genetically modified via lentiviral transduction with a construct encoding for constitutive expression of GFP, generating the cell line U937-GFP. In turn this cell line was transduced with vectors encoding for a shRNA targeting GFP or a non-relevant shRNA as negative control, to generate the cell lines U937-GFP/shRNA-GFP and U937-GFP/shRNA-NR, respectively. The three U937 derived cell lines were characterized in terms of GFP expression by Western blot, as well as their ability to be infected by *Leishmania* braziliensis. One-way ANOVA and Sidak test were applied for statistical analysis using STATA 13 software. Results showed a reduction of 88.9% in GFP levels in the cell line U937-GFP/shRNA-GFP while there was no change in GFP expression in the negative control U937-GFP/shRNA-NR. In functional terms, the U937-GFP cell line showed reduced infection rate as compared with the parental cell line U937. Interestingly, this infection parameter was reconstituted to parental levels, in the U937-GFP/shRNA-GFP cell line, but remained low in the negative control U937-GFP/shRNA-NR still expressing GFP.This suggests that expression of GFP is responsible for the reduction in infection rate observed in the U937-GFP cell line. Caution should therefore be considered when using GFP exogenous expression in this cellular model of human macrophages.

542

GENETIC DIVERSITY IN *LEISHMANIA DONOVANI* FROM SRI LANKA: USE OF MINICIRCLE DNA FOOTPRINT ASSAY

K.K.G.D.U. L. Kariyawasam[1], Tasaduq H. Wani[2], H.V.Y. D. Siriwardena[1], A. Selvapandiyan[2], Hira L. Nakhasi[3], Nadira D. Karunaweera[1]

[1]Faculty of Medicine, University of Colombo, Colombo, Sri Lanka, [2]Institute of Molecular Medicine, New Delhi, India, [3]Division of Emerging and Transfusion Transmitted Diseases, Food and Drug Administration, Bethesda, MD, United States

Leishmaniasis comprises a variety of syndromes primarily due to at least 16 species and subspecies of *Leishmania.* In humans, the clinical spectrum ranges from asymptomatic infections to high mortality, with three distinct forms being classically described: visceral (VL), cutaneous (CL) and mucocutaneous (MCL). Leishmaniasis is a recently established disease in Sri Lanka with over 3000 cases of CL distributed island-wide with a few visceral and mucosal cases reported during the past decade. A genetically distinct variant of the usually visceralizing *L. donovani* is the causative agent and has been studied here using mitochondrial minicircle footprint assay to further understand its genetic status. Extracted parasite DNA from skin lesions of 34 CL patients and bone marrow aspirates of 4 VL patients were subjected to kDNA minicircle PCR followed by its comparative sequence analysis through dendrogram using 6 reference *Leishmania* species as previously reported. Sri Lankan isolates from skin lesions made a separate cluster within other known *L.donovani*. There

were 4 distinct subclusters seen within the Sri Lankan group with isolates that demonstrated poor response to standard drug (intra-lesional sodium stibogluconate) forming a separate subcluster. No specific clustering of clinical isolates based on their geographical origins across Sri Lanka was apparent in the dendrogram. Distinct genetic mutations with specific functional characteristics are likely to induce drug resistance. However, correlations between *L.donovani* minicircle based strain specific sequence heterogeneity and distinct clinical characteristics needs further investigation. Table of Contents

543

APPLICATION OF MOLECULAR TECHNIQUE FOR THE DETECTION OF VISCERAL LEISHMANIASIS IN BLOOD DONORS IN ENDEMIC AREA FOR LEISHMANIASIS IN FORTALEZA-CEARÁ, BRAZIL

Daniela C. Monteiro[1], Raissa Fontes Matos[1], Claudenia Costa Praciano[1], **Danielle Malta Lima**[2], Margarida Maria de Pompeu[1]

[1]Universidade Federal do Ceará, Fortaleza, Brazil, [2]Universidade de Fortaleza, Fortaleza, Brazil

Leishmaniasis is caused by protozoa of the genus *Leishmania* and is endemic in 98 countries, affecting the poorest mainly in developing countries. In 1991 the World Health Organization (WHO) raised the issue that there is the possibility of transmission of *Leishmania* by blood donors. The conditions of storage of blood products allow the survival and maintenance of infectivity of *Leishmania* in blood banks and to date, serologic screening is not done in blood donors in Brazil. In areas of transmission, asymptomatic carriers represent a large contingent, it is important to evaluate the possibility of blood products are a potential risk for transmission of the parasite. In this study, buffy coat of blood donors of the Center of Hematology Ceará (Hemoce), were analyzed by conventional PCR to look for circulating *Leishmania* and PCR positive sample were randomized to perform sequencing for confirmation of the parasite. From May to November 2011, 351 samples of buffy coat of blood donors were analyzed. All of them had a negative serology for Chagas Disease, Syphilis, HIV I and II, HTLV I and II, Hepatitis B and C. For the conventional PCR reaction, specific primers (150 and 152) for the genus *Leishmania* were used, described by Oliveira, 2005, referring to a sequence of the minicircle fragments kDNA with 110pb. The presence and integrity of the human DNA was verified by amplifying the β-globin gene, generating a fragment of 252 bp described by Pizzuto, 2001. DNA of *Leishmania* was detected in peripheral blood from 15/351 (4.3%) donors. The sequencing of 6/15 (40%) of the positive PCR confirmed that the isolated gene is belonging to the genus *Leishmania*. The results indicate the presence of *Leishmania* in blood donors and this implies in potential risk of transmission by blood transfusion depending on the parasite load. The role of asymptomatic donors infected with *Leishmania* in the chain of transmission is still not well established, so the possibility of transmission of the parasite by asymptomatic carriers must be better evaluated.

544

MOLECULAR CHARACTERIZATION AND DRUG SUSCEPTIBILITY OF *TRYPANOSOMA CRUZI* ISOLATES DERIVED FROM INFECTED HUMANS OF ARGENTINA

Luz P. Quebrada Palacio, Mariela N. González, Bibiana Volta, Alina E. Perrone, Yolanda Hernández Vasquez, Jacqueline Búa, Miriam Postan

Instituto Nacional de Parasitologia, Ciudad Autonoma de Buenos Aires, Argentina

The genotype of *Trypanosoma cruzi* was related to the transmission cycle and geographic region. Herein, we studied the phenotype and susceptibility to trypanocidal agents of 5 *T. cruzi* populations recently isolated from 3 chronically infected adults (AR-SE23C, BOL-FC10A and AR-FC553) and 2 children with congenital infection (AR-FC202113 and AR-FC195205) living in Argentina. Molecular analysis showed that all isolates belonged to DTU V. For drug sensibility studies, epimastigotes were cultured in LIT media (106/mL) and treated with 0.38-150 μM of Benznidazol (BZ), Nifurtimox (NX), Pentamindine isethionate (PENT) and dihidroartemisinin (DH) during 72 h; SylvioX10/4 clone was used as reference strain. AR-SE23C, AR-FC553 and AR-FC202113 isolates were as susceptible to BZ (IC50= 3.35±0.5; 7.66±0.0 and 2.25±1.0 μM respectively) and NX (IC50=5.06 ±0.2; 5.54±0.1 and 1.21±0.2 μM, respectively) as Sylvio-X10/4 (IC50= 4.22±0.4 and 5.29±1.3 μM for BZ and NX, respectively). In contrast, significantly higher dose of BZ (73.08±4.3 and 40.62±3.1 μM) was required to reach IC50 for BOL-FC10A and AR-FC195205 isolates, respectively, and of NX (14.11±2.3 μM) for AR-FC195205 isolate. AR-SE23C, AR-FC553, AR-FC202113 and AR-FC195205 were similarly susceptible to PENT, with ID50 ranging from 2.68±2.1 to 4.09 ± 0.1 μM; instead, 10.27±1.7 μM of this compound were necessary to reach IC50 for BOL-FC10A. All isolates were similarly resistant to DHIC50 as compared to BZ, NX y PENT. Preliminary studies on C3H/He mice infected with AR-SE23C trypomastigotes and treated with 100mg/kg/day BZ and NX during 30 days showed that conventional treatment is effective to diminish mortality rate and parasitaemia levels. Nevertheless, histopatological analysis revealed that neither drug was effective to clear tissue parasites and ameliorate the inflammatory process generated in the acute phase. In conclusion, we here confirm the predominant circulation of *T. cruzi* DTU V in Argentina, which includes subpopulations with a wide range of susceptibility to trypanocidal agents *in vitro*. MINCYT/FONCyT-PICT 2010-2148

545

UTILITY OF DIFFERENT CLINICAL SAMPLES OF *LEISHMANIA (VIANNIA) BRAZILIENSIS* LESIONS TO CONDUCT MICROSATELLITE ANALYSIS

Erika S. Perez-Velez[1], Maxy B. De los Santos[2], Juan F. Sanchez[2], Marianela Ore[3], Michael B. Deshotel[4], G. Christian Baldeviano[2], Kimberly Edgel[5], Andres G. Lescano[2]

[1]Universidad Nacional Mayor de San Marcos, Lima, Peru, [2]U.S. Naval Medical Research Unit - 6, Lima, Peru, [3]Comando de Salud del Ejercito, Lima, Peru, [4]Department of Tropical Medicine, Tulane University, New Orleans, LA, United States, [5]Department of Malaria, Naval Medical Research Center, Silver Spring, MD, United States

Leishmania (Viannia) braziliensis (LVB) is the main cause of cutaneous and mucocutaneous Leishmaniasis in the Americas. Microsatellites are DNA sequences repeated consecutively and present in abundance in non-coding regions of the eukaryotic genome. The variation in the number of repeats creates different alleles, allowing estimation of the genetic diversity of populations and gene flow. DNA from in vitro culture has been commonly used in Leishmania microsatellite analyses because of the quality of DNA and ease of standardization. However, parasite culture is difficult to obtain in field conditions. Other clinical samples are easier to collect, transport and maintain, but their use in population diversity studies has not been evaluated. Therefore, we assessed the viability and repeatability of microsatellite analyses based on different clinical samples from the same patients. We studied 22 cutaneous Leishmaniasis cases by LVB confirmed by nested real time polymerase chain reaction (PCR) and tested 53 clinical samples including cultures (22), biopsies (13), filter paper imprints (9) and scrapings using lancets (9). Samples were collected in Lima and six other cities of Peru during one year. Genomic DNA was extracted using the Qiagen DNeasy Blood and Tissue kit. Nine previously described microsatellite markers were tested by PCR and fragments amplified were analyzed to identify alleles using capillary electrophoresis in a 3130xl Genetic Analyzer (AB). We tested if all clinical samples amplified the nine microsatellite markers equally frequently and if PCR products from biopsies, lancets and filter papers showed the same allele peaks (± 2 base pairs) as PCR products from cultures. On average, cultures amplified significantly more markers than the other samples (8.4 versus 5.6 to 6.3) but all clinical samples showed the same alleles peaks as cultures for the nine markers in >90% of the cases. Seven markers had the same alleles in >95% of the cases. Culture strains of LVB amplify more microsatellite

markers compared to other clinical samples, but the alleles identified are similar across all type of samples. Considering that cultures are difficult to obtain in field conditions, the use of other clinical samples may allow a better characterization of the population structure and genetic diversity in this important parasite.

546

UNDERSTANDING THE MOLECULAR EPIDEMIOLOGY OF *TRYPANOSOMA CRUZI* I IN NORTH AMERICA: NEW ORLEANS FIRST APPROACH

Claudia P. Herrera, Meredith H. Licon, Samuel Jameson B. Jameson, Pierre Buekens, Dawn M. Wesson

[1]Tulane University, New Orleans, LA, United States

The most current nomenclature of *Trypanosoma cruzi* recognizes six discrete typing units (DTUs), TcI-VI and a seventh one named Tcbat. TcI is highly heterogeneous and can be divided into 5 distinct subgroups TcIa-TcIe, based on differential amplification of the miniexon intergenic region. Though *T. cruzi* is endemic to Latin America, it has a marked presence in the United States. Despite the presence of an adequate vector for *T. cruzi* in Triatoma sanguisuga and one of the United States' few areas with an autochthonous case of human Chagas disease, there exist few studies exploring the genetic variability of Southeastern Louisiana's *T. cruzi* parasite populations particularly with respect to the TcI haplotypes. In the presented study, 60 rodent hosts and 12 T. sanguisuga vectors were captured from the site of Louisiana's local human *T. cruzi* infection. DNA extractions were prepared from rodent tissues and from cultures established from vector feces. *T. cruzi* prevalence was determined by diagnostic PCR, subsequent PCR genotyping methods allowed for detection of specific TcI sub-genotypes. Amplification of *T. cruzi* satellite DNA revealed 76.6% of infection among sampled rodents. Twenty two *T. cruzi*-positive rodents could be genotyped by differential amplification of the mini-exon and 19 (86.4%) were found to be TcI. Eight samples of feces from vector (66.6%) were trypanosomatid- positive by direct microscopic observation. Six strains were successfully isolated and genotyped as TcI. All TcI samples were further typed as haplotype TcIa. The findings presented here corroborate existing literature on North American *T. cruzi* genetic distribution as well as the current proposed geographical distribution of the TcI haplotypes. Further studies are required to fully assess the broader applicability of this study; however, it provides an epidemiological snap-shot of the sampled region, enhancing our current understanding of regional *T. cruzi* phylogeography.

547

GENETIC EXCHANGE IN *LEISHMANIA TROPICA*

Stefano Iantorno[1], Kashinath Ghosh[2], Audrey Romano[2], James Cotton[3], Alain Debrabant[2], Phil Lawyer[2], Michael Grigg[2], Matt Berriman[3], David Sacks[2]

[1]National Institutes of Health/University of Cambridge, Washington, DC, United States, [2]National Institutes of Health, Bethesda, MD, United States, [3]Wellcome Trust Sanger Institute, Cambridge, United Kingdom

Leishmaniasis is a parasitic disease caused by protozoan intracellular parasites of the genus *Leishmania*. *Leishmania* species can show different clinical presentations, ranging from non-lethal cutaneous and mucocutaneous Leishmaniasis, which typically leave disfiguring scars, to visceral leishmaniasis, which can be lethal if left untreated. While traditionally thought to propagate asexually, hybridization has been unequivocally demonstrated to occur in the sand fly stages for L. major, and evidence has also been found more recently in L. donovani. L. tropica is an emerging Old World species that is responsible for severe cutaneous disease throughout its range, from India to Northern Africa. From a sample set of 36 isolates, 25 nuclear markers and 3 kinetoplastid DNA markers were amplified and sequenced for multi-locus genotype analysis (MLSA). The preliminary sequence data shows extensive heterozygosity, indicative of potential ongoing hybridization occurring in this species. From this set, 4 strains have been selected for introduction of three different drug resistance markers. Selection of double-drug resistant hybrids was performed by culturing midgut homogenates of co-infected sand flies in the presence of both drugs. Here we present genetic evidence illustrating patterns of inheritance and gene exchange in this Old World species.

548

DIVERSITY OF *TRYPANOSOMA CRUZI* INFECTION IN PATIENTS CO-INFECTED WITH HIV AND CHAGAS DISEASE

Natalie M. Bowman[1], Daniel Clark[2], Jeong Choi[2], Gerson Galdos Cardenas[3], Mauricio Dorn[4], Omar Gandirilla[5], Anne Palumbo[5], Lisbeth Ferrufino[6], Leny Sanchez[2], Lousia Messenger[7], Jorge L. Flores Franco[4], Ronny P. Colanzi Zeballos[6], Steven R. Meshnick[8], Caryn Bern[9], Robert H. Gilman[3], Jonathan J. Juliano[1]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]AB Prisma, Lima, Peru, [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [4]Hospital San Juan de Dios, Santa Cruz, Plurinational State of Bolivia, [5]AB Prisma, Santa Cruz, Plurinational State of Bolivia, [6]Universidad Catolica Boliviana San Pablo, Santa Cruz, Plurinational State of Bolivia, [7]London School of Tropical Medicine and Hygiene, London, United Kingdom, [8]University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC, United States, [9]University of California San Francisco, San Francisco, CA, United States

Chagas disease, caused by the protozoan *Trypanosoma cruzi*, causes an unusual and severe neurological syndrome in immunocompromised patients such as those infected with HIV. We characterized the genetic diversity of *T. cruzi* infection in several HIV-infected Bolivian patients with high-level parasitemia. Samples were derived from HIV-positive patients recruited into an epidemiological study of HIV/Chagas disease coinfection in Santa Cruz, Bolivia, and DNA was extracted from whole blood or guanidine-preserved samples using QiaCube extraction robot. *T. cruzi* infection was confirmed with RT-PCR. We used nested PCR to amplify a 327-base pair fragment of the polymorphic TcSC5D gene, which has previously been used for strain typing, and amplicon deep sequenced the region using an Ion Torrent PGM. We determined multiplicity of infection and genotyped the strains of *T. cruzi* using deep sequencing and conventional restriction fragment length polymorphism (RFLP) methods. Sequences were clustered to predict genotypes using a heuristic clustering algorithm. Within-host and within-population diversity indices were calculated using EstimateS. We have shown that deep sequencing of *T. cruzi* from clinical samples is possible. Furthermore, we have documented polyclonal infections in HIV-coinfected patients, which may have implications for the pathogenesis of the disease in this population. Further studies will examine parasite diversity in a larger sample size and compare *T. cruzi* strains found in blood and cerebrospinal fluid.

549

ARTINM ADMINISTRATION DURING ACUTE EXPERIMENTAL INFECTION WITH *TRYPANOSOMA CRUZI* PROMOTES PROTECTION AGAINST THE PARASITE

Camila B. Miguel[1], Thiago Aparecido Da Silva[2], Wellington Francisco Rodrigues[1], Maria Cristina Roque-Barreira[2], Javier Emilio Lazo Chica[1]

[1]Universidade Federal do Triângulo Mineiro, Uberaba, Brazil, [2]Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil

Chagas disease is caused by infection with *Trypanosoma cruzi*, and the profile of the host immune response is essential for the infection control. ArtinM, from *Artocarpus heterophyllus*, a lectin that binds to the manotriose, modulates immunity toward Th1 axis and confers resistance to intracellular pathogens. Then, we analyzed the ArtinM immunomodulatory property during acute experimental infection with *T. cruzi*: Male BALB/c mice (10 mice/group) were used at 10 weeks-old, and the infection was realized with *T.cruzi* (3000 trypomastigotes; "Colombian

strain"). The ArtinM administration (0.5µg/100µL; i.p.) was performed before infection (-5, -4 and -3 days) and post–infection (5, 10 and 15 days), and as negative control was used the vehicle (saline), followed by groups: Saline control (SC), Saline infected (SI) and Lectin infected (LI). Parasitemia was determined at 7th, 14th and 21th days post-infection. The hemogram, reticulocyte count, cytokines measurement (plasma and heart), histological analysis of heart (inflammatory infiltrate and nests of amastigotes) were performed after 23 days of infection. Inflammatory infiltrate was measured in H&E stained heart sections, through the point's grid with predetermined area that express the area (%) of inflammatory infiltrate. This method was used to determinate the number of nests of *T.cruzi* by immunohistochemistry. IL group shows significant decrease in parasitemia at 14th and 21th days of infection. IS and IL showed increased reticulocytes, total leukocytes, neutrophils, lymphocytes and monocytes, and the hematimetrics parameters were decreased, ArtinM administration promotes a significant reduction in the inflammatory infiltrate (% of area) and nests of *T.cruzi* in the heart. Moreover, low levels of IL-12 p40, IFN-γ and TNF-α were found after ArtinM treatment, and IL-10 production was elevated, verified in plasma and heart. Thus, ArtinM administration demonstrates a protective effect against T.cruzi during acute experimental infection.

550

INFLUENCE OF LOAD PARASITIC IN INFLAMMATORY RESPONSE AND DEVELOPMENT OF INTESTINAL INJURY IN MICE INFECTED WITH *TRYPANOSOMA CRUZI*

Javier Emilio Lazo Chica, Bruna Perez Vasquez, Camila Miguel, Thais Perez Vasques, Maria Tays Mendes, Carlo Jose Freire de Oliveira, Wellington Francisco Rodrigues

Universidade Federal Do Triângulo Mineiro, Uberaba, Brazil

The acute phase of Chagas' disease is reported to be multifactorial; among the forms of evolution of the most common disease have cardiac and digestive forms, this last in the esophagus and colon. Therefore, this study aims to evaluate variations of antigenic concentrations in the intestinal lesions in mice with acute *Trypanosoma cruzi* infection. We used 40 male mice C57BL/6WT divided into control group (A) and infected groups with $3x10^2$ (B), $3x10^3$ (C) and $3x10^4$ (D) forms trypomastigotes of strain "Y" of *T. cruzi*. From a total of eight groups, four were euthanized after 7 (S) days of infection, and the remaining groups after14 (F) days. The parasitemia was performed daily from day 3 after infection until the time of euthanasia. In the morphometric analysis, we verified the area myositis, ganglionitis and periganglionitis in relation the area of the muscle layer of the colon. Also analyzed the width and thickness of the muscular layer and of the colon mucosa. By immunohistochemistry, we detected the presence of *T. cruzi* nests. The quantification of the cytokines were performed by CBA. In results, there was an increase in parasitemia dependent on the concentration of inoculum between the different groups. Immunohistochemistry showed antigenic labeling in all animals of the group DF and in no animal of the BS group. The periganglionitis, ganglionitis and myositis were growing between the groups (significant differences), except to groups C and D, seven and fourteen days. The mucosal thickness showed no differences between groups. The width of the colon did not change between groups, except for DF, where there was enlargement. The thickness of the muscle layer is not changed in groups euthanized at 7 days, but after 14 days of infection in CF was hypertrophy and a reduction in DF. In the results of the cytokines, there was a noticeable change in the highest inoculum having an IL-2 after 7 and 14 days, and IL-6, IFN-g and TNF-a only 14 days, in which IL-10 did not differ significant between groups. In conclusion, the parasite load and the time of infection directly influence in the number of nests of *T.cruzi* present in the colon, but not interfere with the mucosal thickness. There is a hypertrophy of the muscle layer in CF and decreased thickness of muscle in DF at the expense of enlargement of the colon, with alterations significants in production of citokines proinflammatory.

551

LABORATORY FINDINGS OF ACUTE INFECTION WITH *TRYPANOSOMA CRUZI* FOR DIFFERENT CONCENTRATIONS OF THE STRAIN "Y" IN THE INOCULUM

Paulo Ricardo Dos Santos, Wellington Francisco Rodrigues, Camila Miguel, Patricia Kellen Martins Oliveira Brito, Carlo Jose Freire de Oliveira, Javier Emilio Lazo Chica

Universidade Federal do Triângulo Mineiro, Uberaba, Brazil

Hematological and urinary parameters are often used for screening and confirmation of various diseases. In Chaga's diseases highlighted the clinical and laboratory findings are the result of a multifactorial infection with *Trypanosoma cruzi*. Moreover, recent studies demonstrate that variations of the antigenic concentrations may influence the course of disease, as well as the functional response of the infected organism. This way, this study aimed to report the laboratory findings in acute experimental T. cruzi infection with different inoculums through hematological and urinary parameters. Mice C57BL/6, males, aged 8 to 10 weeks, weighing 20 to 25g were infected with different parasite loads ($3x10^2$, $3x10^3$ and $3x10^4$) through strain "Y" of *T. cruzi*. The curve of parasitemia was performed until the 12th day after infection. Hematological parameters were evaluated using the complete blood count (automated method - ABX MICROS 60) on days 6, 9 and 12 after infection. The analysis of urinary parameters was performed through physicochemical analysis and urine sediment after 9 days of infection. The onset of parasitemia curve varied with the concentration of parasites in the inoculums on the 3rd, 4th and 5th days, for high, medium and low, respectively. The period of peak occurred for the high, medium and low inoculums on days 10, 9 and 8 respectively after infection. Any assessment of the blood count (red blood count, reticulocyte count, white cells and platelets) and urinary parameters (sediment), we observed changes in the high-inoculums when compared with the control ($p < 0.05$). However laboratory evaluation by hemogram and urine routine are able to detect modifications, dependent on the parasitic load.

552

EVALUATION OF METABOLITES NITROGEN IN MICE CBL57BL/6 INFECTED WITH DIFFERENT INOCULA OF STRAIN "COLOMBIAN" OF *TRYPANOSOMA CRUZI*

Patricia Kellen M. Brito[1], Camila Miguel[1], Wellington Francisco Rodrigues[1], Ricardo Cambraia Parreira[2], Paulo Ricardo Dos Santos[1], Carlo Jose Freire de Oliveira[1], Javier Emilio Lazo Chica[1]

[1]Universidade Federal do Triângulo Mineiro, Uberaba, Brazil, [2]Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

Chagas' disease is a neglected tropical disease caused by *Trypanosoma cruzi*. Through increased catabolism, is associated also with renal disorders. This study aimed to evaluate the nitrogenous wastes from C57BL/6 mice infected with different inocula of *T. cruzi*. 70 mice male C57BL/6 were used at 12 weeks of age, weighing between 20 and 25g. The *T. cruzi* infection was performed with an inoculum of $3x10^2$, $3x10^3$ and $3x10^4$ trypomastigotes of strain "Colombian". The animals were divided in groups: non-infected control (GC); Infected with 300 forms (GIA); Infected with 3,000 forms (GIB) and Infected with 30,000 forms (GIC), both for 22 and for 31 days. Held parasitemia every 3 days, the 24-hour urine collection in individual metabolic cages and blood collection. Urea levels were made by ultra-violet method and creatinine by colorimetric kinetic method, both in blood and in urine 24 hours a spectrophotometer. There was a decrease in urinary volume on the GIC after 22 days of infection and GIB, GIC 31 days after infection, beyond the weight decrease of the GIC group of animals on both days. For the BUN there was an increase to of levels GIC (22 days) and GIC and GIA (31 days). Levels of urinary urea reduced in the group GIB (22 days) and in GIC (31 days). The plasma creatinine showed a tendency to increase in all groups after 22 days of infection, whereas after 31 days there was a reduction in the levels for GIB and GIC. Decrease in urinary creatinine levels in groups GIB and GIC

(22 days) and normal values for all groups 31 days. The BUN / creatinine ratio was elevated in GIC (22 days) and GIA and GIB (31 days). The levels creatinine clearance had decreased for GIB and GIC (22 days) while the group at 31 days showed normal levels. Conclusion: We conclude that there are changes in nitrogenous wastes in C57BL/6 mice infected with *T. cruzi*, dependent on the concentration of the inoculum.

553

POPULATION-BASED SURVEILLANCE STUDY OF INFLUENZA AND COMMUNITY-ACQUIRED PNEUMONIA MORBIDITY SYNERGISM IN UKRAINE

Sergii Soloviov, Iryna Dzyublyk

P.L. Shupyk National Medical Academy of Postgraduate Education, Kiev, Ukraine

Historically, research of infectious diseases has focused on infections with single pathogens. However, infections with pathogens often occur in the context of other pre-existing viral and bacterial infections or pathological conditions. Clinically, this is of particular relevance for co-infections with pathogen *Streptococcus pneumoniae* and influenza virus, which both are the important cause of global morbidity and mortality in the world. However, the analysis of incidence data, representing the possible synergy between community-acquired pneumonia (CAP), which possible causative agent is S. pneumoniae, influenza and other respiratory diseases (RD) (chronic bronchitis, asthma, etc.) has never been presented before in Ukraine. The aim of the research was the model-based study of the official incidence data to identify the possible relationship between CAP, RD and influenza morbidity rates during 2007-2011 epidemic seasons in Ukraine via mathematical modeling. The official incidence data, published annually by Influenza Control Center and FG Yanovsky National Institute of Phthisiology and Pulmonology, was analyzed. As a result it was proposed conceptual synergy model of CAP, influenza RD morbidity among the population of Ukraine. The model parameters were found by the program developed in Java and based on the quasi-gradient method. The highest CAP morbidity rate in Ukraine was in 2009-2010 yrs., exceeding corresponding value for 2008 by 24.2 % and for 2011 - by 5 %. The analysis of incidence data showed significantly higher morbidity rates than average in Vinnytsia, Ivano-Frankivsk and Kyiv regions. The mathematical model implied the existence of functional relationship between the incidence of influenza and RD in their influence on the occurrence of complications such as CAP. Results of modeling showed that probably about 20% of CAP among the population of Ukraine occur as a complication after influenza (individually or against the background of bronchitis, asthma and other pathological conditions related to RD). In conclusion, it was proposed the meaningful approach to modeling that takes into account the functional relationship between RD and influenza cases that led to CAP and show strong association between these diseases and necessity of intensive preventive strategies for its control.

554

THE FIRST ANTIGENIC/ANTIVIRAL CHARACTERIZATION OF INFLUENZA VIRUS ISOLATES RECEIVED DURING AUGUST 2009-SEPTEMBER 2012 FROM INFLUENZA SENTINELS SURVEILLANCE (ISS) IN ETHIOPIA

Gelila D. Tiyo

Ethiopian Public Health Institute, Addis Ababa, Ethiopia

Influenza is a respiratory disease caused by RNA viruses that affects birds and mammals. The vaccinations offered every flu season target types A and B Influenza Viruses. Detection of Influenza virus using Real Time-PCR technique started in Ethiopian National Influenza Reference Laboratory during the A (H1N1) 2009 pandemic and since then laboratory sends influenza positive samples to WHO collaborating center for Influenza at CDC Atlanta, for further antigenic characterization and drug sensitivity tests. A total of 145 samples that tested positive for influenza during August 2009-September 2012 were sent to CDC Atlanta for further antigenic characterization and drug sensitivity tests. In the reference Laboratory, the Influenza positive Isolates were characterized antigenically using hemagglutination inhibition test with a panel of post ferret antisera, and also tested for functional neuraminidase inhibition assay to assess susceptibility of the viruses to the neuraminidase inhibitors oseltamavir and zanamivir drugs. The results of 136(93.8%) isolates were available till September, 2012. 48.5 %(66) of the isolates were A/CALIFORNIA/07/2009-LIKE (H1N1) viruses,12.5 %(17) of the isolates were B/BRISBANE/60/2008-LIKE viruses, 4.4 %(6) of the isolates were A/PERTH/16/2009-LIKE (H3N2) GP viruses, 4.4 %(6) of the isolates were B/WISCONSIN/01/2010-LIKE viruses, 1.5 %(2) of the isolates were B/FLORIDA/04/2006-LIKE viruses, 2.2 %(3) of the isolates were B/VICTORIA/02/87,2.2 %(3) of the isolates were B/YAMAGATA/16L88 LINEAGE BY PCR, which is similar to B/Wisconsin/01/2010 virus. 21.3 %(29) of the isolates were unable to grow on the cell culture and was difficult for the characterization. 2.9 %(4) of the isolates were totally negative for Influenza Virus both by real time -PCR and cell culture techniques. All Influenza Positive Isolates were sensitive to Oseltamivir and Zanamivir on functional neuraminidase inhibition assay to assess susceptibility of the Influenza viruses to the neuraminidase inhibitors oseltamavir and zanamivir. All antigenically characterized Influenza Isolates were compatible with Influenza vaccine recommended by WHO. It also showed that diverse types of Influenza strains are circulating in Ethiopia. The inability of some positive isolates to grow in cell culture may possibly relate to sample collection, storage and sample transportation issues and need evaluations and improvements.

555

SIMULATION MODELLING OF SOCIAL PROTECTION INTERVENTIONS FOR TUBERCULOSIS (TB) AND CHRONIC AIRWAY DISEASES (CAD) IN MALAWI

Elvis S. Gama

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Catastrophic health care expenditure has emerged as one of the concerns among global health practitioners particularly in low income countries. To address this concern, social protection has been used as a framework to address healthcare related poverty and vulnerability in these countries. This has been followed by a growing number of governments in low income countries developing and adapting social protection strategies in their health plans. Similarly, there is also a rising interest in social protection among global health and health system researchers. Despite global interests and adoption of health care related social protection interventions in low income countries, the implementation, uptake, equity and effectiveness of current social protection interventions are limited by; inadequate prevailing national health budgets which are often donor dependent; policies prevalent within the wider health sector and failure of integration of social protection intervention by government agencies and the health sector in general. The research was aimed at finding ways of improving social protection interventions aimed at protecting the poor and vulnerable populations in Malawi, especially those affected by chronic airway diseases (CAD) and TB. The overall objective was to improve delivery of CAD and TB services, by successfully engaging policy makers, healthcare providers and various stakeholders, in the generation of new evidence about effective ways to strengthen the provision, uptake, equity and effectiveness of social protection mechanisms in TB and CAD treatment. Simulation modelling, econometric analysis and process evaluation studies were used to evaluate the process and impact of existing social protection intervention and generate new knowledge. Preliminary results indicate that, the involvement as partners of major stakeholders directly responsible for social protection policy and interventions will ensure policy relevance of this research and its continued impact beyond the life of the research project.

556

PNEUMOCOCCAL SURFACE PROTEIN A (PSPA) BASED PNEUMONIA VACCINE SHOWING ENHANCED PROTECTIVE IMMUNITY WHEN CONJUGATED TO VI POLYSACCHARIDE FROM *SALMONELLA TYPHI*

Neha Kothari

IVI, Seoul, Republic of Korea

Phase I data from trials using protein based pneumococcal vaccine antigens show poor immune response in humans. To address this problem protein antigen need to be presented in a way to induce a high immune response and subsequent protection in humans. We have developed a conjugate vaccine in which PspA family 1 and family 2 proteins from *Streptococcus pneumonia* are conjugated to Vi capsular polysaccharide from *Salmonella typhi*. The conjugation technology strongly boosts protective immune responses against *Streptococcus pneumonia* and *Salmonella typhi*.MethodWe have optimized a scalable high yielding method for fermentation and purification of Vi polysaccharide and two protein antigens of PspA Family. We have developed a method for conjugation of PspA Family 1 and 2 proteins with Vi polysaccharide and tested conjugates by ELISA for their immune response in mice. We have done preliminary challenge studies of the vaccine with pathogenic strains of *Streptococcus pneumoniae* to check vaccine efficacy.Results A series of Vi-PspA conjugates are prepared and tested in mice. A poor anti-PspA response was obtained when un-conjugated PspA was used as antigen but when conjugated to Vi a substantial increase in the responses were obtained. Immunized mice with selected Vi-PspA1 conjugate show 70-80% protection in the preliminary challenge studies done with Streptococcus pneumoniae. Further challenge studies with a Vi-PspA1 and Vi-PspA2 combination vaccine will be performed to enhance the protective immunity against both pathogens.ConclusionThe Vi-PspA production and conjugation process is developed for the purpose of Scale up and making a cost effective vaccine targeting developing countries.

557

ELABORATE HEALTH RECORDS SURVEILLANCE FOR TB CASE FINDING IN AN INFANT COHORT STUDY IN PREPARATION FOR PHASE THREE TB VACCINE TRIALS IN WESTERN KENYA

Joseph O. Opole

KEMRI/Centers for Disease Control and Prevention, Kisumu, Kenya

Finding every case of Tuberculosis in infant vaccine trials is important for efficacy endpoints. Health record surveillance could be a key source of TB suspects and therefore, TB Cases. We therefore, sought to evaluate the yield of health records surveillance for TB case finding in an infant cohort in preparation for vaccine trials.The study was a prospective, observational, cohort study. After enrolment, TB cases listed in TB registers were searched and matched with Health and Demographic Surveillance System data base to confirm residency and compared with the study database, if there was a march,further confirmation was done. Potential TB suspects were also generated from: Inpatient Department, TB laboratory,Patient Support Centre and X ray records.TB cases were found through both the active and passive detection systems. Interestingly the active case finding through scheduled TB screening follow-up visits generated only 8 cases (14.0%), whereas various "passive" surveillance systems located within the health system itself identified 72% of all TB cases. 12% of TB cases were diagnosed post mortem via verbal autopsy. In contrast to other infant cohort studies, incentivized individual health seeking behavior of parents likely played a significant role in increasing the effectiveness of the passive system. Elaborate Health records surveillance for TB case finding is labor and resource intensive, however, is likely to be more useful than routine TB screening to capture each endpoint in vaccine efficacy trials. Improvements are needed to reduce the proportion of TB cases detected post mortem

558

SPECIES AND SEROTYPE DIVERSITY OF HUMAN RHINOVIRUSES FROM PATIENTS PRESENTING WITH INFLUENZA-LIKE ILLNESS IN KENYA IN 2008

Sylvia Milanoi[1], Wallace Bulimo[1], Benjamin Opot[1], Silvanos Opanda[1], George Gachara[2]

[1]United States Medical Research Unit-Kenya, Nairobi, Kenya, [2]Kenyatta University, Nairobi, Kenya

Human Rhinoviruses (HRVs) are the most common causative agents of respiratory infections. They are highly diverse (>100 serotypes) and display a high degree of sequence variation among individual serotypes. This occurs as a result of frequent recombination events and point mutations.There is paucity of information about the genetic diversity of HRV strains circulating in Kenya. In this study we analyzed HRV strains identified in samples collected across the country in 2008 to provide an insight into their genetic characteristics. 517 randomly selected archived samples from the ongoing country-wide influenza surveillance protocol were used in this study. These were nasopharyngeal specimens collected from persons > 2 months, who attended outpatient clinics in the year 2008 in hospitals located in 8 different regions in Kenya presenting with influenza like illnesses. Real-time RT-PCR was used to identify HRV and VP4/VP2 genomic region amplified followed by sequencing. The resulting nucleotide sequences of Kenyan HRV viruses were compared to those of homotypic prototypes to determine serotypic identities. Screening by real time RT-PCR detected HRV in 131 (25%) of the samples. Of these 33 (25%) amplified successfully by conventional RT-PCR, of which upon nucleotide sequencing 17 (50%) yielded usable sequences. Phylogenetic analysis based on the VP4/VP2 genomic region of Kenyan HRV strains, relative to HRV prototypes retrieved from Genbank, revealed separation of the sequences into three main clusters corresponding to HRV A, B and C species. Majority of the Kenyan strains (n=10) belonged to HRVA species and were identified as HRV A29, A47, A1 (n=2), A56, A49, A30, A106, A20 and A45 serotypes. Two Kenyan strains belonged to HRV B species. One of these was identified as HRV B6 while the other was segregated from the other strains. Only one Kenyan strain belonged to HRV C species and was identified as HRV C2. This study demonstrates circulation of Human Rhinoviruses in Kenya. It also shows their species and serotype diversity.Findings from this study suggest that HRV A strains played a key role in human respiratory infections in Kenya. Knowledge about circulating HRV strains is important as it may help guide development of therapeutic strategies against infections caused by these viruses.

559

NEURAMINIDASE INHIBITOR SUSCEPTIBILITY OF INFLUENZA A ISOLATES OBTAINED IN KENYA, 2008-2011

Meshack Wadegu, Wallace Bulimo, Rachel Achilla, Silvanos Mukunzi, Janet Majanja, Janet Nyambura, Benjamin Opot, James Njiri, Steven Ocholla, Rodney Coldren

United States Army Medical Research Unit-Kenya, Nairobi, Kenya

Neuraminidase inhibitors mainly oseltamivir and zanamivir function both as prophylactic and treatment agents. Currently there exists no data on antiviral susceptibility profile of influenza A isolates circulating within the Eastern African region. Here we characterized the antiviral susceptibility of the 2008-2011 influenza A viruses circulating in Kenya. RNA was extracted from virus isolates followed by PCR amplification of NA gene segments. Nucleotide sequencing of the NA amplicons was carried out using the BigDye chemistry prior to analyses using a suite of bioinformatics tools. IC50 values were determined using curve fitting software, Grafit 7.0. Out of 836 influenza A viruses 108 isolates were analyzed for markers of resistance to NA inhibitors. 7 of the 11, 2008 seasonal influenza A/H1N1 isolates depicted oseltamivir resistant marker H275Y while all 33 influenza A/H3N2 isolates had H275 hence were sensitive to oseltamivir. Similarly, genetic analyses of the A (H1N1) pdm09 strains in 2009 and 2010 showed that all had H275 marker. All the 14 influenza A/H3N2

isolates of 2011 had H275 marker. A total of 28 isolates were analysed for phenotypic susceptibility assay. The mean zanamivir IC_{50}s were 1.75nM, 2.53nM and 1.84nM for the subtypes H1N1, pH1N1 and H3N2 respectively. Most of the 2008-2009 (8) sH1N1 analysed showed highly reduced sensitivity to oseltamivir. The IC50s in the fluorescent assay ranged from 73nM-984nM. Pandemic A/H1N1 strains obtained between 2009-2011 indicated oseltamivir IC50 ranges of 1.60nM-6.32nM categorised as normal sensitivity. All the 8, influenza A/H3N2 isolates obtained between 2008-2011 were sensitive to oseltamivir with the IC50s ranging between 0.16nM and 0.94nM. The 2011, WHO range and median IC50 values for oseltamivir carboxylate were 0.4nM-10nM and 0.5nM; 0.1nM-5nM and 0.2nM; 0.2nM-10nM and 0.6nM for wild types sH1N1, sH3N2 and pH1N1 respectively. The 2011, WHO range and median IC50 values for oseltamivir carboxylate were 257nM-3455nM and 458.2nM; 132nm-2179nM and 191.3nM for mutant types sH1N1 and pH1N1 respectively. The WHO IC_{50} values for zanamivir both for mutant and wildtype strains ranged between 0.2nM-3nM for all subtypes with no significant difference between the mutant and wildtype strains for each subtype. H275Y mutation increased the IC50 in the 2008-2009 sH1N1 isolates by 50-100 fold. Resistance to NAI was found to be both drug and virus subtype specific.

560

CHALLENGES FACING TB CONTROL IN INDIA FOCUSING ON THE ROLE OF XPERT MTB/RIF IN THE PUBLIC AND PRIVATE SECTOR

Ruvandhi Nathavitharana

Beth Israel Deaconess Medical Center, Boston, MA, United States

Globally, tuberculosis (TB) remains a major public health issue with an estimated 8.8 million new cases and 1.3 million deaths reported in 2012. In India, this health issue is compounded by the failure to diagnose cases in the national surveillance system and the high incidence of TB drug resistance. Of the estimated 3 million cases missed by national notification systems globally, 31% were in India. Drug resistance is of increasing concern with 3.6% of newly diagnosed TB cases and 20% of previously treated patients having multidrug-resistant TB (MDR-TB), defined as Mycobacterium tuberculosis resistant to isoniazid and rifampin, with or without resistance to other first-line drugs. There were an estimated 99 000 cases of MDR-TB in India in 2009, including those outside the Revised National TB Control Program (RNTCP). The Indian RNTCP introduced the Programmatic Management of Drug Resistant TB (PMDT) to address the needs of this patient population and is rapidly scaling up its services. A key issue in the management of MDR-TB is timely and accurate diagnosis (including drug susceptibility testing). There is interest in the promise of point-of-care (POC) diagnostics for diseases of global health importance but a need for better appreciation of the POC testing process moving beyond the technology alone. One area of progress is the global scale up of Xpert MTB/RIF (Cepheid Inc.), a molecular test for TB that also enables rapid detection of rifampin resistance. However, a greater understanding of role that the large, unregulated private health care sector plays in providing TB care is essential. The Initiative for Promoting Affordable Quality TB Tests (IPAQT) endorses the use of WHO approved tests such as Xpert MTB/RIF in private labs at affordable rates. Focus group interviews were conducted with health care providers from the public and private health care sectors, the latter at sites involved with the IPAQT initiative, to ascertain current practices and perceptions and attitudes to inclusion Xpert MTB/RIF in the TB diagnostic algorithm. Perceived barriers to implementation include delays in obtaining results and cost.

561

TUBERCULOSIS AND WAR IN THE U.S. MILITARY

James Mancuso

Uniformed Services University of the Health Sciences, Bethesda, MD, United States

Studies which have demonstrated increased morbidity and mortality from tuberculosis (TB) during war have largely focused on civilian populations. TB also has a long and well-established association with the military, but the association of increased TB risk during armed conflict is less certain. The purpose of this study was to examine the U.S. military experience in times of war and armed conflict to better understand its impact on the risk of TB. This historical study estimates the risk of TB infection, disease and mortality during these conflicts, comparing those serving overseas with civilian and military populations remaining in the U.S. TB rates in the U.S. Army declined dramatically over the period from 1885 to 2012, from a high of 1,168 per 100,000 in 1917 to a low of 0.4 per 100,000 in 2012. Army rates were generally considerably lower than civilian rates, due in large part to the "healthy soldier" effect. Other than World War I, armed conflict showed very little impact on declining TB trends. Some of the focal risk groups known to have higher rates of TB included U.S. and enemy prisoners of war (POWs), the foreign born, and others found to be infected at induction into the military. Although the risk of TB in the U.S. military largely reflects that of the underlying U.S. population, the military also has unique exposures to tuberculosis during times of armed conflict. In order to protect the health of these troops and conserve military fighting strength, these unique exposures require additional surveillance and control measures.

562

FEASIBILITY ASSESSMENT FOR ESTABLISHING SURVEILLANCE FOR EMERGING RESPIRATORY VIRUSES IN NON-PUBLIC HOSPITALS IN KENYA, 2013

Joan Karanja[1], Wences Arvelo[2], Phillip Muthoka[3], Daniel Kaburu[1], Rosalia Kalani[3], Henry Njuguna[2], Josh Mott[2]

[1]Field Epidemiology and Laboratory Training Program, Kenya, Nairobi, Kenya, [2]Centers for Disease Control and Prevention, Nairobi, Kenya, [3]Disease Surveillance and Response Unit, Ministry of Health, Kenya, Nairobi, Kenya

Influenza A H7N9 and Middle East respiratory syndrome coronavirus (MERS-CoV) are emerging respiratory viral infections with pandemic potential which can result in high fatality. As of March 2014, 395 cases and 125 deaths of influenza A H7N9 and 206 cases and 86 deaths of MERS-CoV had been reported worldwide. Kenya's high volume of international travel poses a risk for introduction of these pathogens. Public hospitals in Kenya conduct severe acute respiratory illnesses (SARI) surveillance. Little is known about SARI surveillance in non-pubic hospitals where majority of foreigners and travelers are likely to seek care. We conducted an assessment to determine the capacity of non-public hospitals to conduct SARI surveillance and to identify hospitals for implementation of MERS-CoV and influenza A H7N9 surveillance. In November 2013, we interviewed managers from 44 non-public hospitals in Nairobi and Mombasa counties. Respiratory illness accounted for a range of 0-18 admissions per day. A surveillance focal person was present in 32 (73%) hospitals, of whom 10 (23%) had received training in the last 12 months. Although requested by the Ministry of Health, national reporting of SARI was done in 18 (41%) hospitals; 12 (67%) reported monthly and 6 (33%) weekly. Among 28 (64%) hospitals which recorded patients' nationalities, foreigners accounted for a median of 10% (range: 0.5%-80%) of patients. Nasopharyngeal (NP) and oropharyngeal (OP) swab specimens were collected for clinical diagnosis in 27 (61%) and 29 (66%) hospitals respectively. In the last 12 months, 10 (23%) hospitals had trained their staff on NP/OP specimen collection, packaging, storage and transport to reference laboratories, and 27 (63%) had training on use of personal protective equipment. Non-public hospitals are an

important source of medical care for foreigners and the basic capacity for surveillance exists. These hospitals will be sensitized on the importance of national reporting of priority conditions, and some will be targeted for implementation of MERS-CoV and influenza A H7N9 surveillance.

563

THE ACCURACY OF NON SEVERE PNEUMONIA DIAGNOSIS IN TANZANIAN CHILDREN: THE VALIDITY OF THE RESPIRATORY RATE

Florida J. Muro[1], Nicole Zamsaeur[2], Hannah Wangai[3], George Mtove[4], Jim Todd[1], Raimos Olomi[1], Hugh Reyburn[2]

[1]Kilimanjaro Christian Medical University College, Moshi, United Republic of Tanzania, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Joint Malaria Project - KCMC, Moshi, United Republic of Tanzania, [4]National Institute of Medical Research, Amani Centre, Tanga, United Republic of Tanzania

Integrated Management of Childhood Illness (IMCI) guidelines recommend antibiotic treatment to all children presenting with cough or difficult breathing and an increased respiratory rate for their age. However, many pneumonia diagnoses in resource poor settings are done on the bases of sub-optimal condition; the environment may not be conducive for counting respiratory rates. We conducted a study to determine if respiratory rate was likely to be transiently raised by a number of contextual factors in a busy clinic leading to overuse of antibiotics. Respiratory rates were recorded in children aged 2 - 59 months presenting with cough or difficulty breathing to one of the two busy outpatient clinics and then repeated at 10 minute intervals over 1 hour in a quiet setting. A total of 167 children were enrolled with a mean age of 7.1 (SD±2.9) months in infant and 27.6 (SD±2.8) months in the older age group. The mean respiratory rate declined from 37.5 breaths per minute (bpm) at clinic to 35.8 bpm initial reading at quiet room and 35.0 bpm final reading ($p<0.02$). This resulted in 11(85%) being mis-classified with non severe pneumonia in infants and 2(13%) in older children. In a logistic model age group (infant or older child) was the only risk factor associated with over-diagnosis of non severe pneumonia. Over-diagnosis of non severe pneumonia in a busy clinic is significant and it tends to vary with age. Changing the respiratory rates cut-offs to higher threshold reduced the proportion of non-severe pneumonia mis-classification in infants. These findings have public health impact in managing these children as antibiotic over-use accelerates high levels of resistance. More studies of the accuracy and utility of respiratory rate as an indication for antibiotics are needed, especially as vaccines against bacterial pneumonia are introduced to many resource poor countries.

564

MICROSCOPIC OBSERVATION DRUG SUSCEPTIBILITY (MODS): A RAPID DIAGNOSIS OF PULMONARY TUBERCULOSIS IN HIV/AIDS PATIENTS IN RESOURCE-SCARCE BOLIVIA

Melissa Reimer-McAtee[1], Meredith Lora[2], Robert Gilman[3], Daniel Lozano Beltran[4], Caryn Bern[2], Monica Pajuelo[5], Faustino Torrico[4]

[1]Tulane University, New Orleans, LA, United States, [2]University of San Francisco California, San Francisco, CA, United States, [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [4]Universidad San Simon, Cochabamba, Plurinational State of Bolivia, [5]Universidad Peruano Cayetano Heredia, Lima, Peru

The need for fast, reliable diagnosis of *Mycobacterium tuberculosis* (Mtb) infection is particularly acute in patients with HIV. This study examined the rates of tuberculosis (TB) and multidrug-resistant tuberculosis (MDRTB) in HIV patients in Bolivia. It evaluated the Microscopic Observation Drug Susceptibility rapid liquid culture (MODS) versus traditional Ziehl-Neelsen staining (ZN) and Lowenstein Jensen culture (LJ), and assessed the value of the string test and induced sputum in sputum-scarce individuals. The presence of Mtb in sputum of 107 HIV-positive patients was evaluated by ZN, LJ, and MODS. Mtb detection by string test was evaluated by MODS in 92 of these. The TB-HIV co-infection rate of HIV patients with respiratory symptoms by sputum sample was 44.9% (48/107). The mortality of TB+ hospitalized patients was 51.4% (18/35) and of ambulatory patients was 30.7% (4/13). The rate of MDRTB was 9%. Of 48 sputum samples positive by any diagnostic method, 63% were positive by ZN, 79% by LJ, and 96% by MODS. Median time to positive culture was 10 days by MODS versus 34 days by LJ ($p<0.0001$). In pts not able to produce sputum without induction, the string test had a sensitivity of 82% compared to induced sputum. Of the ten patients unable to produce a sputum sample, four were TB-positive by the string test. MODS was faster and more sensitive for Mtb detection when compared to LJ, and these differences are more pronounced in smear-negative patients, who are at the greatest risk for missed diagnoses. The string test, in conjunction with MODS, is a valuable diagnostic technique for HIV positive sputum-scarce patients. Nine percent of our patients had MDRTB, which reinforces the need for rapid detection of antibiotic sensitivity testing in HIV patients in Bolivia.

565

HEALTH RISK ASSESSMENT OF PESTICIDES AND POLYCHLORINATED BIPHENYLS CONTAMINATIONS IN DAIRY PRODUCTS FROM SELECTED FARMS IN GREATER ACCRA REGION, GHANA

Douglas Amoako

University of Ghana, Accra, Ghana

The residual concentrations of synthetic chemicals such as organochlorines pesticides (OCPs), pyrethroids and polychlorinated biphenyls (PCBs) in dairy products (milk, yoghurt, cheese) from selected farms in Ghana were analyzed using Gas Chromatography (GC). A total of 50 samples of dairy products (9 cheese, 25 cow milk and 16 yoghurt) were analyzed for OCPs, pyrethroids and PCBs. Of the numerous pesticides evaluated, detectable levels of OCPs (β-HCH, endrin, heptachlor, endosulfan, p'p-DDT and methoxychlor);Pyrethroids (permethrin, allethrin, cypermethrin and deltamethrin) and PCBs (18, 28, 52,101, 153, 138, and 180) were found in all the dairy product samples analysed. Milk samples were found to be the most contaminated with respect to the OCPs and the levels ranged between 0.0001µg/ml and 0.0407µg/ml. β-HCH was the highest OCP with concentration of 0.0407µg/ml while Cyfluthrin was the highest synthetic pyrethroids recorded in yoghurt sample(0.0318µg/ml).The highest PCB 18 (2,2,5-Trichlorobiphenyl) recorded (0.2668µg/ml) in yoghurt samples. Data obtained from the field regarding safe use of pesticides and symptoms among farmers was very high. The estimated dose for γ-chlordane(8.5x10-5µg/ml), endrin(0.0114 µg/ml)p'p'-DDT(8.5x10-5µg/ml),DDE(8.5x10-5µg/ml),heptachlor(2.8x105µg/ml),dieldrin(6.8x10-5µg/ml) do not pose a direct hazard to human health, although present in milk samples since the values were lower than toxic threshold as well as,reference,doses (γchlordane: 0.0005 µg/ml; endrin:0.20µg/ml; p'p'DDT:0.50 µg/ml; DDE:0.50µg/ml; Heptachlor: 0.0001 µg/ml; and Dieldrin:0.005 µg/ml) and may indicate minimum risk to human. However, β-HCH(0.0375 µg/ml), endosulfan(0.0142 µg/ml), methoxychlor(0.0746 µg/ml) and PCBs (0.0498 µg/ml) levels exceeded the reference doses of (β-HCH:0.003 µg/ml, endosulfan:0.006 µg/ml, methoxychlor:0.005 µg/ml and PCBs:0.002 µg/ml) in children between the ages of 0-1 year, 1-11 years and adults indicating a great potential for systemic toxicity in all age groups especially children who are considered to be the most vulnerable population

566

DESCRIPTIVE CHARACTERIZATION OF CHOLERA OUTBREAK CAUSED BY BREAK DOWN OF PUBLIC PIPE BORNE WATER, OKE ALAAFIA COMMUNITY, OYO STATE SOUTHWESTERN NIGERIA SEPTEMBER 2013

Elizabeth B. Adedire[1], Abass O. Gbolahan[2], Ikeoluwapo O. Ajayi[3], Aishat B. Usman[1], Olufunmilayo O. Fawole[1]

[1]Nigerian Field Epidemiology and Laboratory Training Program, FCT Abuja, Nigeria, [2]Ministry of Health, Ibadan, Oyo State, Nigeria, [3]University of Ibadan, Ibadan, Oyo State, Nigeria

Cholera is an acute illness with profuse watery diarrhea caused by *Vibro cholerae* serotypes 01 or 0139. In Nigeria, frequent outbreaks do occur. Effective interventions to control these outbreaks require the identification of the source and risk factors for infection. In August 2013, an outbreak of cholera occurred in Egbeda LGA. We investigated the outbreak to determine its magnitude, source, possible risk factors and initiate control measure. We reviewed cholera case-based line lists from health facilities, hospital records and conducted active case search for cases. We defined a suspected case as any resident of Egbeda, two years or above, with acute watery diarrhoea with or without vomiting between 26th August and 10th September, 2013. We used structured questionnaire to collect data on demographic characteristics, clinical informations, and risk factors. Data were analyzed with Epi-info software and Microsoft excel. Environmental assessment of water sources, water sampling, latrine use and waste disposal methods. We collected and analyzed 5 stool samples as well as 5 well water samples There were a total of 28 cases and 7 deaths case fatality rate of 25%. Twenty seven (96.4%) of cases were from Oke-alaafia community. Median age of cases 10.5yrs (range 2-65yrs); five of the deaths occurred among children 0-5years;(71.4%). Seventeen of the cases were males (60.7%). Major sources of water were wells (38.5%), 61.4% of respondents had no toilet facilities hence indiscriminate defaecation was common. Open dumping was the commonest (80.8%) waste disposal method. Vibrio cholerae 01 was isolated in 3 (60%) of stool samples analyzed. The outbreak probably occurred as result of drinking water from contaminated sources of water such as wells following break down of public pipe borne water. Chlorination of wells was done and we conducted intensive health education of Community members on proper storage and household water treatment.

567

BACTERIOLOGICAL QUALITY OF WELL WATER USED FOR DRINKING PURPOSES IN GAROUA, NORTH CAMEROON

Moussa Djaouda[1], Thomas Njiné[2], Song Liang[3], Daniel Ebang Menye[1], Bouba Gaké[4], Serge Hubert Zébazé Togouet[2], Moïse Nola[2]

[1]University of Maroua, Maroua, Cameroon, [2]University of Yaoundé 1, Yaoundé, Cameroon, [3]University of Florida, Gainesville, FL, United States, [4]Centre Pasteur du Cameroun, Annexe de Garoua, Garoua, Cameroon

Groundwater serves as a major source of drinking water in North Cameroon. Water quality is an important determinant of human health, considering in particular waterborne diseases in local communities. This study aimed at assessing the bacteriological quality and potential sources of well water contamination in Garoua, a metropolis of North Cameroon. The water quality of 23 wells was assessed through commonly used microbiological tests. Also analysed were physicochemical parameters of the water. For each well, monthly sampling was performed during 10 months; physical characteristics of sampling sites were documented and potential sources of contamination were identified. Results showed that the abundance of heterotrophic aerobic and mesophilic bacteria (HAMB) and bacterial bio-indicators in well water in Garoua all exceeded the WHO's drinking water standards. Total coliforms were present in all well water samples at high concentrations ($5.0x10^2$ to $4.8x10^4$ CFU/100 ml of water). The water harbours relatively high concentrations of faecal coliforms ($1.2x10^2$ to $2.3x10^4$ CFU/100 ml of water). *Escherichia coli* and faecal streptococci concentrations showed high spatial and seasonal variations from one well to another. The physicochemical analysis showed that, in 52.17% of wells, water was acidic with various mineralization. The principal component analysis (PCA) pointed out that seasonality had less influence on the majority of measured water parameters (pH, electrical conductivity, total dissolved solids, and salinity) than the location of the well water point. Human wastes from the traditional latrines system extensively used in this area and animal manure might have contaminated the wells.

568

WATER RESOURCES DEVELOPMENTS IN ETHIOPIA: POTENTIAL BENEFITS AND NEGATIVE IMPACTS ON THE ENVIRONMENT, VECTOR-BORNE DISEASES AND FOOD SECURITY

Delenasaw Yewhalaw

Jimma University, Jimma, Ethiopia

To satisfy the growing demand for electricity, Ethiopia plans to increase its electricity production five-fold between 2010 and 2015, mainly through the construction of dams. A literature review shows that while dams can boost power and agricultural production, promote economic development and facilitate flood control, they can also lead to environmental, ecological and socioeconomic changes. Several case studies show that dams may alter the composition and density of vectors and intermediate host species, increase the incidence of malaria schistosomiasis and possibly lymphatic filariasis and lead to eutrophication of reservoirs, soil erosion and earthquakes. There is evidence that dams and commercial irrigation schemes can increase soil and water degradation, vulnerability to drought and food insecurity in riverine and lacustrine areas downstream of dams. It appears that dams in Ethiopia are also vulnerable to high soil erosion rates and earth quakes. Consequently, the current and proposed large-scale dam construction program in Ethiopia requires in-depth research to improve our understanding of the unintended negative effects of projects and to guide the location, design and implementation of appropriate preventive and remedial programs .

569

EXPLORING ADAPTATION MEASURES FOR INFECTIOUS DISEASES IN MACHALA, ECUADOR

Mercy J. Borbor-Cordova[1], Anna M. Stewart Ibarra[2], Cristina G. Recalde[3], Efrain F. Beltran[4], Raul Mejia[3]

[1]Escuela Superior Politechnica del Litoral, Guayaquil, Ecuador, [2]Upstate Medical University, Syracuse, NY, United States, [3]Instituto Nacional de Meteorologia e Hidrologia, Guayaquil, Ecuador, [4]Ministerio de Salud Publica, Machala, Ecuador

Ecuador is facing new challenges related to climate change impacts in the human health sector, especially for emerging infectious diseases, such as dengue fever. Although the public health sector is investing substantially in measures of surveillance and mosquito control, the effects of climate variability and change are not considered in the current strategies for reducing dengue virus transmission. Effective planning for adaptation measures in the public health sector would require the following: a) a better understanding of climate and environmental interactions with the vector and disease transmission in which a monitoring and surveillance climate-disease program is fundamental b) an evaluation of the effectiveness of current policies and measures in reducing the vector disease including 'what if' analysis in projected scenarios, and finally c) building scientific capacity in research institutions and looking for a permanent dialog with stakeholders and decision makers to discuss the adaptation measures on the climate change impacts on infectious diseases. With this perspective, a consortium of national and international institutions and local public health organizations is developing an interdisciplinary research program on dengue transmission in the city of Machala, Ecuador. This 3-year study will develop predictive models by

linking climate factors with local neighborhood social and environmental conditions to predict when and where dengue outbreaks will occur in Machala, Ecuador. This study aims to link multi-scale climate phenomena to neighborhood-level dengue risk, fundamentally changing how we think about climate-disease dynamics. At the same time, an iterative process of monitoring and evaluation of adaptation measures linked to decision making aids and tools is proposed for developing climate change policy in the public health sector.

570

OCCUPATION-RELATED SELF REPORTED HEALTH PROBLEMS AMONG SOLID WASTE HANDLERS IN A RAPIDLY URBANIZING COASTAL COMMUNITY IN GHANA

James-Paul Kretchy[1], Flemming Konradsen[2], Mawuli Dzodzomenyo[3], Thilde Rheinlander[4], Irene Ayi[5], Julius Fobil[3], Anders Dalsgaard[6]

[1]University of Ghana/University of Copenhagen, Accra, Ghana, [2]University of Copenhagen, Copenhagen, Denmark, [3]University of Ghana School of Public Health, Accra, Ghana, [4]University of Copenhagen School of Global Health, Copenhagen, Denmark, [5]Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana, [6]University of Copenhagen Faculty of Health and Medical Sciences, Copenhagen, Denmark

This study applied a mixed method design to investigate exposure to waste, use of personal protective gear, and self-reported health problems among solid waste handlers in a semi-urban township of southern Ghana. A total of 280 waste handlers were studied representing all types of waste handling practices including sweepers, collectors, transporters and disposers of waste. Most waste handlers engaged in multiple of these activities (69.3%). The most commonly reported health problems were bodily pains (56.4%), headache (38.6%), fever (35.7%), feelings of physical discomfort (28.2%), and diarrhoea (11.4%). In-depth interviews with 22 waste handlers also highlighted eye problems, stomach pains and non-specific symptoms such as stress and tiredness as commonly experienced. There was a correlation between exposure of bare body parts of waste handlers and disease, with a higher likelihood to report fever for those using bare hands during waste handling [odds ratio (OR) = 1.89 (95% C.I 1.37 - 2.56), $p < 0.0001$] and diarrhoea [OR = 6.25 (95% C.I 4.17 - 10.00), $p < 0.0001$] compared with those who used protective gear. Interviews showed that waste handlers generally had basic knowledge about the disease preventive purpose of wearing personal protective measures. However, observations revealed that most waste handlers did not use such measures consistently mainly due to discomforts, impracticalities of wearing it in hot and humid conditions, and not being supplied with protective gear by employers or not being able to invest in it themselves. The study indicates that waste handlers experience a burden of disease which may be consequences of their occupation. Our findings stress that waste handlers in rapidly urbanizing areas need protection against occupational diseases through the wearing of affordable and suitable protective gear. Waste companies and government institutions employing a growing number of waste handlers should train waste handlers in the proper use of protective gear, educate on how to protect their health and to provide such protective gear.

571

VENO-OCCLUSIVE LIVER DISEASE IN CHILDREN AND YOUNG ADULTS: AN EMERGING PROBLEM IN DEVELOPING COUNTRIES

Maria Teresa Giordani[1], Paolo Fabris[1], Alessandro D'alessandro[1], Francesco Paolo Russo[2], Enrico Brunetti[3], Francesca Rinaldi[3], Francesca Tamarozzi[3], Annarosa Floreani[2]

[1]San Bortolo Hospital, Vicenza, Italy, [2]University of Padua, Padua, Italy, [3]University of Pavia, Pavia, Italy

Veno-occlusive liver disease (VOLD) is increasingly reported in young people from developing countries, particularly in the Arabian peninsula and in countries from the former Soviet Union. Many conditions can be associated with this multifactorial disease: parasitic diseases like CE and schistosomiasis, as well as TB, abscesses, cysts or the presence of a membraneous web that obstructs the terminal portion of the inferior vena cava . Poverty, malnutrition, recurrent bacterial infections, and filariasis have been suggested to be predisposing factors for inferior vena cava occlusion in developing countries , whereas myeloproliferative neoplasms, abdominal cancer and oral contraceptives are associated with this syndrome in developed countries. However, environmental toxic causes are described in children and young adult living in rural areas: particularly, pyrrolizidine alkaloids derivatives can cause the disease by microscopically damaging the hepatic venular bed. We report a case of a 25 year old male immigrant from a rural area of Morocco admitted to our Hospital for ascites, mild cholestasis and abdominal pain in October 2013. All tests resulted negatives except quanti FERON gold test, so the patient was unsuccessfully treated for TB for 2 months. The patient clinical conditions and LFT slowly worsened and after 2 inconclusive hepatic biopsies , a third biopsy combined with ultrasound and CT scan findings allowed the diagnosis of VOLD. All commonest causes of VOLD were ruled out, therefore, a toxic cause due to pyrrolizidines alkaloid derivatives was considered. The patient is currently on the wait list for liver orthotopic transplantation due to end-stage liver failure. This finding warrants more attention to the toxic environmental agents in developing countries , as they need to be considered in the differential diagnosis of liver failure also in immigrants to Europe.

572

SOCIAL AND ENVIRONMENTAL DETERMINANTS OF CHILDHOOD DIARRHEAL DISEASE IN MALAWI: A NATIONWIDE, HOUSEHOLD-LEVEL, GEOSPATIAL ANALYSIS

Leslie A. Arney[1], Peter S. Larson[2], Mark L. Wilson[2]

[1]William Davidson Institute, Ann Arbor, MI, United States, [2]University of Michigan School of Public Health, Ann Arbor, MI, United States

Diarrhea continues to be a leading cause of death among children under-five in developing countries. Despite increased access to improved water and sanitation in some settings, many areas of sub-Saharan Africa and Asia have been left behind. The complex and multifactorial nature of associations with childhood diarrheal disease complicate understanding of the more critical determinants of risk. We analyzed data from the 2010 Malawi Demographic and Health Survey to identify risk factors for diarrhea among Malawian children. A hierarchical logistic regression model and a GIS-based raster analysis using inverse distance weighted interpolation were used to evaluate the independent effects of sanitation on childhood diarrheal prevalence, and to characterize the distribution of diarrheal disease and its determinants across Malawi. Approximately one-quarter of all households reported at least one child under-five having diarrhea in the two weeks preceding the survey. Households with an average child age between 6-12 months had six times the odds of childhood diarrhea when compared to households with an average child age less than 5 months. While 79% of households had access to an improved water source, only 12% of households were using an improved sanitation facility, with an additional 11% lacking access to any sanitation facility. Households with no sanitation facility had 45% greater odds of childhood diarrhea as compared to those with an improved and unshared sanitation facility. In multivariable analyses, the significant effect of sanitation on diarrhea prevalence remained after adjusting for child age and maternal education. This study calls renewed attention to the persistent, yet preventable, burden of diarrheal disease among children in Malawi and reveals important District- and sub-District-level variation in the distribution of determinants that could be used to inform the targeting of future interventions.

573

PERVASIVE EXPOSURE TO FECAL CONTAMINATION IN LOW-INCOME NEIGHBORHOODS IN ACCRA, GHANA

Jacqueline M. Hurd, Monique Hennink, Christine Moe, Claire Null, Katherine Robb, Habib Yakubu, Dorothy Peprah

Emory University, Atlanta, GA, United States

Globally, diarrhea contributes to about 800,000 fatalities in children under five each year, and is a primary cause of mortality in developing countries. Rapid urbanization in low-income countries has led to a growing sanitation crisis. A need exists for more effective WASH interventions in low-resource urban environments that can minimize the transmission of feces and reduce the rate of diarrheal illnesses. Effective interventions require evidence-based research that highlights risk behaviors and perceptions of fecal contamination risk in people's daily lives. This study examines the context of fecal contamination during daily activities among residents in low resource urban settings of Accra, Ghana. Qualitative data were collected through 16 focus group discussions to understand the daily behaviors that place people at risk of fecal contamination. Data were collected and analyzed using a grounded theory approach to develop a conceptual framework of the context of fecal contamination in low income neighborhoods of Accra. MaxQDA10 software was used for data analysis. Results show that latrine use is low in these neighborhoods leading to a range of alternative methods of fecal disposal that contribute to fecal contamination throughout neighborhoods. Feces were further spread through refuse dumping, poor refuse collection systems, recreational activities, and occupational tasks of residents. These pathways of fecal contamination underscore the pervasiveness of risk for fecal contamination throughout low-income urban neighborhoods, suggesting the need for multi-pronged interventions that target multiple pathways of feces transmission.

574

NEUROPSYCHOLOGICAL PROFILE OF CHILDREN IN NATIVE COMMUNITIES EXPOSED TO MERCURY CONTAMINATION IN MADRE DE DIOS, PERU

J. Oscar Tarazona-La Torre, E. Terrazas-Nuñez, **J. Antonio Grandez-Urbina**, J. Gonzalo-Rodriguez, R. Pichardo-Rodriguez

Ricardo Palma University, Lima, Peru

Recent studies show that an adequate neuropsychological profile is associated to adequate levels of learning. For this reason we are interested in knowing which cognitive profiles in pediatric populations of native communities exposed to mercury contamination in Madre de Dios, which vulnerable to suffer neurocognitive deficiencies because there are located in areas of difficult access to education and present deficiencies in their basic needs so we have as objective to determinate the Neuropsychological Profile of children in native communities exposed to mercury contamination in Madre de Dios, Peru Transversal study of a representative sample taken from the native communities Ese'eja (Palma Real, Sonene, Inferno) of Madre de Dios, 64 children were evaluated regardless of gender, ages from 3 to 7 years old. For this evaluation we used Neuropsychological Test Maturity, CUMANIN. Parametric Kormogorov-Smirnov test of a sample was applied in order to check for a normal distribution in the study variables. Frequencies established variables, measures of central tendency and dispersion in qualitative variables were used. The test of variance analysis of factor was applied to check for differences between the study groups. The children of Infierno native community were 23,8%(n=15), Palma Real 47,6%(n= 30) and Sonene 28,6% (n=18). The mean age was 5,11 +/- 1,38. The percentiles about different parameters of Neuropsychological Profile of children were inferior of the p50 in Psychomotor 69.50% (n<P50=44), Language Articulatory 66.7%(n<P50=42), Expressive Language 71.40% (n<P50=45), Comprehensive Language 98.4% (n<P50=62), Space structure 69.80% (n<P50=44), Visopercepcion 69.80% (n<P50=44), Iconic Memory 34.9% (n<P50=22), Rhythm 87.3% (n<P50=55), Attention 90.50% (n<P50=57), Verbal Development 88.90% (n<P50=56), Nonverbal Development 77.8% (n<P50=49), Global Development 85.70% (n=<P50=54).The analysis of variance of a factor for Global Neuropsychological Development Profile respect to the three communities was 0.211 for the Verbal Development was 0.229 and Nonverbal Development was 0.248. In the present study we found a large number of children with less than p50 during different parameters of Neuropsychological Profile in other hand there was no difference between groups regarding their Neuropsychological Profile (Verbal and Non-Verbal Development)

575

ASSESSMENT OF THE QUALITATIVE IMMUNE RESPONSE INDUCED BY THE CYD TETRAVALENT DENGUE VACCINE IN HUMAN VOLUNTEERS

Bruno Guy[1], Mark Boaz[2], Antony Byers[3], Aure Saulnier[1], Aravinda de Silva[4], Sandra R. Henein[4], Ralph Baric[5], Scott R. Royal[5], James Crowe[6], Nicholas Jackson[7]

[1]sanofi pasteur, Marcy l'Etoile, France, [2]sanofi pasteur, Swiftwater, PA, United States, [3]sanofi pasteur, Orlando, FL, United States, [4]University of North Carolina School of Medicine, Chapel Hill, NC, United States, [5]University of North Carolina School of Public Health, Chapel Hill, NC, United States, [6]Vanderbilt University Medical Center, Nashville, TN, United States, [7]sanofi pasteur, Lyon, France

Clinical efficacy observed against dengue virus (DENV) serotypes 1, 3 and 4, but not DENV2 in the Phase 2b trial of the CYD tetravalent dengue vaccine candidate (CYD23; Clinicaltrials.gov NCT00842530), contrasted with the similar levels of antibody neutralization levels observed for all 4 serotypes in a Vero cell based $PRNT_{50}$ assay. To further investigate this finding, we assessed the quality of the vaccine-induced antibodies. Sera from a completed clinical trial (NCT 01134263) in volunteers who were flavivirus-seronegative at baseline, were depleted using virus-coated beads, and neutralization was assessed before and after depletion in a flow cytometry-based assay using U937-DC SIGN+ cells. These showed that in this naïve population CYD-TDV vaccination elicited mostly homotypic anti-DENV4 responses, while anti-DENV1, DENV2 and DENV3 responses included a significant heterotypic component. Antibody epitopes were then mapped in a few samples using recombinant DENVs displaying serotype-specific, E protein domain I/II hinge epitopes. Homotypic anti-DENV4 responses were seen to be directed against the DI/II hinge, while anti-DENV3 was not. This serotype-specific quaternary epitope was present and recognized by human monoclonal antibodies (mAbs) on the corresponding CYD-1, 2 and 3 serotypes, while cross-reactive anti-rE DI-II or DIII mAbs recognized all 4 CYD serotypes. Potentially enhancing anti-prM responses were not dominant in vaccinees' sera in Western Blots, and it was also observed from a FcγR+ CV1 cell-based assay (presented by Byers and others at this conference) that vaccine-induced anti-DENV2 responses were not more enhancing than against the other serotypes. In conclusion, our results suggest that despite the presence of key epitopes on the vaccine viruses, qualitative differences exist between vaccine-induced responses against serotype 4 compared to the other 3 serotypes, although it will be necessary to confirm the results obtained here in a larger number of sera. These results demonstrate nevertheless the interest of qualitatively assessing dengue vaccine immunogenicity and how it relates to protection. Studies using post-vaccination sera from clinical trials in endemic areas are ongoing, and results are expected in the coming 2 months

576

INVESTIGATIONS OF THE OBSERVED EFFICACY OF THE CYD TETRAVALENT DENGUE VACCINE IN THE PHASE 2B TRIAL IN RATCHABURI, THAILAND

Nicholas Jackson[1], Mark Boaz[2], Branda Hu[2], Edith Langevin[1], Anthony Byers[3], Ralph Baric[4], Aravinda de Silva[4], James Crowe[5], Thomas Papa[1], Dany De Grave[2], Melanie Saville[1], Jean Lang[1], Bruno Guy[1]

[1]Sanofi Pasteur, Lyon, France, [2]Sanofi Pasteur, Swiftwater, PA, United States, [3]Sanofi Pasteur, Orlando, FL, United States, [4]University of North Carolina School of Medicine, Chapel Hill, NC, United States, [5]Vanderbilt University Medical Center, Nashville, TN, United States

The first efficacy trial (CYD23; Clinicaltrials.gov NCT00842530) of the CYD vaccine showed clinical protection against dengue virus (DENV) serotypes 1, 3, and 4, but not DENV2, while similar antibody neutralization levels (Vero cell based PRNT50 assay) were observed for all four serotypes. Post study investigations of this result included a broad array of analytical and experimental methods in four areas: host, virus, vaccine vector, and novel immunological assays. Antigenic diversity between parental vaccine virus and wild-type CYD23 isolates does not impact neutralization (PRNT50) using serum from either CYD23 vaccinees or placebo recipients, and an assessment of viremia differences between serotypes in CYD23 clinical cases was inconclusive. Pre-existing immunity impacts vaccine immunogenicity and exploratory analysis using logistic regression, suggests a relationship between probability of disease and PRNT titers. Using CV1 cells transfected with FcγRIIa, preliminary studies with post-vaccination sera from baseline-naïve subjects show no evidence of differential neutralization. Serotype-specific antibody depletion studies, and studies using recombinant DENVs displaying serotype-specific E protein domain I/II hinge epitopes show qualitative differences between serotype-specific neutralizing responses, while a Vero-cell based microneutralization assay shows certain discrimination between serotypes. Analyses using serotype-specific monoclonals show that important E protein DI/II hinge region epitopes are displayed on the vaccine viruses, including CYD2. Finally, prior clinical data suggest a relationship between vaccine potency (CCID50) and neutralization, as well as *in vivo* competition between serotypes in different formulations, and no differences in IFNγ responses between serotypes. Additional investigations, including Phase 3 efficacy study data anticipated in the second half of 2014, will deepen our understanding of the results observed in this Phase IIb efficacy trial.

577

LARGE SCALE SAFETY AND IMMUNOGENICITY OF CYD DENGUE VACCINE; RESULTS FROM A RANDOMIZED, PLACEBO-CONTROLLED, MULTICENTER PHASE III EFFICACY TRIAL IN LATIN AMERICA

Luis Angel Villar[1], Gustavo H. Dayan[2], Jose Luis Arredondo[3], Maribel Rivera[4], Rivaldo Cunha[5], Carmen Deseda[6], Humberto Reynales[7], Jose Remes-Troche[8], Maria Selma Neves Costa[9], Javier Morales[10], Luiz Carlos Rey[11], Reynaldo Dietze[12], Kleber Giovanni Luz[13], Gabriel Carrasquilla[14], Enrique Rivas[15], Maria Consuelo Miranda[16], Margarita Cortes[16], Zambrano Betzana[17], Edith Langevin[18], Mark Boaz[2], Nadia Tornieporth[18], Melanie Saville[18], Fernando Noriega[2]

[1]Clinical Epidemiology Unit, School of Medicine, Bucaramanga, Colombia, [2]Sanofi Pasteur, Swiftwater, PA, United States, [3]Instituto Nacional de Pediatria, Mexico City, Mexico, [4]Organización para el Desarrollo y la Investigación Salud en Honduras (ODISH), Tegucigalpa, Honduras, [5]Hospital Universitário/UFMS, Campo Grande, Brazil, [6]Caribbean Travel Medicine Clinic, San Juan, Puerto Rico, [7]Centro de Atención en Investigación Médica, Bogota, Colombia, [8]Instituto de Investigaciones Médico – Biológicas, Veracruz, Mexico, [9]Hospital das Clínicas da Universidade Federal de Goiás, Goiania, Brazil, [10]Clinical Research Puerto Rico, Guayama, Puerto Rico, [11]Federal University of Ceara, Fortaleza, Brazil, [12]Nucleo de Doenças Infecciosas/UFES, Vitória, Brazil, [13]Instituto de Medicina Tropical/UFRN, Vitoria, Brazil, [14]Centro de Estudios de Investigación en Salud (CEIS), Bogota, Colombia, [15]Sanofi Pasteur, Mexico City, Mexico, [16]Sanofi Pasteur, Bogota, Colombia, [17]Sanofi Pasteur, Montevideo, Uruguay, [18]Sanofi Pasteur, Lyon, France

In a phase III study of the efficacy of the recombinant, live, attenuated, CYD tetravalent dengue vaccine (TDV) against virologically-confirmed dengue fever in dengue-endemic areas of five Latin American countries: Brazil, Colombia, Honduras, Mexico, and Puerto Rico (N=20 875). Children and adolescents aged 9-16 were randomized 2:1 to receive 3 injections of CYD-TDV or placebo at study months 0, 6 and 12. Safety and immunogenicity were assessed as secondary objectives. Serious adverse events (SAEs) occurring at any time throughout the study in the whole study population were documented, and reactogencity was described in a representative subset of 2000 children (300-600 per country) who were randomly selected from among those enrolled during the first months of the study. SAEs were reviewed by an independent data monitoring committee. Reactogenicity data included solicited injection site reactions, solicited systemic reactions, and unsolicited adverse events, respectively collected for 7, 14, and 28 days after each vaccination. Immunogenicity was also assessed in this subset of 2000 children using Vero cell-based PRNT50 assays against the four dengue serotypes to test serum collected at enrolment, on D28 after the 2nd and 3rd injections, and at 13 months after the 3rd vaccination. Results of these analyses are expected in September/October and will be presented here for the first time. ClinicalTrials.gov: NCT01374516

578

EFFICACY OF THE CYD DENGUE VACCINE; RESULTS OF A RANDOMIZED, PLACEBO-CONTROLLED, MULTICENTER PHASE III TRIAL IN LATIN AMERICA

Gustavo H. Dayan[1], Luis Angel Villar[2], Jose Luis Arredondo[3], Maribel Rivera[4], Rivaldo Cunha[5], Carmen Deseda[6], Humberto Reynales[7], Jose Remes-Troche[8], Maria Selma Neves Costa[9], Javier Morales[10], Luiz Carlos Rey[11], Reynaldo Dietze[12], Kleber Giovanni Luz[13], Gabriel Carrasquilla[14], Enrique Rivas[15], Maria Consuelo Miranda[16], Margarita Cortes[16], Betzana Zambrano[17], Edith Langevin[18], Mark Boaz[1], Nadia Tornieporth[18], Melanie Saville[18], Fernando Noriega[1]

[1]Sanofi Pasteur, Swiftwater, PA, United States, [2]Clinical Epidemiology Unit, School of Medicine, Bucaramanga, Colombia, [3]Instituto Nacional de Pediatria, Mexico City, Mexico, [4]Organización para el Desarrollo y la Investigación Salud en Honduras, Tegucigalpa, Honduras, [5]Hospital Universitário/UFMS, Campo Grande, Puerto Rico, [6]Caribbean Travel Medicine Clinic, San Juan, Puerto Rico, [7]Centro de Atención en Investigación Médica, Bogota, Colombia, [8]Instituto de Investigaciones Médico – Biológicas, Veracruz, Mexico, [9]Hospital das Clínicas da Universidade Federal de Goiás, Goiania, Brazil, [10]Clinical Research Puerto Rico, Guayama, Puerto Rico, [11]Federal University of Ceara, Fortaleza, Brazil, [12]Nucleo de Doenças Infecciosas/UFES, Vitoria, Brazil, [13]Instituto de Medicina Tropical/UFRN, Vitoria, Brazil, [14]Centro de Estudios de Investigación en Salud, Bogota, Colombia, [15]Sanofi Pasteur, Mexico City, Mexico, [16]Sanofi Pasteur, Bogota, Colombia, [17]Sanofi Pasteur, Montevideo, Uruguay, [18]Sanofi Pasteur, Lyon, France

The protective efficacy afforded by vaccination with the recombinant, live, attenuated, CYD tetravalent dengue vaccine (TDV) against virologically-confirmed dengue fever was evaluated in a phase III trial in dengue-endemic areas of five Latin American countries: Brazil, Colombia, Honduras, Mexico, and Puerto Rico. (N=20 875). Children and adolescents aged 9-16 were enrolled over a 9 month period, randomized 2:1 to receive 3 injections of CYD-TDV or placebo at study months 0, 6 and 12, and were actively followed for febrile illness (temperature ≥38°C for ≥2days) until the 13th month after the 3rd injection; ie for 25 months after the 1st injection. The primary endpoint was efficacy against cases against symptomatic, virologically-confirmed dengue occurring at least 28 days after the 3rd injection, regardless of severity or serotype. Efficacy by serotype was evaluated as a secondary objective. Active surveillance consisted of weekly contacts via phone or home visits to remind parents to consult the trial center or health care center in the event of febrile illness, considered as a suspected dengue case. An acute sample was to be collected within 5 days of fever onset, and a convalescent sample 7-14 days later. Virological confirmation was by dengue qRT-PCR or dengue NS1 antigen ELISA and cases were subsequently serotyped by qRT-PCR. Together with a second phase III efficacy study in >10000 children aged 2-14 in 5 Asian countries conducted in parallel with a similar protocol, this study will provide pivotal data on the efficacy of the CYD-TDV vaccine in different populations and epidemiological settings. Results of our study are expected in September/October and will be presented here for the first time.

579

A SINGLE DOSE OF LIVE ATTENUATED TETRAVALENT DENGUE VACCINE TV005 IS SAFE, IMMUNOGENIC AND HIGHLY INFECTIOUS IN HUMANS

Stephen Whitehead[1], Dan Elwood[2], Cecilia Tibery[2], Catherine Larsson[3], Adrienne Jarvis[3], Beulah Sabundayo[2], Matthew Jo[2], Janece Lovchik[2], Marya Carmolli[3], Ben McElvany[3], Cassandra Ventrone[3], Catherine Luke[1], Kristen Pierce[3], Beth Kirkpatrick[3], Anna Durbin[2]

[1]Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [2]Center for Immunization Research, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]Vaccine Testing Center, College of Medicine, The University of Vermont, Burlington, VT, United States

Dengue virus remains a public health burden in many tropical and subtropical areas of the world. The lack of a vaccine or antiviral therapy and the relative unsustainability of vector control together contribute to the ongoing emergence of dengue disease. The last decade has seen an increase in vaccine development efforts, with live attenuated vaccines making significant progress. Our laboratory at the NIAID has developed live attenuated dengue vaccine candidates shown to be both safe and immunogenic in monovalent and tetravalent studies in humans. These studies have enabled the down-selection of vaccine candidates to an optimal mixture of rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31, and rDEN4Δ30. In admixture TV003, each component is delivered at a potency of 1000 PFU and neutralizing antibody data collected 90 days post-vaccination in flavivirus-naïve adults showed seroconversion to DENV1, DENV2, DENV3, and DENV4 in 92%, 76%, 97%, and 100% of vaccinees, respectively, after a single subcutaneous dose. In this cohort, 74% of vaccinees achieved a tetravalent antibody response. When the potency of the DENV2 component of the vaccine was increased 10-fold in admixture TV005 and administered in the same manner as TV003, frequencies of seroconversion in vaccinees to the individual serotypes 1 - 4 reached a remarkable 92%, 97%, 97%, and 97%, respectively, after a single dose, with 90% of vaccinees achieving a tetravalent antibody response. In both studies, low level vaccine viremia was detected in 70 - 75% of vaccinees and mild asymptomatic vaccine-associated rash was observed in 55 - 68% of vaccinees. Following a second dose of vaccine given 180 days after the first dose, vaccine viremia, rash, or boosts in neutralizing antibody titers were not observed in any vaccinee, indicating that sterilizing immunity was elicited following the first dose. Importantly, the data suggest that admixtures such as TV005 can be administered as a single dose. This is unprecedented among dengue vaccines and has positive implications for vaccine safety, compliance, cost, and dose sparing.

580

PHASE I CLINICAL TESTING OF A RECOMBINANT SUBUNIT VACCINE FOR DENGUE

Susan Manoff[1], Michele Sausser[1], Tyler Finn[1], Amy Russell[1], Brynne Tannenbaum[1], David Radley[1], Donna Hyatt[1], Christine Roberts[1], Andrew Bett[2], Jason Martin[1], Beth-Ann Coller[1]

[1]Merck and Company, North Wales, PA, United States, [2]Merck and Company, West Point, PA, United States

A recombinant subunit vaccine is being developed to prevent disease associated with dengue virus infection. The vaccine candidate comprises truncated dengue envelope proteins (DEN-80E) from all four serotypes produced in Drosophila S2 cells. The vaccine is being evaluated in a Phase I clinical trial in 98 healthy, flavivirus-naïve, adults in Australia. The study is a randomized, placebo-controlled, dose escalation study which evaluates 3 different dosage levels of the tetravalent DEN-80E. The formulations evaluated include non-adjuvanted, aluminum hydroxide adjuvanted, and two different dosage levels of ISCOMATRIX™ adjuvanted vaccine. Volunteers received three doses of vaccine at a 0, 1, 2 month schedule. Safety is being followed throughout the study and immunogenicity is

being assessed pre-dose, post-dose 1, post-dose 2, and 1, 6, and 12 months post-dose 3. The primary endpoint for immunogenicity is based on assessment of virus neutralizing antibody responses with analysis of seroconversion and geometric mean titers. Subjects have now completed their follow-up through the primary endpoint (1 month post-dose 3) and the immunogenicity and safety data through this time point will be presented. Subjects are continuing through the long term follow-up stage of the protocol.

581

SAFETY AND IMMUNOGENICITY OF TAKEDA'S LIVE ATTENUATED DENGUE VACCINE CANDIDATE IN A PHASE I STUDY CONDUCTED IN FLAVIVIRUS-NAÏVE HUMAN VOLUNTEERS

Sarah George[1], Aurelia Haller[2], Claire Huang[3], Mimi Wong[4], Tina Dube[4], Jorge Osorio[2], Dan T. Stinchcomb[2]

[1]St. Louis University, St. Louis, MO, United States, [2]Takeda Vaccines, Inc., Madison, WI, United States, [3]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [4]EMMES Corporation, Rockville, MD, United States

We conducted a phase I, randomized, double-blind dose-escalation study of two different formulations of Takeda's live attenuated dengue vaccine candidate in 72 healthy flavivirus-naïve adults at the Saint Louis University VTEU (NCT01110551). Volunteers received two doses of the tetravalent dengue vaccine candidate 90 days apart and were followed for safety and immunogenicity. Serum samples were collected after each dose to measure vaccine virus replication by qRT-PCR and virus isolation and to measure neutralizing antibodies to wild-type DENV. In this phase 1 study, the vaccine was well-tolerated and no serious vaccine-associated adverse events occurred. Low levels of vaccine viral RNA were detected after prime in 46% of individuals that received the lower dose formulation and in 79% of individuals that received the higher dose formulation. Both vaccine formulations induced seroconversion rates of 67-100% to each of the 4 serotypes of DENV, and more than 90% of subjects who received two doses of the high dose formulation seroconverted to three or more DENV (trivalent response). In summary, these findings highlight the good tolerability and immunogenicity of Takeda's live attenuated dengue vaccine in flavivirus-naïve healthy volunteers and support further development and clinical testing of the candidate dengue vaccine.

582

BURDEN, RISK FACTORS AND CHARACTERIZATION OF ROTAVIRUS AMONG CHILDREN WITH MODERATE-TO-SEVERE DIARRHEA IN RURAL WESTERN KENYA–2008-2011: THE GLOBAL ENTERIC MULTICENTER STUDY (GEMS)

Richard Omore[1], Jacqueline E. Tate[2], Ciara E. O'Reilly[2], John Williamson[3], Tracy Ayers[2], Fenny Moke[1], Benjamin J. Ochieng[1], Joseph Oundo[3], Michele B. Parsons[2], Cheryl A. Bopp[2], Tamer H. Farag[4], Karen L. Kotloff[4], James P. Nataro[5], Sandra Panchalingam[4], Myron M. Levine[4], John Vulule[1], Kayla F. Laserson[6], Pekka J. Nuorti[7], Eric D. Mintz[2], Robert F. Breiman[8]

[1]Kenya Medical Research Institute/Centers for Disease Control and Prevention, Kisumu, Kenya, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Centers for Disease Control and Prevention, Nairobi, Kenya, [4]University of Maryland, Center for Vaccine Development, Baltimore, MD, United States, [5]University of Virginia School of Medicine, Charlottesville, VA, United States, [6]India Epidemic Intelligence Service Programme, Centers for Disease Control and Prevention, Delhi, India, [7]University of Tampere School of Health Sciences, Tampere, Finland, [8]Global Health Institute, Emory University, Atlanta, GA, United States

Rotavirus is the most common cause of severe diarrhea among children worldwide. Data on risk factors for morbidity are limited. We analyzed data from children <5 years old seeking health care with moderate-to-severe diarrhea (MSD) and enrolled as cases in the Global Enteric Multicenter Study (GEMS) site in Nyanza Province, Kenya. An MSD case was defined as a child with a diarrheal illness <7 days duration comprising ≥3 loose stools in 24 hrs and ≥1 of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization. Rotavirus VP6 antigen was detected in stools by the ProSpecT ELISA rotavirus kit. Demographic and clinical information were collected at enrollment and during a ~60-day follow-up visit. We used logistic regression to evaluate factors associated with rotavirus among children with MSD compared with non-rotavirus MSD. From January 31, 2008 to January 29, 2011, 1,476 cases were enrolled; rotavirus was detected in 217 (14.7%); among cases aged 0-<6, 6-11, 12-23 and 24-59 months old, rotavirus was detected in 24.0% (59/246), 18.3% (78/427), 15.6% (64/410) and 4.1 % (16/393), respectively. Compared to cases without rotavirus, cases with rotavirus infection were more likely to: be 0-11 months old (65.6% vs. 44.7%, OR= 6.08, 95% confidence interval (CI) [3.80-9.72]), be female (48.7% vs. 41.4%, OR= 1.34, 95%CI [1.06-1.70]), and present with vomiting ≥3 times/24hrs (69.9% vs. 45.1%, OR= 2.83 95%CI [2.19-3.65]), sunken eyes (97.1% vs. 92.2%, OR= 2.85 95%CI [1.45-5.59]), lethargy (18.7% vs.11.3%, OR= 1.80 95%CI [1.31-2.47]) and restlessness (72.7% vs. 58.2%, OR= 1.92 95%CI [1.47-2.49]). Among children with MSD, rotavirus was more prevalent among infants and significantly associated with severe diarrheal illness in our study setting. Our findings support the Kenya Ministry of Health plan to introduce rotavirus vaccine.

583

THE THRESHOLD OF ROTAVIRUS SPECIFIC PLASMA IGA AS A CORRELATE OF PROTECTION FROM ROTAVIRUS DIARRHEA IS AGE DEPENDENT

Marya P. Carmolli[1], Beth D. Kirkpatrick[1], Rashidul Haque[2], K. Zaman[2], Josyf C. Mychaleckyj[3], Taufiqur R. Bhuiyan[2], Dorothy M. Dickson[1], Elizabeth R. Colgate[1], Uma Nayak[3], Sean Diehl[1], M. Hossain[2], S. Islam[2], M. I. Uddin[2], Firdausi Qadri[2], William A. Petri[3]

[1]University of Vermont, Burlington, VT, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]University of Virginia, Charlottesville, VA, United States

Optimizing and interpreting immunogenicity data and correlates of protection may help to better identify children in whom rotavirus vaccination is failing in developing countries. Here we use the PROVIDE study to examine the ability of rotavirus specific plasma IgA to correlate with protection from future episodes of rotavirus diarrhea within the first year of life. At the icddr,b site in Mirpur, Dhaka, 700 children were enrolled at birth and followed for 24 months. Bi-weekly surveillance for diarrhea was performed and diarrheal specimens were evaluated for rotavirus by enzyme-linked immunoabsorbent assay (ELISA). Diarrheal disease severity was measured by a modified Vesikari-Ruuska score, with score ≥11 indicating severe diarrhea. Rotavirus plasma IgA was detected by ELISA at weeks 6, 18, and 24. Per published standards, children were considered seropositive if the plasma IgA was ≥20 U/ml. Five percent of children were seropositive at week 6 with a Geometric Mean Titer (GMT) of 1.5 (66.5 for seropositive). Irrespective of rotavirus vaccination status, at weeks 18 and 24, 26% and 37% of the children were seropositive with a GMT of 7.1 and 12.3 (114.1, 139.4 for seropositive) respectively. Seropositivity at 18 and 24 weeks of age was correlated with up to 85% and 87% protection from future episodes of rotavirus diarrhea, respectively. At six weeks, seropositivity as previously defined was not associated with protection against future episodes of diarrhea. Increasing the cut-off to 40 U/ml at six weeks increased the proportion protected to 86% however the sample size was small. Our data show that regardless of natural exposure or vaccine-induced responses, rotavirus specific plasma IgA levels at ≥20 U/mL at weeks 18 and 24 serves as a correlate of protection from rotavirus diarrhea in the first year of life. If measured at 6 weeks of age, higher IgA levels are needed to indicate protection from future episodes of rotavirus diarrhea. After unmasking, analysis will assess plasma IgA as an effective correlate of protection in rotavirus-vaccinated children.

584

PATHOGEN-SPECIFIC MORTALITY AMONG INFANTS AND YOUNG CHILDREN WITH MODERATE-TO-SEVERE DIARRHEA - WESTERN KENYA, 2008-2011

Allison T. Walker[1], Richard Omore[2], Fenny Moke[2], Benjamin Ochieng[2], Tamer Farag[3], Dilruba Nasrin[3], Sandra Panchalingam[3], James P. Nataro[3], Karen L. Kotloff[3], Myron M. Levine[3], Xin Lu[1], Joseph Oundo[4], Michele B. Parsons[1], Cheryl Bopp[1], Kayla Laserson[1], Eric Mintz[1], Robert F. Breiman[4], Ciara O'Reilly[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Kenya Medical Research Institute/Centers for Disease Control and Prevention, Kisumu, Kenya, [3]Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, [4]Centers for Disease Control and Prevention-Kenya, Kisumu, Kenya

Diarrhea is a leading cause of childhood morbidity and mortality in sub-Saharan Africa; one in ten child deaths in the first 5 years of life is due to diarrheal disease. We assessed pathogen-specific mortality following an episode of moderate-to-severe diarrhea (MSD) in children enrolled in the Global Enteric Multicenter Study in western Kenya. We recruited children <5 years old presenting to sentinel health facilities with MSD. At enrollment each child was assessed clinically, and provided anthropometric data and a stool sample to identify enteropathogens. Survival status was determined at 60 days. We calculated unadjusted exact odds ratios (OR) and 95% confidence intervals (CI) using simple logistic regression models. From 2008 to 2011, 1,476 children with MSD were enrolled; 52 (3.5%) died. Nineteen (37%) children died at health facilities (7 at enrollment) and 33 (63%) died at home. Case-fatality rates by age stratum were 4.5% (<12 months), 3.1% (12-23 months), and 2.3% (24-59 months). Pathogens associated with increased risk of case-fatality were *Shigella dysenteriae* (OR: 7.2; CI: 1.3-27.8), non-typhoidal *Salmonella* (OR: 2.8; CI: 1.0-6.6), typical enteropathogenic *Escherichia coli* (OR: 2.6; CI: 1.1-5.5), and enterotoxigenic *E. coli* producing heat-stable toxin (OR: 2.5; CI: 1.1-5.2). Children who died were more likely to be underweight (weight-for-age z score < -2: OR 12.6; CI: 6.4-26.8), wasted (weight-for-length/height z score < -2: OR 8.2; CI: 4.4-15.1), and stunted (length/height-for-age z score < -2: OR 4.2; CI: 2.3-7.9) at enrollment. Malnutrition and four bacterial pathogens for which no vaccines are available were associated with increased risk of mortality from MSD. In the near-term, optimizing nutritional status and water, sanitation, and hygiene interventions are urgent priorities for childhood diarrheal mortality reduction in Kenya.

585

PATHOGEN-SPECIFIC ETIOLOGY AND BURDEN OF COMMUNITY DIARRHEA IN THE FIRST TWO YEARS OF LIFE IN DEVELOPING COUNTRIES: RESULTS FROM THE MAL-ED MULTISITE COHORT STUDY

James A. Platts-Mills[1], Benjamin J. McCormick[2], Monica McGrath[2], Alexandre Havt[3], Aldo A. Lima[3], Ila F. Lima[3], Tahmeed Ahmed[4], Dinesh Mondal[4], Rashidul Haque[4], Sudhir Babji[5], Dinesh Hariraju[5], Gagandeep Kang[5], Bishnu Rajamajhi[6], Ladaporn Bodhidatta[6], Carl J. Mason[6], Pablo P. Yori[7], Mery Siguas Salas[8], Margaret Kosek[8], Shahida Qureshi[9], Furqan Kabir[9], Aneeta Hotwani[9], Sadia Shakoor[9], Anita K. Zaidi[9], Amidou Samie[10], Brenda Mufamadi[10], Pascal Bessong[10], Caroline Amour[11], Jean Gratz[1], Dennis Lang[2], Mark Miller[2], Eric R. Houpt[1]

[1]University of Virginia, Charlottesville, VA, United States, [2]Fogarty International Center, National Institutes of Health, Bethesda, MD, United States, [3]Clinical Research Unit and Institute of Biomedicine, Federal University of Ceara, Fortaleza, Brazil, [4]Division of Nutrition and Food Security, International Centers for Diarrheal Disease Research, Matlab, Bangladesh, [5]Christian Medical College, Vellore, India, [6]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [7]Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, [8]Asociación Benéfica PRISMA, Iquitos, Peru, [9]Aga Khan University, Karachi, Pakistan, [10]University of Venda, Thohoyandou, South Africa, [11]Haydom Lutheran Hospital, Haydom, United Republic of Tanzania

Studies of diarrheal etiology in developing countries have historically focused on children presenting with severe symptoms to health centers and thus best describe pathogens associated with severe diarrhea. However, the etiologies of community diarrhea may be different. MAL-ED is a multisite birth cohort study with intensive community surveillance for diarrhea as well as collection of monthly asymptomatic stool specimens from eight sites in South America, Africa and Asia. A total of 7,068 diarrheal and 22,599 non-diarrheal control specimens from 2,073 children aged 0-24 months were comprehensively tested for a broad range of enteropathogens. In the first year of life, Campylobacter, rotavirus, ST-producing enterotoxigenic E.coli (ST-ETEC), Cryptosporidium and astrovirus were associated with the highest burdens of diarrhea in descending order of attributable fraction. In the second year of life, diarrhea attributable to Shigella was more prominent while diarrhea attributable to Campylobacter decreased. There was substantial site-to-site variation, such that 6 distinct pathogens had the highest burden of diarrhea for at least one combination of site and age. Cryptosporidium, rotavirus, ST-ETEC and Shigella were associated with more severe diarrheal episodes. This study reveals substantial heterogeneity in the pathogen-specific burden of community diarrhea including an unexpectedly high burden of disease associated with Campylobacter and astrovirus.

586

CHANGES IN GUT MICROBIOME COMPOSITION DURING DIARRHEA EPISODES IN NICARAGUAN CHILDREN

Sylvia Becker-Dreps[1], M. Andrea Azcarate-Peril[1], Samuel Vilchez[2]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]Universidad Nacional Autonoma de Nicaragua, Leon, Leon, Nicaragua

The gut microbiome, the collection of bacteria in the human gastrointestinal tract, plays an important role in human health. Understanding how the gut microbiome is affected by diarrhea episodes may help explain alterations in intestinal function among children in low-income settings. This study examined the gut microbiome of Nicaraguan children during diarrhea episodes and while free of diarrhea for at least 2 months. 16S amplicon sequencing was performed to determine changes in the gut bacterial microbiome during diarrhea episodes. Sequencing data analysis was done using Qiime (Caporaso, 2010). Rarefaction analysis and diversity estimates were carried out to compare the overall diversity of microbiota in diarrheal versus healthy stools. Principal Coordinate

Analysis of amplicon sequences (Hamady, 2010) was used to compare clustering by state (diarrheal vs. healthy). In all, 74 stools were provided by 27 children who were enrolled in a one-year cohort study of diarrhea etiologies. These children had a mean age of 21 months (range: 1 to 45 months), 49% were female, 63% were breastfed, and 10% had received an antibiotic during the diarrhea episode. A total of 593,509 sequences (an average of 7,707 reads per sample) were assigned to 7,880 operational taxonomic units at ≥97% similarity, clustering into 237 genera, 115 families, 54 orders, 28 classes, and 14 phyla. Diarrheal and healthy stools had statistically significant differences ($p<0.05$) for the phyla Firmicutes, Bacteroidetes, and Actinobacteria. Also, as compared to healthy stools, diarrheal stools had a greater relative abundance of the taxa Enterobacteriaceae (13.0% diarrheal vs. 7.9% healthy) and Streptophyta (9.4% vs. 3.3%), and a lower relative abundance of the taxa Ruminococcaceae (9.8% vs. 21.0 %) and Cyanobacteria YS2 (2.1% vs. 9.7%). Phylogenetic diversity did not differ significantly between diarrheal vs. healthy stools. Principal Coordinate Analysis showed clustering by state (diarrheal vs. healthy samples) indicating an overall perturbation of the microbiota in diarrheal stools.

587

MARKERS OF OPV FAILURE: INFLAMMATORY AND NUTRITIONAL ENVIRONMENT CRITICAL MEDIATORS OF VACCINE RESPONSE

Caitlin Naylor[1], Rashidul Haque[2], Jennie Ma[1], Miao Lu[1], Mami Taniuchi[1], Eric Houpt[1], William Weldon[3], Mark Pallansch[3], Uma Nayak[1], Beth Kirkpatrick[4], William Petri[1]

[1]University of Virginia, Charlottesville, VA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]University of Vermont, Burlington, VT, United States

The oral polio vaccine (OPV) shows reduced immunogenicity in low income countries; children often have lower antibody titers in response to the vaccine, but the biological cause is unknown. We are measuring OPV responses in infants in an urban slum of Dhaka, Bangladesh. While OPV2 had only a 1.2% failure rate, OPV1 and OPV3 failed in 6.5% and 9.9% respectively as measured by serum neutralizing antibody responses at 18 weeks of age. We hypothesized that nonpolio enterovirus infection at the time of immunization could interfere with OPV. We found that the presence of a nonpolio enterovirus at the time of the 14 week immunization was associated with failure of OPV1 and OPV3 at 18 weeks (p=.005 & .003). We further hypothesized that nonpolio enterovirus infection was interfering by inducing an innate antiviral response in the gut that prevented OPV replication. In support of this, up to a quarter of OPV failures were associated with the inability to culture OPV1 or OPV3 virus from stool samples 24 hours after the first OPV dose at 6 weeks of age (chi-square probability=.01 & .07). In addition to these analyses, blood, stool, and urine samples from the children have been tested for over 20 biomarkers of immunity, inflammation and nutritional status. We performed univariate linear regression analyses to evaluate the association of each biomarker with vaccine performance using serum antibody titers. Markers of poor intestinal health reg1B and mannitol (p=.0002 & .044) were negatively associated with vaccine performance. Serum ferritin and IL-5 (p =.028 & .029) levels, markers of systemic inflammation, were also negatively associated with vaccine performance. Serum zinc concentration and WAZ at 18 weeks of age (p=.034 & .006) positively correlated with increased antibody titers. These results indicate that competing nonpolio enterovirus infections and a chronic inflammatory response likely serve to hinder initial OPV replication and the ability to elicit an effective immune response. Further support for this hypothesis was obtained by measuring fecal excretion of vaccine virus after the final OPV dose as a measure of mucosal immunity. We found that high levels IL-5 positively trended with increased virus excretion (p = .065). We concluded that the majority of the children in the cohort exhibited high levels of inflammatory markers, resulting in an enteric environment of chronic inflammation and infection that left little room for an OPV response.

588

IMMUNOGENICITY IN MICE OF A CHOLERA CONJUGATE VACCINE CONTAINING *VIBRIO CHOLERAE* O1 INABA O-SPECIFIC POLYSACCHARIDE (OSP) AND A RECOMBINANT TETANUS TOXIN HEAVY CHAIN FRAGMENT

Richelle C. Charles[1], Grace Eckhoff[2], Meagan K. Bufano[1], Peng Xu[3], Md. Murshid Alam[1], Anuj Kalsy[1], Yanan Yu[1], Ying Wu-Freeman[1], Tania Sultana[1], Md. Rasheduzzaman Rashu[1], Daniel Leung[1], Regina C. LaRocque[1], Jason B. Harris[1], Stephen B. Calderwood[1], Firdausi Qadri[4], W. F. Vann[5], Pavol Kováč[3], **Edward T. Ryan**[1]

[1]Massachusetts General Hospital, Boston, MA, United States, [2]Harvard Medical School, Boston, MA, United States, [3]National Institute of Diabetes and Digestive and Kidney Diseases, LBC, National Institutes of Health, Bethesda, MD, United States, [4]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [5]CBER, Food and Drug Administration, Laboratory of Bacterial Toxins, Bethesda, MD, United States

Immunity against cholera is serogroup-specific and serogroup specificity is determined by the O-specific polysaccharide (OSP) of Vibrio cholerae lipopolysaccharide (LPS). Here, we describe a cholera conjugate vaccine containing Inaba OSP from a well-characterized source strain isolated in 2007 from a patient with cholera in Bangladesh. The OSP was conjugated via its core oligosaccharide to a recombinant tetanus toxin heavy chain fragment (rTThc) using squaric acid chemistry. We administered the vaccine intramuscularly to mice in the presence and absence of immunoadjuvantative alum. We immunized mice at day 0, 21 and 42. Immunization with OSP:TThc induced detectable anti-OSP IgG responses after one immunization. There was a trend toward higher immune responses in the presence of alum. Although vibriocidal responses were detectable in mice receiving OSP:TThc, they were low-level compared to what has historically been reported following oral whole-cell cholera immunization. OSP:TTHc induced memory B cell responses targeting OSP as well as TT. Importantly, serum from mice immunized with OSP:TThc and alum protected against lethal challenge in mice orally challenged with wild-type V. cholerae. These results suggest that a cholera conjugate vaccine containing Inaba OSP can be highly immunogenic for inducing anti-OSP responses and that such anti-OSP-based immunity can be protective even in the absence of significant vibriocidal responses. These results suggest that OSP:TThc may warrant further development as a cholera conjugate vaccine.

589

SALIVARY ANTIBODIES TO WB123 PROVIDE A NON-INVASIVE TOOL FOR ASSESSMENT OF ONGOING *WUCHERERIA BANCROFTI* TRANSMISSION

Joseph Kubofcik[1], Abdallah Diallo[2], Lamine Soumaoro[2], Michel E. Coulibaly[2], Yaya I. Coulibaly[2], Thomas B. Nutman[1]

[1]Laboratory of Parasitic Diseases, Bethesda, MD, United States, [2]NIAID-Mali ICER, Bamako, Mali

Significant progress has been made toward the global goal to eliminate lymphatic filariasis (LF) by 2020 though the tools for monitoring control success and certification of transmission interruption need to be refined further. WHO guidelines for transmission assessment surveys (TAS) will guide decisions about stopping mass drug administration (MDA); the tools for post MDA surveillance, however, are likely to involve antibody testing --likely based on antibodies to Wb123, a *Wuchereria bancrofti* (Wb)-specific antigen that is expressed early in parasite development and has been shown to be a sensitive and specific marker of exposure to Wb infective stage larvae (L3). Although serum/plasma based IgG4 anti-W123 testing has already proved useful in assessing prevalences in target (6-7 year olds) populations, and a rapid diagnostic test (RTD) for these same IgG4 anti-Wb123 antibodies is currently under development for use with whole blood, both require at a minimum a finger prick. To development a

non-invasive methodology for the measurement of antibodies to Wb123, a commercially available salivary collection device was used to collect saliva from a group of 321 6-7 year olds from 5 villages in Mali 3 years after cessation of 7 annual rounds of MDA with albendazole and ivermectin. At the same time, dried blood spots were collected for antibody elution and whole blood was used for measurement of circulating filarial antigen by ICT. Prevalences of serum IgG4 anti-Wb123 was performed by ELISA and compared to salivary IgG and IgG4 anti-Wb123 performed by luciferase immunoprecipitation assay systems (LIPS). While the prevalence of CFA was 4.5% (16/321) and for IgG4 serum anti-Wb123 from dried blood spots was 4.0% (13/321), the salivary antibody prevalences were higher with IgG anti-Wb123 being 8.4% (27/321) and for the more specific IgG4 anti-Wb123 being 6.5% (21/321). Our data suggest that saliva is a rich source of specific antibody to Wb123 and may provide a convenient, sensitive and non-invasive alternative for antibody surveillance following cessation of MDA.

590

A COMPARISON OF TWO RAPID TESTS FOR DETECTING FILARIAL ANTIGENEMIA IN LOW PREVALENCE SETTINGS IN SRI LANKA

Thishan C. Yahathugoda[1], **Ramakrishna U. Rao**[2], Mirani V. Weerasooriya[1], Gary J. Weil[2]

[1]University of Ruhuna, Galle, Sri Lanka, [2]Washington University School of Medicine, St. Louis, MO, United States

Filarial antigen tests are useful for mapping the distribution of *Wuchereria bancrofti* infections and for detecting areas with persistent infections following mass drug administration (MDA). Prior studies have shown that the new Alere™ Filariasis Test Strip (Strip) has better analytical sensitivity than the BinaxNOW® Filariasis card test (Card), and the Strip detected 26% more positives than the Card in field studies performed in a highly endemic area in Liberia. This study compared the performance of the Strip and Card in 2 areas in Southern Sri Lanka with low level persistence of filariasis some 7 years following that country's intensive MDA program. The study design called for testing ~400 people > 8 years of age in each site (cluster sampling of households). Card and Strip tests were performed in parallel in the field with finger prick blood samples; test results were scored at 10 and 30 min and at 12 hr. The Strip detected many more positives at 10 min than the Card (28/389 versus 13/390 in study site A and 50/462 versus 13/462 in study site B). With one exception, all blood samples positive by Card were also positive by Strip. Semi-quantitative test scores based on the intensity of "T" lines tended to be higher by Strip than by Card. Thus filarial antigenemia rates by Strip can be much higher than those by Card test in the post-MDA setting, when filarial antigen levels tend to be low, and this could affect the outcome of transmission assessment surveys. It was not uncommon for Strips to turn from negative to positive between 10 and 30 min (5.6 % of samples tested), and this rarely occurred with the Card (0.36%). However, many Cards that were negative at 30 min were positive at 12 hr (14.3%), and this was less common with Strip tests (4.0%). These results underline the importance of taking care to read filarial antigen test results at 10 minutes according to the manufacturer's instructions. The improved sensitivity, lower cost, and longer shelf life of the Test Strip favor its use over the Card test for filariasis elimination programs.

591

INTEGRATED MAPPING OF LYMPHATIC FILARIASIS AND PODOCONIOSIS IN ETHIOPIA: RESULT OBTAINED FROM A NATIONWIDE SURVEY

Heven Sime Firew[1], Ashenafi Assefa Bahita[1], Kalkidan Mekete Begashaw[1], Abeba Reda[1], Gemechu Tadesse Leta[1], Amha Kebede Hailemikael[1], Asrat Hailu Mekuria[2], Kebede Deribe Kassaye[3], Simon Brooker[4], Gail Davey[3], Moses Bockarie[5], Maria Rebollo Polo[5]

[1]Ethiopian Public Health Institute, Addis Ababa, Ethiopia, [2]Addis Ababa University Faculty of Medicine, Addis Ababa, Ethiopia, [3]BSMS, London, United Kingdom, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom, [5]Liverpool School of Tropical Medicine, London, United Kingdom

Lymphatic Filariasis (LF) is endemic in Ethiopia, while efforts to eliminate LF and management of podoconiosis are continuing; there is no complete map that details the geographical distribution of LF and podoconiosis in Ethiopia. The Federal Ministry of Health has recognized LF and podoconiosis among priorities for control in the recent national master plan for the control of NTDS, and indicated that a nationwide mapping is the loop-hole. Considering clinical similarities and both diseases have the same target group for mapping the survey integrated the two diseases. Thus, this survey aimed to generate a complete map of LF & podoconiosis distribution in Ethiopia. The study was conducted between June to September 2013, based on WHO guideline for mapping of LF. Equal number of male and female age ≥15 years were randomly selected. Data was collected using Smartphone and server was hosted by the Task Force for Global Health. 100 µl of blood was collected from each consented/assented individual and tested for circulating W. banchrofti antigen using Immunochromatographic tests (ICT).Differentiation between LF and Podoconiosis was made by clinical examination, ICT result and Wb123 antibody tests. A total of 130116 individuals aged between 15 and 100 from 660 unmapped districts were participated on the survey. Demographic data showed 1:1 Female to Male ratio. More than half of the study participants (58.4%) were illiterate. 32.2%study participants reported bed net Utilization. ICT results confirmed the presence of W. banchrofti antigen in 139 (0.1%) of the total study participants. Among 313 (0.24%) of the study participant who showed the development of hydrocoel, only one participant tested positive by ICT for LF infection. 1.8%and 0.6%of the total participant report hematuria and chyluria respectively. Lymphoedema was observed in 6083 (4.7%) of the total participants, of which 5.9% was of upper limbs, 90.7% was of lower limbs, 3.5% was of breast. Of the total lymphoedema of lower limb, 5253 was due to podoconiosis 20 was due to LF and the rest is due to various health problems. The current study revealed 76 districts with at least one ICT positive for the antigen of W. banchrofti. Districts with ≥1% of ICT positive result, considered new endemic districts. The study also revealed massive presence of Podoconosis. The available data will be a direct input for the national and global control and elimination of LF and management of Podoconiosis.

592

CORRELATION BETWEEN HIGH *LOA LOA* MICROFILAREMIA AND LEVELS OF CIRCULATING FILARIAL ANTIGENS USED TO DETECT *WUCHERERIA BANCROFTI* INFECTION

Sébastien Pion[1], Céline Montavon[1], Cédric J. Chesnais[1], Joseph Kamgno[2], Samuel Wanji[3], Michel Boussinesq[1]

[1]Institut de recherche pour le Développement, Montpellier, France, [2]Center for Research on Filariasis and Other Tropical Diseases, Yaoundé, Cameroon, [3]Research Foundation in Tropical Diseases and the Environment, Buea, Cameroon

The methods of choice to detect infection with *Wuchereria bancrofti* are tests detecting circulating filarial antigens (CFA): the Og4C3 ELISA and the field-friendly immunochromatographic card test (ICT) which has been widely used to map lymphatic filariasis (LF) and assess the impact

of mass treatments. During a LF survey conducted in 26 villages in South Cameroon (1805 individuals tested), we noted that none of those 52 subjects who had a positive ICT presented *W. bancrofti* microfilariae (mf) in their night blood smear, whereas 91% had *Loa loa* mf. As blood smears were also prepared by day for all the individuals, we could analyze the association between ICT positivity and *Loa* microfilaremia. At village level, the prevalence of ICT positivity was strongly correlated with the prevalence of diurnal *Loa* microfilaremia (r=0.61, p=0.001). Among the ICT-positive subjects, 96% presented *Loa* mf by day (arithmetic mean: 36,977 mf/mL). The association between ICT positivity and *Loa* mf density was assessed using multivariate logistic regression adjusting on individual characteristics (age, sex, *Mansonella perstans* mf density). Five groups were defined: one including the amicrofilaremics (reference group) and four with increasing mf densities (divided into quartiles). Odds-ratios [95% confidence interval] associated with ICT positivity were 85 [18-294] and 315 [72-1373] in the two groups with the highest *Loa* microfilaremias (3061-12,120 mf/mL and >12,120 mf/mL). We also compared the results of Og4C3 ELISA tests, using dried blood spots, between ICT negative subjects with no *Loa* mf and ICT negatives with >20,000 *Loa* mf/mL. Optical density values obtained with samples from the latter group were higher than those obtained in the former (mean 0.44 vs 0.25, p<0.001). Our results suggest that high *Loa* mf density may cause false positivity with CFA detection tests (in that case LF mapping based on ICT would have to be redone in *Loa*-endemic areas) or is associated with amicrofilaremic *W. bancrofti* infection. Antibody detection tests will be applied on our samples to resolve the issue.

593

ELIMINATION OF ONCHOCERCIASIS IN AFRICA: DO WE HAVE STRATEGIES AND TOOLS? UGANDA CASE ANALYSIS

Thomson Luroni Lakwo[1], Edridah Muheki Tukahebwa[1], Peace Habomugisha[2], Ambrose Onapa[3], Harriet Namwanje[3], Ephraim Tukesiga[4], David Oguttu[1], Rolf Garms[5], Moses Katabarwa[6], Stella Agunyo[3]

[1]Vector Control Division, Ministry of Health Uganda, Kampala, Uganda, [2]The Carter Center, Kampala, Uganda, [3]RTI International, Kampala, Uganda, [4]Kabarole District Local Government, Kabarole, Uganda, [5]Bernard Nocht Institute for Tropical Medicine, Hamburg, Germany, [6]The Carter Center, Atlanta, GA, United States

Onchocerciasis is a neglected parasitic disease that infects at least 37 million people in Africa. The first major attempt to control onchocerciasis was launched in West Africa in 1974 with vector control as the only strategy. However, the advent of Ivermectin (Mectizan®) in 1987, provided opportunities for other endemic African countries to join the war against onchocerciasis. Uganda has had a long history of onchocerciasis control and elimination dating back to the 1950s. Control of onchocerciasis using annual mass treatment with Ivermectin started in 1992. However, annual treatments alone were not able to interrupt transmission of onchocerciasis in some foci. The Ministry of Health adopted new strategies to address this challenge, hence the initiation of Simulium spp. vector control and semi-annual treatments. New sensitive M&E tools have been developed to delineate areas where transmission has been interrupted. Impact assessments conducted in 2004 revealed a reduction of microfilaria prevalence in most of the foci. Assessments were based on skin snip surveys; serology (Ov16), pool screening of flies using PCR and crab collections and examinations for infestation with immature stages of the vector. Based on the results in 2007, an elimination policy was adopted in some foci. Since then, interruption of onchocerciasis transmission has been achieved in a total of 7 foci, where the vectors have also been eliminated. This translates to 1,354,390 treatments halted with more than 1.2 million people living in areas where transmission has been interrupted. With a combination of strategies and available evaluation tools, Uganda has demonstrated that it is feasible to achieve onchocerciasis elimination in Africa.

594

MONITORING TRANSMISSION OF *WUCHERERIA BANCROFTI* POST-MDA IN GHANA

Irene Offei Owusu[1], Dziedzom de Souza[2], Charles Narh[2], Michael David Wilson[2], Daniel Boakye[2], Francis Anto[1], Moses Bockarie[3], John Gyapong[1]

[1]School of Public Health, University of Ghana, Legon, Accra, Ghana, [2]Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana, [3]Liverpool School of Tropical Medicine, University of Liverpool, Liverpool, United Kingdom

The WHO recommends using Transmission Assessment Surveys (TAS) as a primary tool for deciding when to stop Mass Drug Administration (MDA) and for post-MDA surveillance in lymphatic filariasis (LF) elimination programs. In Awutu Senya, Effutu, Agona East and West districts of Ghana, MDA was stopped in 2010 after 9 rounds, and TAS conducted. These areas have passed the TAS and thus the need for post-MDA surveillance to monitor recrudescence. The aim of this study is to monitor transmission of *Wuchereria bancrofti* Post-MDA in Ghana using periodic surveys. Two surveys were conducted, school-based and household surveys. A xenomonitoring study was also undertaken alongside the surveys using Indoor Residual Spraying (IRS) technique. A total of 1,708 children (aged 6-10 years) from selected schools and 1,214 (aged 11-60 years) community members participated in the school-based and household surveys respectively. Daytime finger-prick blood samples were collected from all consenting participants and tested using ICT kit and OG4C3 (ELISA). Mosquitoes were captured from 324 households, and LAMP assay performed to detect *W. bancrofti* parasites. Preliminary results show that prevalence of LF in humans is fairly stable [2010=0.13%, 2012=0%, 2013=0.06%] among 6-10 year old children and the general population [2010=1.17%, 2012=1.00%, 2013=0.08%]. A total of 3347 mosquitoes were captured; 2038 *Anopheles* spp. (2002 *An. gambiae*, 36 *An. funestus*), 1269 *Culex* spp., 33 *Aedes* spp. and 7 *Mansonia* spp. For each community, *Anopheles* spp. were pooled with an average pool size of 15. Forty-two (30%) out of 139 pools were found to be positive, (35 *An. gambiae* and 7 *An. funestus*). While the surveys in humans revealed very low prevalence of infection, xenomonitoring has proven to be much more sensitive showing a prevalence of 30% in known vectors, especially using the LAMP method in detecting the presence of *W. bancrofti*. Information from this study will provide recommendations on mechanisms for monitoring transmission of LF post-MDA which can be scaled-up both in Ghana and globally.

595

FINANCIAL AND ECONOMIC COSTS FOR CONTROL, ELIMINATION AND ERADICATION OF RIVER BLINDNESS IN AFRICA

Young Eun Kim[1], Elisa Sicuri[2], Fabrizio Tediosi[1]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]CRESIB, Barcelona, Spain

Onchocerciasis, also known as river blindness, has been a serious public health problem in Africa; however, the transmission of the disease has been suppressed with successful mass drug administration (MDA) with ivermectin. Also, recent studies in Mali and Senegal proved the feasibility of elimination with ivermectin. These provided momentum for shifting the goal from control to elimination along with the global movement toward elimination of neglected tropical diseases. As part of an Eradication Investment Case for onchocerciasis, financial and economic costs of alternative scenarios of control, elimination, and eradication are estimated using a micro costing approach from a viewpoint of service providers (i.e. ministries of health, WHO, and NGOs). The costs are estimated up to 2045 considering regional elimination in Africa is predicted to be achievable by 2040 employing a micro simulation model for onchocerciasis transmission. Financial costs include costs for MDA and surveillance. Economic costs measure the opportunity costs for community drug

distributors (volunteers) and donated drugs. Unit cost per person living in endemic area is estimated at $1.43 with all costs included and $0.11 with only financial costs. Financial cost of staying in control mode is estimated at $527 million over 2013-2045. Scaling up MDA coverage and implementing regular surveillance to achieve elimination and eradication would cost $440 million -$453 million, the savings being due to decrease in the number of required treatments as MDA is stopped with elimination achieved. Economic costs are estimated to be significantly higher than financial costs, $6.3 billion for the control scenario and $3.1 billion - $3.3 billion for the scenarios of elimination and eradication over the same time horizon. The results suggest scaling up MDA coverage and implementing regular surveillance to achieve elimination and eradication, after initial resource-intensive efforts, would allow substantial financial and economic savings in the long term.

596

EXPLORING THE RELATIONSHIP BETWEEN ACCESS TO WATER, SANITATION AND HYGIENE AND SOIL-TRANSMITTED HELMINTH INFECTION: A DEMONSTRATION OF TWO RECURSIVE PARTITIONING TOOLS

Katherine Gass[1], David Addiss[1], Matthew C. Freeman[2]

[1]Task Force for Global Health, Decatur, GA, United States, [2]Rollins School of Public Health, Emory University, Atlanta, GA, United States

Soil-transmitted helminths (STH) - a class of parasites that affect billions of people - can be mitigated using mass drug administration, though reinfection following treatment occurs within a few months. Improvements to water, sanitation and hygiene (WASH) likely provide sustained benefit, but few rigorous studies have evaluated the specific WASH components most influential in reducing infection. There is a need for alternative analytic approaches to help identify, characterize and further refine the WASH components that are most important to STH reinfection in order to improve WASH interventions for control of STH. In this paper, we introduce two recursive partitioning approaches: classification and regression trees (C&RT) and conditional inference trees (CIT). Utilizing data from a school-based randomized control trial in Kenya, we conduct an assessment of the school- and household-level WASH components and demographic indicators that contribute to reinfection of pupils 10 months following deworming treatment. We show how C&RT and CIT can be used to identify the WASH components most predictive of and associated with STH infection. In addition, we demonstrate how both tools can be used to identify complex interactions between WASH indicators and sub-populations that may be particularly susceptible to STH reinfection, both of which are difficult to identify using traditional epidemiological methods. We discuss the relative merits and weaknesses of each approach and make recommendations for their use as tools to enhance STH control programs.

597

IMPACT OF WASH AND ALBENDAZOLE DISTRIBUTION ON INFECTION WITH SOIL-TRANSMITTED HELMINTHS IN TIMOR-LESTE: INITIAL RESULTS OF A CLUSTER RANDOMIZED TRIAL

Susana V. Nery[1], Suzy Campbell[1], Stacey Llewllyn[2], Ross Andrews[3], Darren Gray[4], Rebecca Traub[5], James S. McCarthy[2], Archie Clements[6]

[1]The University of Queensland, Brisbane, Australia, [2]Queensland Institute of Medical Research, Brisbane, Australia, [3]Charles Darwin University, Darwin, Australia, [4]Australian National University, Canberra, Australia, [5]University of Melbourne, Melbourne, Australia, [6]The Australian National University, Brisbane, Australia

Soil-transmitted helminths (STH) infect more than two billion people worldwide, causing considerable morbidity, including malnutrition and anaemia. STH infections are most prevalent in communities where adequate water and sanitation are lacking and hygiene behaviour is poor. Deworming programmes with anthelminthic drugs are highly effective in reducing morbidity but rapid reinfection occurs if there is no change in the environment. Therefore, provision of water, sanitation and hygiene (WASH) programs is of critical importance in the sustainable control of STHs. "WASH for Worms" is a cluster randomised controlled trial assessing the impact of a community-based WASH intervention, implemented by WaterAid Australia, on infection with intestinal parasites following mass albendazole (ALB) chemotherapy in villages in Timor-Leste. In this trial, initiated in 2012, twelve intervention villages receive the WASH programme and ALB treatments every six months. Twelve control villages receive only the six-monthly ALB. All villages are followed-up for two years after the first ALB distribution. Infection prevalence and intensity is measured by a modified qPCR. The results for STH infection levels at baseline and after the first follow-up in the first 16 villages enrolled will be presented together with results of an anthelmintic efficacy trial using a single dose of ALB on STHs conducted in 8 villages. At baseline the prevalence of STHs in the first eight villages was high, with more than 90% of participants infected with at least one STH (assessed by PCR) mostly comprising Necator americanus (75.3%) followed by Ascaris lumbricoides (63.6%). At the first follow-up in 8 of the villages, it was possible to detect an additional benefit of the WASH intervention compared to deworming alone. This trial is the first reported RCT evaluating the impact of integrated WASH and deworming programmes on infection with STHs; and will provide essential evidence for scaling up integrated programmes for STH control.

598

ASSOCIATION OF WATER QUALITY WITH SOIL-TRANSMITTED HELMINTHIASIS, DIARRHEA AND INFLUENZA-LIKE ILLNESS IN NUEVA SANTA ROSA, GUATEMALA -- 2010

Almea Matanock[1], Xin Lu[1], Victoria M. Cuéllar[1], Patricia Juliao[1], Maricruz Alvarez[2], Andrew Thornton[1], Jaymin C. Patel[1], Fredy Muñoz[2], Gerard Lopez[2], Lissette Reyes[3], Wences Arvelo[1], Beatriz López[2], Kimberly Lindblade[1], Sharon L. Roy[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Universidad del Valle de Guatemala, Guatemala City, Guatemala, [3]Área de Salud de Santa Rosa, Ministerio de Salud Pública y Asistencia Social, Cuilapa, Guatemala

Improved water quality is essential in reducing diarrhea. Its impacts on influenza-like illness (ILI) and the soil-transmitted helminths (STH) *Ascaris* and *Trichuris* are less well described, though some data link them to water, sanitation, and hygiene. To assess their association with water quality, we conducted a cross-sectional survey in Nueva Santa Rosa, Guatemala among persons >1 year old in randomly selected households (HH). A stool sample was tested by Fecal Parasite Concentrator and Kato-Katz method for STH. Diarrhea and ILI were identified by self-reported symptoms in the past week. We explored associations between *Escherichia coli*-positive drinking water (water quality) and disease outcomes using exact logistic regression models. Median unbiased estimates (MUE) are reported when maximum likelihood estimates did not exist. We interviewed 920 persons from 204 HH. Water results were available for 778 persons; 84% (650/778) lived in HH with *E. coli*-positive water. Among persons in HH with water quality testing, 12.4% (76/611) tested positive for *Ascaris* and/or *Trichuris*, 9.4% (73/778) had diarrhea, and 13.5% (105/778) had ILI. Univariable analysis showed an association between water quality and STH (OR 8.9, CI 2.3–76.2), but not with diarrhea or ILI. There was no difference in water treatment practices between HH with and without diarrhea to explain the lack of association between water quality and diarrhea. In stratified analyses, *E. coli*-positive water remained associated with STH among persons ≥15 years (MUE 13.4, CI 2.9–infinite) and those living in densely populated areas (≥1,000 persons/km^2) (OR 15.1, CI 2.5–614.7). The lack of association between water quality and diarrhea was unexpected, as was the association between water quality and STH, since STH has been viewed primarily as a sanitation and hygiene issue. Waterborne transmission and effects of water treatment on STH should be investigated. If a causal relationship is found, practices such as household water treatment, might be useful adjuncts to sanitation, hygiene, and deworming in STH control programs.

599

IMPROVEMENT OF WATER, SANITATION AND HYGIENE IN TWO URBAN SLUMS IN UGANDA THROUGH COMMUNITY PROACTIVE AND SUSTAINABLE INTERVENTIONS

Rawlance Ndejjo[1], David Musoke[1], David Carpenter[2], Simon Kasasa[1], William Bazeyo[1], John C. Ssempebwa[1]

[1]Makerere University School of Public Health, Kampala, Uganda, [2]University at Albany, New York, New York, NY, United States

In urban slum settlements in Uganda, the major risk factor for water borne diseases such as diarrhea is contaminated drinking water due to poor latrine status, low safe water coverage, and poor domestic and personal hygiene practices. To address the challenges affecting these areas, a project aimed at improving the water, sanitation and hygiene (WASH) status in 2 urban slum communities in Uganda through community proactive and sustainable interventions was implemented. The 2 slums involved were in Kampala and Mukono in the central region of the country. To establish the WASH status of the communities before project implementation, a baseline survey was carried out among 213 households. The survey involved both quantitative and qualitative methods. Several interventions with full community participation were then implemented to improve the situation. The interventions included: community sensitization on WASH, promotion of hand washing using the tippy tap technology, supporting clean-up exercises in the community, providing advisory roles in WASH, supporting health clubs in primary schools, training community members in a short course in WASH, capacity building of youth in WASH, promoting drinking safe water through household chlorination and home improvement campaigns. After the project implementation period, a final evaluation survey was carried out among 300 households. This survey involved both quantitative and qualitative data collection methods. Latrine coverage improved from 86.0% to 98.7%. Piped water usage improved from 38.0% to 86.0% with a reduction in the use of unprotected sources from 30.0% to 2.3%. Treatment of drinking water improved from 95.3% to 99.7% with more households (96.0%) boiling their water from 94.0% who did so at the baseline. Indiscriminate disposal of solid waste reduced from 18.0% to 2.0% and satisfaction with solid waste management services increased from 40.0 to 91.8%. Drainage around homes improved from 57.0% to 86.0% while presence of soak pits at households increased from 2.3% to 10.0%. Improvements in the latrine statuses, environmental hygiene and waste management practices were also registered. There were significant improvements in the WASH status of these communities after the implementation of several multi-faceted interventions. Urban slums can benefit from WASH interventions when communities are fully involved and with a focus on capacity building.

600

SOME CHILDREN WITH ACCESS TO IMPROVED WATER AND SANITATION DEMONSTRATE BETTER GROWTH: FINDINGS FROM THE YOUNG LIVES COHORT STUDY IN ETHIOPIA, INDIA, PERU AND VIETNAM

Debbie Humphries[1], Kirk A. Dearden[2], Whitney Schott[3], Benjamin Crookston[4], Mary E. Penny[5], Jere Behrman[3]

[1]Yale School of Public Health, New Haven, CT, United States, [2]Boston University School of Public Health, Boston, MA, United States, [3]University of Pennsylvania, Philadelphia, PA, United States, [4]Brigham Young University, Provo, UT, United States, [5]Instituto de Investigacion Nutricional, Lima, Peru

Child undernutrition is widespread in developing countries and is perceived to have large costs over the lifecycle. We used data on 8,062 children from Ethiopia, India, Peru and Vietnam at ages 1, 5, and 8 yrs from the Young Lives cohort to estimate multivariate logit regressions for associations between household and community-level access to improved water and sanitation, and stunting and underweight at these three ages. Improved drinking water was associated with stunting only in Peru, where access at age 5 yrs decreased risks of stunting at age 5 yrs (OR: 0.68; $p<0.05$). In Ethiopia access to improved sanitation facilities (facilities) at age 1 yr was associated with decreased odds of stunting at 1 yr, 5 yrs and 8 yrs (OR: 0.59, 0.63, 0.64; $p<0.05$). In India and Peru, access to improved facilities at 1 yr was significantly associated with stunting at age 5 yrs only (OR: 0.61, 0.75, $p<0.05$). Improved facilities access when children were 5 yrs old was associated with stunting only at age 5 in Peru (OR: 0.71; $p<0.05$) and age 8 in Vietnam (OR: 0.64; $p<0.05$). Improved facilities at age 8 was not associated with stunting at age 8 in any country. Improved drinking water at 1 yr was significantly associated with underweight at age 8 in India (OR: 0.52; $p<0.05$); for all other time points and countries, improved drinking water was not significantly associated with underweight. Facilities quality at age 1 yr was associated with lower odds of being underweight in Peru at 1 yr (OR: 0.59, $p<0.05$) and in India at age 5 yrs (OR: 0.60, $p<0.05$). There were no other significant associations between facilities and underweight. The combination of significant associations with quality of sanitation facilities and reduced undernutrition and the numerous insignificant associations suggest the importance of further investigating mediating factors that affect whether interventions to improve sanitation reduce concurrent and long-term child malnutrition.

601

INTEGRATING WATER TREATMENT INTO ANTENATAL CARE: IMPACT ON USE OF MATERNAL HEALTH SERVICES AND HOUSEHOLD WATER TREATMENT AMONG MOTHERS -- UGANDA, 2013

Almea Matanock[1], Tara Anderson[1], Miriam Mutabazi[2], Lilian Likicho[2], Thomas Emeetai[2], Celia Kakande[2], Xin Lu[1], Tracy Ayers[1], Robert Quick[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Management Sciences for Health, STRIDES for Family Health, Kampala, Uganda

In Uganda, high maternal and neonatal mortality rates reflect underutilization of reproductive health services; high diarrhea risk among children results from poor access to safe water. To incentivize household water treatment and reproductive health service use, water treatment kits (buckets and 30 sachets of flocculant-disinfectant powder) were provided at first antenatal visits. Refills of 30 sachets each were distributed at follow-up antenatal (ANC) visits, health facility deliveries, and postnatal check-ups. We evaluated this intervention through cross-sectional surveys of a random sample of women from participating health facilities who received reproductive health care in 2013 after project launch (intervention group) and in 2012 before project launch (pre-intervention/comparison group). We used the Chi-Square test statistic to compare groups. We surveyed 226 intervention group women and 207 comparison group women. A higher percentage of intervention than comparison group women reported treating drinking water on the day of the survey (31.7 vs 19.7%, P=0.01), and had detectable chlorine residual, an objective measure of treatment, in their stored drinking water (13.5% vs 3.4%, P<0.01). Of 226 intervention group women, 222 (98.2%) received water treatment kits. Although 96.8% of intervention group women had ≥2 ANC visits (median 4 visits, range 1-5), only 101 (45.5%) received one or more sachet refills. There was no difference in the percentage of women in intervention and comparison groups that reported ≥4 ANC visits (66.2 vs 68.5%, P=0.61) or health facility deliveries (67.8 vs 74.3, P=0.14). Intervention group women were more likely than comparison group women to treat their drinking water, but had similar low use of reproductive health services. Although water treatment kit coverage at first antenatal visit was high, inadequate distribution of, or low demand for, refill sachets may have contributed to limited program impact.

602

IMPACT OF A SCHOOL-BASED WATER, SANITATION AND HYGIENE PROGRAM ON DIARRHEA, RESPIRATORY INFECTIONS AND ABSENTEEISM: A LONGITUDINAL EVALUATION

Victoria Trinies, Anna Chard, Howard Chang, **Matthew Freeman**

Emory University, Atlanta, GA, United States

School-based water, sanitation and hygiene (WASH) programs can lead to improvements on pupil health and attendance, but few rigorous studies are available. We conducted a quasi-experimental, longitudinal impact evaluation of a comprehensive school-based WASH program in Mali that provided schools with latrines, water points, drinking and handwashing stations, cleaning supplies, hygiene promotion, and training on management and governance. We randomly selected 100 primary schools participating in the WASH program and matched them to 100 control schools that were not participating in the program based on location, school population, and the presence of latrines and water points before the start of the program. Unannounced visits were conducted between February 2013 and June 2014, for a total of seven visits per school. At each visit we conducted a roll-call of all pupils in the school and asked 40 pupils to provide a one-week recall of absence, diarrhea, and symptoms of respiratory infections. We evaluated schools for adherence to the program. We will employ random effects longitudinal regression analysis with data clustered at the school level to examine the association between participation in the WASH program and roll-call absence, self-reported absence, self-reported diarrhea, and self-reported symptoms of respiratory infections. The study is powered to detect a 20% reduction in pupil absence and a 30% reduction in diarrhea. Data will be analyzed after the final round of data collection in June 2014.

603

A CHIMERIC *PLASMODIUM VIVAX* CSP TAILORED TO ENHANCE THE CELLULAR IMMUNE RESPONSE

Jairo A. Fonseca, Monica Cabrera-Mora, Balwan Singh, Alberto Moreno

Emory Vaccine Center, Atlanta, GA, United States

Plasmodium vivax is the most widespread species of *Plasmodium* representing 50% of the malaria cases occurring outside sub-Saharan Africa. Existing control measures have significantly reduced the burden of malaria in the past 10 years. However, these measures are not effective against hypnozoites a dormant stage form responsible of *P. vivax* relapse infections. An effective vaccine is therefore essential to the control and eradication of *P. vivax*. A well characterized vaccine candidate is the circumsporozoite protein (CSP). Anti-CSP antibodies have the ability to inhibit the invasion of hepatocytes by *P. vivax* sporozoites *in vitro*. Cellular immune responses against CSP have also been correlated with protection. However, preclinical and clinical data of *P. vivax* CSP based vaccines have shown limited success in inducing cellular immune responses. Based on our reported data on the use of chimeric *P. yoelii* proteins to enhance cellular reactivity, we decided to design a recombinant chimeric protein based on the *P. vivax* CSP. In this study we tested the capacity of a recombinant protein chimera containing immunogenic domains that preserved the protein topology described for *P. yoelii*. The chimeric protein was expressed in soluble form with high yield. The proper configuration and antigenic integrity of the protein were defined by western blot analysis and ELISA. Groups of six different strains of mice were used to test immunogenicity. The chimeric protein was able to induce robust antibody responses in all the mice strains tested against the immunogen. Interestingly, synthetic peptides representing the allelic forms of the *P. vivax* CSP were also recognized to a similar extent regardless of the mouse strain. Cellular immune responses were investigated by IFN-y and IL-4 ELISPOT test as well as intracellular cytokine staining measured using flow cytometry. The immunization regimen resulted in robust production of IFN-y, IL-2 and TNF-a by CD4+ and CD8+ T cells. The fine specificity of the cellular immune responses induced by immunization with the chimeric *P. vivax* CSP will be discussed.

604

INHIBITION OF *PLASMODIUM VIVAX* INVASION OF RED BLOOD CELLS USING ANTI-DUFFY BINDING PROTEIN ANTIBODIES

Brenden P. Jenks[1], D'Arbra Blankenship[1], Vincent R. Gerbasi[2], G. Christian Baldeviano[2], Christopher L. King[1], Andres G. Lescano[2], Brian T. Grimberg[1]

[1]Case Western Reserve University School of Medicine, Cleveland, OH, United States, [2]U.S. Naval Medical Research Unit - 6, Lima, Peru

It has long been established that the malaria parasite, *Plasmodium vivax*, depends on the interaction of its Duffy Binding Protein (DBP) with the erythrocyte Duffy antigen (either FyA, FyB) for host cell invasion. Recent evidence has indicated that patients who are FyAFyA homozygotes are far less likely to experience severe vivax malaria and that DBP protein has a lower binding affinity for this version of the Duffy receptor. As most vaccine resources and strategies concentrate on the parasite's use of the Duffy Binding Protein, it is important to determine if current studies of the FyB::DBP interaction should remain the sole interaction for vaccine development or if we need to explore the FyA::DBP more closely. The focus of this study was to examine the ability of *P. vivax* to invade red blood cells *in vitro* and to determine if inhibition of the parasite's invasion of FyAFyA red blood cells differs from FyBFyB cells. Reticulocyte enriched blood samples of varied Duffy-positive FyBFyB and FyAFyA homozygotes were exposed to different strains of *P. vivax* with genetic variations of DBP (*P. vivax* strains Nicaragua, Indonesia, and Thailand). New invasions were measured using flow cytometry and selective staining techniques, with and without anti-DBP blocking antibodies. The results showed that the blocking ability of the anti-DBP antibodies was highly dependent upon the parasite strains used. Anti-DBP antibodies directed against the DBP Sal 1 variant showed a 97.0% inhibition of invasion into FyAFyA red blood cells by *P. vivax* Indonesia, but only a 24.8% inhibition into FyBFyB red blood cells by the same strain. Conversely, anti-DBP antibodies showed 1.4% inhibition and 12.7% inhibition of *P. vivax* Nicaragua invasion into FyAFyA and FyBFyB red blood cells, respectively. Invasion by *P. vivax* Thailand was not inhibited in either Fy genotype. Also, anti-DBP antibodies showed highly varied efficacy against invasion by all strains into Duffy-negative (Fy0Fy0) red blood cells. These results demonstrate that differences in the parasite's DBP polymorphisms may play a large role in invasion success in the absence of other complex genetic factors between donors. This work indicates that both the genotype of the *P. vivax* and the Fy genotype of the host are potentially important considerations in the development of vaccine candidates.

605

ANTIBODIES TO A HYPOTHETICAL FALCIPARUM MALARIA ANTIGEN (PF3D7_1134300) INHIBIT ERYTHROCYTE INVASION

Ian C. Michelow[1], Dipak K. Raj[1], Christina Nixon[1], Christian P. Nixon[1], Sara Nelson[1], Sunthorn Pond-Tor[1], Joanna Glanz[1], Kai Fricke[1], Jennifer Friedman[1], Michal Fried[2], Patrick E. Duffy[2], Jonathan D. Kurtis[1]

[1]Center for International Health Research, Rhode Island Hospital, Medical School of Brown University, Providence, RI, United States, [2]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Washington, DC, United States

We previously screened a *Plasmodium falciparum* 3D7 strain blood stage cDNA phage display library using a differential approach and identified three antigens encoded by PF3D7_1335100 [MSP-7], PF3D7_1021800 [PfSEA-1] and PF3D7_1134300 that are uniquely recognized by antibodies in plasma from resistant Tanzanian children but not by those from

susceptible children. Resistance was defined by a median parasite density of zero (IQR, 25) documented in monthly blood smears obtained from children between 2 and 3.5 years. The current work characterizes the effector function of murine polyclonal antibodies directed against the immunorelevant portion of PF3D7_1134300. In silico analysis (PlasmoDB.org) predicts that the 6684 bp gene encodes a 263 kDa phospho-protein, contains no introns and has rodent syntenic orthologs. We cloned the immunorelevant region (nt 1,337,023-1,338,945) as well as three overlapping constituent fragments into a eukaryotic expression plasmid (VR2001) and immunized mice to generate antisera. To confirm that PF3D7_1134300 encodes a parasite protein, we probed *P. falciparum* 3D7 infected and uninfected erythrocytes with antisera, which recognized the relevant full length protein only in infected erythrocytes. We performed growth inhibition assays (GIA) by synchronizing 3D7 parasites three times with sorbitol and cultivating them to obtain mature trophozoites that were plated at 1.5% parasitemia (hematocrit 1.0%). Trophozoites were cultured in the presence of heat-inactivated and pre-adsorbed PF3D7_1134300 anti-sera or pre-immune mouse sera for 24 hours and ring stage parasites were enumerated. Anti-sera directed against the antigen of interest and its constituent fragments inhibited parasite invasion by 31-53% compared to controls (all $P < 0.01$). Based on the observation that extra-cellular merozoites appeared to aggregate in the presence of anti-sera, we are conducting studies to explore the mechanism of invasion arrest. Our data suggest that PF3D7_1134300 may be a novel vaccine candidate for pediatric falciparum malaria.

606

FROM EXPERIMENTAL HUMAN MALARIA INFECTION TO A CHEMICALLY ATTENUATED *PLASMODIUM FALCIPARUM* WHOLE PARASITE BLOOD-STAGE VACCINE

Danielle I. Stanisic[1], James Fink[2], John Gerrard[2], Lana Sundac[2], Xue Q. Liu[1], Jolien Pingnet[1], Ingrid B. Rodriguez[1], Moses Lee[3], Terry Spithill[4], Chris B. Davis[1], Eric Hanssen[5], Jo-Anne Chan[6], Christine Langer[6], James G. Beeson[6], Stephen L. Hoffman[7], Michael F. Good[1]

[1]Griffith University, Southport, Australia, [2]Gold Coast University Hospital, Southport, Australia, [3]Hope College, Holland, MI, United States, [4]LaTrobe University, Bundoora, Australia, [5]University of Melbourne, Melbourne, Australia, [6]Burnet Institute, Melbourne, Australia, [7]Sanaria Inc., Gaithersburg, MD, United States

Malaria is a leading cause of morbidity and mortality attributable to infectious disease. The possibility of a malaria vaccine was first realized in the 1940s, yet a vaccine capable of inducing long lasting immunity remains elusive. We have shown that a chemically attenuated whole parasite blood-stage vaccine, consisting of ring stage malaria parasites attenuated with the Cyclopropylpyrroloindole analogue Tafuramycin-A, offers profound protection in rodent models. To evaluate this vaccine approach in humans, suitable reagents and systems were developed. Two malaria cell banks, consisting of human red blood cells infected with *Plasmodium falciparum* NF54 or 7G8, were manufactured by *in vitro* cultivation of the malaria parasites in a GMP compliant facility. Clinical studies were undertaken to assess the safety and infectivity of these cell banks in malaria naïve human volunteers. The cell banks were well tolerated, however differential infectivity was observed between the 2 cell banks, with a significantly higher dose of NF54 required to initiate a blood stage infection. Phenotypic analysis of the malaria parasites in the two cell banks prior to participant inoculation revealed a profound difference. Unlike the 7G8 parasites, the NF54 parasites were unable to adhere to CD36 and there was an absence of knobs on the parasitised erythrocyte surface. Interestingly, when infectivity of the NF54 cell bank was demonstrated, parasitised erythrocytes obtained *ex vivo* from the study participants were able to adhere to CD36 and knob expression was observed. Following these studies, the *P. falciparum* 7G8 cell bank was selected for the manufacture and evaluation of a chemically attenuated blood-stage malaria vaccine in humans. Malaria naïve volunteers will be inoculated with a single dose of 3×10^7 *P. falciparum* 7G8 parasitised erythrocytes, attenuated with Tafuramycin-A, to evaluate its' safety and immunogenicity in humans. The results from this study will be available in the second half of 2014 and will be presented. This is the first evaluation of a whole parasite blood-stage malaria vaccine approach in humans.

607

HUMANIZED HLA MICE ARE PROTECTED AGAINST CHALLENGE WITH *PLASMODIUM FALCIPARUM* SPOROZOITES (PFSPZ) WHEN IMMUNIZED WITH IRRADIATED PFSPZ OR LIVE PFSPZ UNDER CHLOROQUINE COVER BUT NOT BY IMMUNIZATION WITH HUMAN ADENOVIRUS 5-VECTORED *PLASMODIUM FALCIPARUM* ENCODING AMA1 AND CSP

Sai P. Majji[1], Wathsala Wijayalath[1], Soumya Shashikumar[1], Sandra Inoue[1], Andrea Akyeampong[1], Thomas Richie[1], Teodor-Doru Brumeanu[2], Sofia Casares[1]

[1]Naval Medical Research Center/Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Uniformed Services University of Health Sciences, Bethesda, MD, United States

Development of humanized mouse models that are able to act as surrogate human immune system is a highly pursued goal for testing human vaccine candidates, generating fully human monoclonal antibodies and studying the development of human immune system. Previously we have demonstrated that humanized mice expressing HLA molecules in NOD.RagKO.IL2RgcKO background (DRAG/DRAGA) that were infused with HLA matched human hematopoietic stem cells develop a functional human immune system and respond to vaccination. Humanized HLA mice also develop human hepatocytes, kupffer cells, liver endothelial cells and erythrocytes and sustain the vertebrate life cycle of *P. falciparum*. Herein we show that humanized mice are protected against liver stage *Plasmodium falciparum* infection upon immunization with irradiated Pfspz or live sporozoites under chloroquine cover. In contrast, humanized HLA mice were not protected by immunization with human adenovirus 5-vectored *P. falciparum* NMRC-M3V-Ad-PfCA encoding AMA1 and CSP constructs. Humanized HLA mice represent a new pre-clinical model for testing vaccines against malaria.

608

IDENTIFICATION OF A CORE GROUP OF *PLASMODIUM FALCIPARUM* MHC CLASS I PEPTIDES SEQUENCED FROM INFECTED LIVER CELLS

Kimberly Edgel[1], Cathy Zou[1], Sri Hadiwidjojo[1], Jessica Bolton[1], Joao Aguiar[1], Emily Smith[1], Parimal Samir[2], Xinnan Niu[2], Andrew Link[2], Eileen Villasante[1], **Vincent R. Gerbasi**[1]

[1]Naval Medical Research Center, Silver Spring, MD, United States, [2]Vanderbilt University Medical School, Nashville, TN, United States

Sterile protection from *Plasmodium falciparum* (Pf) infection can be achieved by developing potent cell mediated immunity against malaria liver stages. Liver stage immunity has been established in human clinical trials using two different but similar approaches: 1. Immunization with radiation-attenuated-sporozoites (RAS) and 2. infection-treatment-vaccination (ITV). The model for RAS immunization suggests that attenuated sporozoites enter hepatocytes, initiate development, and then die leaving behind parasite material for degradation and presentation on MHC Class I receptors. During ITV human volunteers are challenged with live sporozoites under chloroquine coverage resulting in complete hepatocyte invasion and development. Both RAS and ITV immunization suggest that liver stage immunity is mediated by Class I presentation of Pf peptides by either aborted liver stage development or during successful merozoite development. A long standing goal in the malaria vaccine field is to identify the Pf antigens that induce liver stage immunity. Towards this goal we have worked to identify Pf Class I peptides presented from *in vitro* infected human hepatocytes by direct peptide sequencing. We have identified a core group of 12 Pf proteins sequenced from three genetically

distinct primary human hepatocyte sources infected with sporozoites. Among this core group of Pf proteins we have identified a subset that induces IFN-γ secretion from PBMCs of RAS-protected volunteers. In our future experiments we will test this core group of liver stage antigens as vaccine candidates in malaria challenge models.

609

IMMUNODOMINANCE AND VACCINE DEVELOPMENT: NEW INSIGHTS FROM PROTEOME-WIDE PROFILING OF T CELL AND ANTIBODY RESPONSES TO MALARIA

Carla Proietti[1], Angela Trieu[1], Joanne Roddick[1], Bruno Douradinha[1], Leanne Robinson[2], Ivo Mueller[2], Peter Siba[2], Lutz Krause[1], John Sidney[3], Alessandro Sette[3], **Denise Doolan**[1]

[1]QIMR Berghofer Medical Research Institute, Brisbane, Australia, [2]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [3]La Jolla Institute for Allergy and Immunology, La Jolla, CA, United States

For many infectious diseases, the development of effective vaccines based on immunodominant antigens has thus far not been successful. Immunodominance is the phenomenon whereby pathogen-specific immune responses target only a small fraction of the full range of possible antigens and epitopes. It is a central feature of immunity but what distinguishes effective immune targets from less effective targets is not obvious. In experimental infections with simple organisms, immunodominance results in only a few specificities dominating the host's response. Large pathogens such as the *Plasmodium* parasite represent a far greater challenge because of the complexity and scale of potential immune targets. We have generated unique datasets of proteome-wide T cell responses and antibody responses to *Plasmodium* using omics-scale technology platforms, including protein microarrays and epitope prediction algorithms, with specimens from humans experimentally or naturally exposed to malaria. We have integrated these datasets to develop metrics of immunological, structural and genetic parameters associated with antigen immunodominance in the context of a complex parasite. We establish that the repertoire of T cell reactive antigens is largely distinct from the repertoire of antibody reactive antigens, and that T cell target antigens are more conserved as compared with antibody targets. We further establish that the immunodominance hierarchy for antibody responses is influenced by structural or functional properties that differ from those that underlie the hierarchy for T cells. By defining the degree to which immunodominance shapes immunity against *Plasmodium* and identifying antigens and epitopes that represent key targets of protective immunity in humans, our studies have direct outcomes for vaccine development. These also data further our understanding of host-pathogen interactions.

610

PLASMODIUM FALCIPARUM ACTIVATION OF B-CATENIN MEDIATES THE DISRUPTION OF INTERENDOTHELIAL CELL JUNCTIONS: A ROLE IN CEREBRAL MALARIA

Julio Gallego-Delgado[1], Upal Basu-Roy[1], Maureen C. Ty[1], Matilde Alique[2], Cristina Fernandez-Arias[1], Alexandru Movila[1], Samuel C. Wassmer[1], Marta Ruiz-Ortega[2], Ana Rodriguez[1]

[1]New York University Langone Medical Center, New York, NY, United States, [2]IIS-Fundación Jiménez Díaz, Madrid, Spain

A high proportion of deaths by *Plasmodium falciparum* is caused by cerebral malaria, the most profound syndrome of severe malaria that is fatal within 24-72 hours in 20% of the cases. The disruption of the blood brain barrier (BBB), characteristic of cerebral malaria, causes diffusion of blood cells and serum into the brain tissue leading to coma and damage to the nervous system. The mechanisms underlying this process are largely unknown but the need for adjunct therapy to rescue patients from death is mandatory due the high rate of mortality. Using monolayers of human brain microvascular endothelial cells (HBMECs), we have observed that incubation with erythrocytes infected with *Plasmodium falciparum* promotes the disruption of interendothelial cell junctions (IEJs) between these cells. This process is mediated by the delocalization of β-Catenin from the adherent junctions to the nuclei and the activation of the Tcf/LEF pathway, which leads to the detachment of the HBMECs. Infecting HBMECs with a lentivirus carrying a dominant negative mutant tcf4 vector, we were able to inhibit the detachment of HBMECs induced by *P. falciparum*. Compounds that inhibit the activation of the β-Catenin pathway also inhibit the disruption of IEJs and detachment of HBMECs induced by *P. falciparum* and have a protective effect against experimental cerebral malaria in mice. Our findings describe an unprecedented role for β-Catenin in the maintenance of the BBB integrity in the setting of cerebral malaria and open new perspectives for the treatment of the disease.

611

MULTIPRONGED PROTEOMICS AND METABOLOMICS ANALYSIS OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX* INDUCED ALTERATIONS IN HUMANS TO DECIPHER DISEASE PATHOGENESIS AND IDENTIFY SURROGATE MARKERS

Sandipan Ray[1], Sandip K. Patel[1], Vipin Kumar[1], Apoorva Venkatesh[1], Nithya Gogtay[2], Gangadhar Chatterjee[3], Urmila Thatte[2], Kunal Sehgal[4], Arunansu Talukdar[5], Sanjay K. Kochar[6], Dhanpat K. Kochar[6], Rapole Srikanth[7], Santosh G. Varma[3], Swati Patankar[1], Sanjeeva Srivastava[1]

[1]Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai, India, [2]Departments of Clinical Pharmacology, Seth GS Medical College and KEM Hospital, Mumbai, India, [3]Department of Biochemistry, Government Grant Medical College and Sir JJ Group of Hospitals, Mumbai, India, [4]PD Hinduja National Hospital and Medical Research Centre, Mumbai, India, [5]Medicine Department, Medical College Hospital Kolkata, Kolkata, India, [6]Department of Medicine, Malaria Research Center, S.P. Medical College, Bikaner, India, [7]Proteomics Laboratory, National Centre for Cell Science, Pune, India

In this study serum samples from severe and non-severe falciparum and vivax malaria patients and healthy controls from different endemic regions of India were investigated using multiple quantitative proteomics approaches and results were validated in larger clinical cohorts by using WB, ELISA and surface plasmon resonance-based measurements. Additionally, metabolomics analysis was performed using LC-MS/MS to identify the altered metabolites and associated pathways in malaria. Specificity of the identified serum markers was evaluated by analysis of dengue fever and leptospirosis patients as febrile disease controls. In quantitative proteomic analysis, 67 and 82 differentially expressed ($p < 0.05$) serum proteins were identified in falciparum and vivax malaria respectively, and almost half of these proteins were commonly modulated in both of the plasmodial infections. Using LC-MS coupled with multivariate statistical data analysis approaches over 3000 serum metabolites were screened among which nearly 200 exhibited altered serum level in the malaria patients. Functional pathway analysis involving the identified proteins and metabolites revealed the modulation of different vital physiological pathways including acute phase response signaling, amino acid and lipid metabolism, chemokine and cytokine signaling, complement cascades and blood coagulation in malaria. Different hematological, liver and renal function parameters were also measured in malaria patients and controls. Hemoglobin level and platelet count found to be significantly lower ($p < 0.01$) in the malaria patients. ROC curve analysis demonstrated Serum amyloid A, Apolipoprotein E and Haptoglobin as efficient predictor proteins (AUC > 0.90) for malaria detection at an early stage of infection. Expression levels of these three serum proteins also exhibited good correlation with parasite count ($r > 0.5$; $p < 0.001$). Interestingly, analysis of longitudinal cohorts (early febrile, defervescence and convalescent stages) indicated cyclic alterations in the expression levels of Haptoglobin, Retinol binding protein, ApoE and Apo-A1 during the different stages of the infection, which could serve as indictors of the disease progression. Our findings may open up new

opportunities for the early detection and prognosis of malaria as well as could provide better understanding of disease pathogenesis and host responses in malaria.

612

THE ROLE OF ENDOTHELIN-1 IN THE VASCULAR PATHOBIOLOGY OF CEREBRAL MALARIA

Brandi D. Freeman, Yuri Chaves Martins, Mahalia S. Desruisseaux

Albert Einstein College of Medicine, Bronx, NY, United States

Cerebral malaria (CM) is a serious complication of *Plasmodium falciparum* infection associated with cerebral vasculopathy, high mortality, and adverse neurological sequelae. The vasoactive peptide, endothelin-1 (ET-1), has been shown to mediate blood-brain barrier (BBB) permeability, inflammation, and vascular tone, and may be important in CM pathogenesis. We previously reported that ET-1 was important in regulating cerebral blood flow, brain microvascular hemorrhage and mortality in our experimental CM (ECM) model. These actions were mediated by ET-1 activation of the endothelin A (ET_A) receptor. To test the hypothesis that ET-1 is involved in the pathological process of ECM, we investigated ET_A receptor mediated signaling in mice infected with *P. berghei* ANKA (PbA). ET_A receptor blockers (ET_ARB) significantly improved survival in ECM mice. In addition, ET_ARB enhanced vascular integrity during PbA infection. BBB permeability, and protein levels of angiopoietin-2 and VCAM-1 were significantly lower in ECM mice treated with ET_ARB than in mice treated with saline. In addition, ET_ARB prevented the ECM-induced decrease in angiopoietin-1 in PbA-infected mice. CM is associated with astrogliosis in both human disease and in experimental models. Our preliminary data indicate that astrogliosis is associated with abnormal protein levels of connexin 43 (Cx43), a gap junction protein critical in gliosis and BBB integrity. ET_ARB prevented the PbA-induced dysregulation of Cx43. We hypothesized that ET-1 mediated vascular dysfunction in ECM potentially by regulating neuroinflammation and Cx43 expression. In this regard, we observed a reduction in the activation of JNK in the brains of mice with ECM. JNK, a downstream substrate of ET-1, has been demonstrated to regulate Cx43 expression and function, and is important in CM. Our data indicate that ET-1 may mediate the vascular pathology and neuroinflammation in ECM via regulation of JNK signaling and subsequent Cx43 dysregulation. The ET-1 pathway may thus be a potential therapeutic target as an adjunct in the treatment of human CM.

613

PLASMODIUM FALCIPARUM IN AOTUS: A NOVEL MODEL FOR PLACENTAL MALARIA

Ankur Sharma, Adovi Akue, Lynn Lambert, Sachy Orr-Gonzales, Jessica Hinderer, Dariyen Carter, Tom Thomas, John Bacher, Michal Fried, Patrick E. Duffy

National Institutes of Health, Rockville, MD, United States

Pregnant women are infected with a distinct *Plasmodium falciparum* (Pf) phenotype that binds to chondroitin sulfate A (CSA) and grows to high density in the placenta. Placental sequestration is linked to severe pathologies in the mother and offspring, leading to tens or hundreds of thousands of deaths each year. CSA-binding parasites express VAR2CSA, a distinctly structured member of the Pf erythrocyte membrane protein 1 (PfEMP1) variant surface antigen family that is now the leading target for vaccine development. Further research progress is hindered in the absence of an animal model that recapitulates the features of human placental malaria. Previous work has shown that Pf can infect and sequester in brain, heart and spleen of non-pregnant Aotus, and we sought to establish a model of placental malaria using this parasite-monkey combination. We infected pregnant Aotus (n= 2) with CSA-binding Pf-CS2 during the 3rd trimester and observed a pronounced sequestration of parasites in placental intervillous spaces, with ~30-fold higher parasite density in placental versus peripheral blood. Most placental parasites were mature blood stages, while peripheral samples were exclusively young ring stages. Aotus (n= 1) infected with non-CSA-binding parasite lines (CAMP; FVO) before pregnancy developed recrudescences during subsequent pregnancy, similar to the experience of women. CS2 parasites collected from pregnant Aotus express VAR2CSA on the infected erythrocyte (IE) surface, and bind specifically to CSA. Similar to immune multigravid women, a monkey infected with Pf-CS2 parasites over successive pregnancies acquired antibodies against VAR2CSA, and purified antibodies blocked the binding of several maternal parasite isolates to CSA. In summary, Pf infections in pregnant Aotus monkeys recapitulate all the prominent features of malaria infection and immunity in pregnant women, and can be useful for basic mechanistic studies as well as preclinical studies to qualify candidate pregnancy malaria vaccines.

614

SPLENIC PATHOLOGY SUPPORTS THE ACCELERATION OF SEVERE MALARIA THROUGH HIV INFECTION IN PEDIATRIC PATIENTS

Regina Joice[1], Charles Frantzreb[1], Alana Pradhan[2], Clarissa Valim[1], Javier G. Ogembo[3], Joyce Fingeroth[3], Karl B. Seydel[4], Steve Kamiza[5], Dyann F. Wirth[1], Manoj Duraisingh[1], Malcolm E. Molyneux[6], Terrie E. Taylor[4], Matthias Marti[1], Danny A. Milner, Jr.[2]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Brigham and Women's Hospital, Boston, MA, United States, [3]Beth Israel Deaconess Hospital, Boston, MA, United States, [4]Michigan State University, East Lansing, MI, United States, [5]Queen Elizabeth Hospital, Blantyre, Malawi, [6]Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi

A number of cohort studies have demonstrated that HIV-1 infection correlates with severe *Plasmodium falciparum* malaria (higher parasitemia, more clinical malaria complications, and higher case fatality rate), and *in vitro* studies have shown that HIV-1 impairs phagocytosis of parasitized erythrocytes by macrophages. As macrophage-mediated clearance of parasites in the spleen plays an important role in the clearance of parasites during malaria infection, we hypothesized that histological analysis of the spleen might further elucidate the mechanisms by which HIV-malaria co-infection accelerates progression to severe malaria. As part of a post-mortem study in Blantyre, Malawi of parasitemic comatose children, we systematically surveyed malaria parasites across organs in patients with and without HIV. The overall HIV seropositivity rate in the cohort was 21%. Using a combination of histology, immunohistochemistry and qRT-PCR, we found that HIV-positive patients had significantly higher parasite loads in brain, heart, gut, spleen and skin. The difference was most pronounced in the spleen where the difference in the mean number of parasites was highly significant (320 ± 139 vs 81 ± 20 parasites in 10 high power fields, $p = 0.0018$). In a focused histological analysis of the spleen, we found that HIV-positive cases had higher levels of "free" parasitized erythrocytes, i.e. those not engulfed by macrophages, while phagocytosed parasites could be readily observed in HIV-negative cases. These HIV-specific effects were significant, even across a wide range of values for peripheral parasitemia, hematocrit, and final histologic diagnosis (cerebral malaria or non-malarial coma). These data suggest that HIV infection enhances parasite sequestration in many different organ systems, and specifically in the spleen, where phagocytosis of parasites is impaired. These findings bridge *in vitro* mechanistic data and *in vivo* association study data to further elucidate the process by which HIV accelerates progression to severe malaria.

615

CHILDREN WITH CM MANIFEST MARKED DIFFERENCES IN GLOBAL STRESS RESPONSES WHICH ARE ASSOCIATED WITH CEREBRAL PARASITE SEQUESTRATION AND UNDERLYING PATHOPHYSIOLOGY

Catherine Manix Feintuch[1], Karl Seydel[2], Alex Saidi[3], Adam Goldman-Yassen[1], Ryung Kim[1], Terrie Taylor[2], Johanna Daily[1]

[1]Albert Einstein College of Medicine, Bronx, NY, United States, [2]Michigan State University, East Lansing, MI, United States, [3]Blantyre Malaria Project, Blantyre, Malawi

Cerebral Malaria (CM), a severe complication of *Plasmodium falciparum* infection, is associated with a high rate of mortality and neurological sequelae. The WHO defines CM as a single clinical entity of coma and peripheral blood malaria parasitemia; however, disease heterogeneity has been suggested by the identification of brain sequestration (RET+) in some but not all patients with CM. RET+CM is associated with brain microvascular pathology, parasite sequestration, coagulopathy and heightened inflammation. To explore potential mechanistic diversity in factors resulting in CM, we carried out unsupervised clustering on whole blood transcriptomes from 98 children with CM and identified three transcriptional clusters. One cluster was significantly associated with hyperparasitemia ($p<0.0001$) and another cluster with lack of brain sequestered parasites (RET-) ($p=0.028$). To further define host features associated with parasite sequestration, we then compared transcriptional profiles between samples associated with brain sequestered parasites (RET+) to lack of brain sequestrated parasites (RET-) taking parasite load into account. Gene sets associated with RET+CM reflected heightened inflammation (cytokine activity GO.000512), changes in neutrophil (neutrophil chemotaxis GO.0030593) and platelet (alpha granules GO.0031093) biology and dysregulation of coagulation (blood coagulation GO.0007597). Elevated plasma inflammatory cytokines and neutrophil proteins were found in RET+ samples, consistent with the RNA data. Surprisingly, neutrophils isolated from RET+ patients at the time of infection displayed impaired chemotaxis towards IL-8. In contrast, the transcriptional profiles of children with RET-CM were associated with upregulation of non-inflammatory stress pathways (protein catabolism pathways: GO.0006511; DNA repair pathways: GO.0006270) and type I interferon. Our data suggests host response diversity despite similar clinical presentations of WHO defined CM. Irrespective of parasite load, we found that children with cerebral parasite sequestration demonstrate evidence of neutrophil activation and coagulopathy. Further study of how neutrophil and platelet biology is involved in sequestration and why children with RET-CM have distinct host profiles could reveal novel strategies to prevent brain sequestration in CM and improve clinical outcomes.

616

PHENOTYPIC VARIATION IN THE ABILITY OF *PLASMODIUM KNOWLESI* LAB AND FIELD STRAINS FOR THE INVASION OF HUMAN RED BLOOD CELLS

Caeul Lim[1], Elsa Hansen[1], Angela Siner[2], Caroline O. Buckee[1], Balbir Singh[2], Manoj T. Duraisingh[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Malaria Research Center, Kuching, Malaysia

Plasmodium knowlesi, primarily a simian parasite, is now the major cause of human malaria in Malaysian Borneo. While the majority of the cases are uncomplicated, clinical profiles similar to that of severe *P. falciparum* cases occur in a small subset of infected patients, with high parasitemia as the only risk factor consistently associated with disease severity. However, there has been little investigation to understand what causes such disparity in parasitemia. Recently, we showed that *P. knowlesi* H strain, a primate adapted lab strain, preferentially invades very young human red blood cells (RBCs). This strain however can gradually adapt to proliferate at a much higher efficiency in human blood. To expand this observation to other *P. knowlesi* strains, we have established *in vitro* culture rhesus RBCs 3 additional *P. knowlesi* strains. RBC age-dependent invasion assays and proliferation assays demonstrate that these lab stains exhibit different capacity in invading human RBCs. We are currently carrying adaptation experiments for these additional lines. To determine whether variations observed in the lab are also found in the field, we investigated the human RBC tropism of 32 field strains isolated from patients admitted to Kapit Hospital, Sarawak, Malaysia. The *in vivo* preference determination of 26 of them shows that a majority of the strains have a skew for invading younger RBCs (18/26). Indeed, 5 isolates display a very strong preference in invading reticulocytes. *In vitro* invasion assays performed with 9 isolates (parasite density greater than 10,000/ul) confirm that field strains differ significantly in the range of human RBCs they can invade. Together, these results suggest a significant variation in the ability of *P. knowlesi* strains to invade human RBCs,underlying a possible mechanism for the virulence of the parasite in human infections.

617

CHARACTERIZATION OF THE EXPANDED ACYL CO-A SYNTHETASE GENE FAMILY

Selina Bopp, Allison Demas, Pamela Magistrado, Sarah Volkman, Ulf Ribacke, Dyann Wirth

Harvard School of Public Health, Boston, MA, United States

The plasticity of the *Plasmodium falciparum* genome allows this malaria parasite to adapt quickly to selective pressures encountered in the human host by the acquisition of single nucleotide polymorphisms, recombination or gene duplications. One example of such duplication events is the acyl Co-A synthetase (ACS) gene family where four conserved orthologs of ACS are predicted to perform classical ACS function while nine paralogs have expanded and diverged from the *Pf*ACS9 ortholog and their function remains unknown. ACSs activate fatty acids (FA), which can then be used for protein modification, phospholipid biosynthesis, FA elongation, and beta-oxidation. In *P. falciparum* the majority of FA are taken up from the host environment. Long-range haplotype analysis suggests that members of this gene family are under recent positive selection. To address the biological function of the individual members of the *Pf*ACS family, we tagged individual genes with an HA tag and a protein degradation domain resulting in inducible protein knockdowns. These modifications allowed us to identify different expression patterns and distinct cellular localization of individual ACSs that could be a result of neo-functionalization. PfACS5 and PfACS1a were exported to the host cell cytosol and membrane periphery while PfACS9 remained within the parasite. Comparison of growth rates for wildtype and PfACS1a, PfACS4, PfACS5 and PfACS9 knockdown parasites in complete media did not differ and we could not detect any changes in gene expression of any other family member by quantitative RT-PCR. However, growth defects could be detected for PfACS5 knock down parasites in media containing restricted glucose and solely palmitic and oleic acid. We hypothesize that the expansion and recent positive selection of the *Pf*ACS gene family are the consequence of metabolic pressures driving parasite evolution, and understanding FA metabolism will give us insight into key metabolic pathways that might serve as potential targets for novel antimalarials.

618

MUTATIONAL STUDIES FOR FUNCTIONAL CHARACTERIZATION OF ATP SYNTHASE COMPLEX IN *PLASMODIUM FALCIPARUM*

April M. Pershing, Praveen Balabaskaran Nina, Michael W. Mather, Akhil B. Vaidya

Drexel University College of Medicine, Philadelphia, PA, United States

The energy-converting rotary nanomotor ATP synthase is a central player in the bioenergetics of most organisms. However, several studies have found that blood stages of *Plasmodium falciparum* rely on glycolysis as its major source of ATP rather than oxidative phosphorylation, suggesting dispensability of ATP synthase. Yet, we have been unable to disrupt the

genes encoding the β and γ subunits of the ATP synthase complex in *P. falciparum* blood stages, raising the possibility that non-enzymatic functions of the ATP synthase may be essential. To address this possibility, we aimed to design a catalytically inert but structurally intact ATP synthase complex. A conserved residue in the catalytic site of the β subunit was identified as an initial target for mutagenesis. A merodiploid line expressing a tagged mutated β subunit at an ectopic site was generated in Dd2attB and NF54attB parasites. In theory, this should reduce the overall ATP synthase catalytic activity by about 87.5%. Although the mutant subunit was correctly trafficked to the mitochondrion in both lines, the transgenic parasites grew at the same rate as the parental lines, indicating that the ectopically expressed mutant subunits did not have a dominant negative effect. Mitochondria from the NF54attB merodiploid line were isolated and initial blue native page results indicated the mutant form of the protein being incorporated into the complex. Given that no dominant negative effect was seen with the merodiploid lines, a synthetic gene to replace the endogenous gene with a mutated version through single allelic exchange has been designed and will be transfected into the sexually competent NF54 line. We are furthermore attempting to knockdown the mRNA of the β subunit with a ribozyme system. These studies will help determine the role of ATP synthase in erythrocytic and insect stages of *P. falciparum*.

619

AN ALGORITHM FOR ASCERTAINING THE *VAR* GENE REPERTOIRE IN *PLASMODIUM FALCIPARUM* FIELD SAMPLES

Mark Travassos[1], Joana C. Silva[2], Elliott F. Drábek[2], Arthur L. Delcher[3], Qi Su[2], Antoine Dara[1], Drissa Coulibaly[4], Modibo Daou[4], Ahmadou Dembele[4], Issa Diarra[4], Abdoulaye K. Kone[4], Bourema Kouriba[4], Matthew B. Laurens[1], Amadou Niangaly[4], Karim Traore[4], Youssouf Tolo[4], Claire M. Fraser[2], Mahamadou A. Thera[4], Abdoulaye A. Djimde[4], Ogobara K. Doumbo[4], Christopher V. Plowe[1]

[1]Howard Hughes Medical Institute/Center for Vaccine Development, Baltimore, MD, United States, [2]Institute for Genome Sciences, Baltimore, MD, United States, [3]McKusick-Nathans Institute of Genetic Medicine, Baltimore, MD, United States, [4]Malaria Research and Training Center, Bamako, Mali

Plasmodium falciparum erythrocyte membrane protein-1 (PfEMP1) proteins are encoded by the *var* gene family, present in 40 to 60 copies per genome. PfEMP1 mediates tissue-specific cytoadherence of infected erythrocytes, allowing parasites to evade clearance by the spleen and cause pathologic effects via sequestration. An infected erythrocyte usually expresses only one PfEMP1 variant on its surface at a time. PfEMP1 molecules undergo clonal antigenic variation that is central to their ability to evade the host immune response. Next generation short-read sequencing data cannot be used to accurately and comprehensively characterize sequence variation of this large, repetitive and highly variable gene family. Each *var* gene consists of two exons, the first of which is longer (2,500 -10,500 bp) and much more variable in length than the second (1,000 - 1,500bp). We developed a novel algorithm to perform targeted assembly of *var* gene exons, based on a combination of Pacific BioSciences (PacBio) and Illumina data. The algorithm takes advantage of patterns of conservation at the boundaries of the single intron, and the different variation profiles of the two exons, to generate exon 1 and exon 2 sequences from a combination of *k*-mer walks using Illumina reads and information from PacBio-based contig assemblies. Our novel algorithm recovers a complement of exons which is similar in number and length distribution to those found in the reference 3D7 genome, suggesting that it captures most of the *var* exons in the genomes of field-derived samples. Using this algorithm, we have characterized the repertoire of *var* genes in 12 clinical samples from Mali. We are determining the constitutive domains and upstream promoters for identified *var*s in each sample and the sequence diversity present. We will compare these results to known *var* repertoires. This new tool will advance efforts to understand the role of PfEMP1 variation in pathogenesis and immune evasion and may aid in the design of diversity-covering malaria vaccines.

620

SUBTLE CHANGES IN *PLASMODIUM FALCIPARUM* INFECTION COMPLEXITY FOLLOWING ENHANCED INTERVENTION IN MALAWI

Tamika J. Sisya[1], Raphael M. Kamn'gona[1], Jimmy A. Vareta[2], Joseph M. Fulakeza[1], Mavuto F. Mukaka[3], Miriam K. Laufer[4], Karl B. Seydel[5], Terrie E. Taylor[5], **Standwell C. Nkhoma**[1]

[1]Malawi-Liverpool-Wellcome Trust, Blantyre, Malawi, [2]Blantyre Malaria Project, Blantyre, Malawi, [3]Johns Hopkins University, Baltimore, MD, United States, [4]Centre for Vaccine Development, University of Maryland, Baltimore, MD, United States, [5]College of Osteopathic Medicine, Michigan State University, East Lansing, MI, United States

With support from the Global Fund, the United States President's Malaria Initiative (PMI) and other cooperating partners, Malawi is implementing a comprehensive malaria control programme involving indoor residual spraying in targeted districts, free distribution of insecticide-treated bed nets to children and pregnant women, and use of the highly effective artemisinin-based combination therapy, CoArtem, as the first-line treatment for malaria. We genotyped 24 genome-wide single nucleotide polymorphisms (SNPs) in *Plasmodium falciparum* infections (n=295) sampled from a single location in Malawi before and after the switch to CoArtem to evaluate the impact of this enhanced malaria control programme. We used the SNP data generated to examine temporal changes in the incidence of infections containing multiple parasite genotypes (MIs), mean number of heterozygous SNPs within MIs, parasite genetic diversity (He), multilocus linkage disequilibrium and effective population size (Ne). While the incidence of MIs, He, multilocus linkage disequilibrium and Ne were unchanged over time, the mean number of heterozygous SNPs in MIs decreased significantly (p=0.0120) from 9±1 in 2006 to 7±1 (95% CI) in 2012. These findings indicate that the genetic diversity of malaria parasites remains high in this area, suggesting that only subtle gains, if any, have been made in reducing malaria transmission. Continued surveillance is required to evaluate the impact of malaria control interventions in this area and the rest of Malawi, and to better target interventions.

621

COMPARATIVE ANALYSIS OF FIELD ISOLATE AND MONKEY-ADAPTED *PLASMODIUM VIVAX* GENOMES

Ernest R. Chan[1], John Barnwell[2], Peter A. Zimmerman[3], David Serre[1]

[1]Cleveland Clinic Foundation, Cleveland, OH, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Case Western Reserve University, Cleveland, OH, United States

Due to the difficulty to culture *Plasmodium vivax in vitro*, significant insights into this important malaria parasite is based upon a history of successful adaptations of human infections to non-human primates. *P. vivax* strains grown in monkeys serve as a renewable source of parasites for studying relapse characteristics, mosquito species compatibilities, drug susceptibility profiles or immune response characteristics for potential vaccine development. However, little is known as to how the adaptation to a new host influences the parasite's genome and, consequently, its biology. Additionally, despite the consistent observation of complexity of infections in clinical isolates, these monkey-adapted strains are typically assumed to consist of a clonal population of a single strain. We describe here the comparative analysis of genome sequences from seven *P. vivax* parasites that have been adapted to monkey hosts with sequences obtained from six field isolate genomes. Our results reveal that the adaptation of parasites into monkey hosts is unlikely to result in any systematic modification of the genome. We also show that monkey-adapted strains are not always

homogenous and that they can still consist of a mixture of strains. Additionally, we describe the analysis of six blood samples collected during the generation of the Mauritania-I and Mauritania-II strains and show that, starting from a single complex infection, different strains became dominant in different monkeys. Overall, our study highlights some of the complications associated with studying monkey-adapted strains but also provide a solid framework for developing better, more controlled studies of this important resource for understanding vivax malaria.

622

ASSESSMENT OF POPULATION STRATIFICATION AND COMPLEXITY OF INFECTION IN CAMBODIAN *PLASMODIUM VIVAX* BY HIGH-THROUGHPUT SEQUENCING

Lindsey R. Friedrich[1], Jean Popovici[2], Ernest R. Chan[1], Didier Menard[3], David Serre[1]

[1]Cleveland Clinic, Cleveland, OH, United States, [2]Institute Pasteur in Cambodia, Phnom Penh, Cambodia, [3]Case Western Reserve University, Cleveland, OH, United States

In Cambodia, malaria is one of the foremost public health problems and its control is a high priority for the government and NGOs. Among the estimated 13.6 million Cambodians, 2.5 million individuals live in forested areas where malaria transmission is highest. With the implementation of extensive control efforts, the number of reported malaria cases has globally decreased since 1997, due to a decrease in falciparum malaria. By contrast, in the same period, the number and proportion of cases attributed specifically to *Plasmodium vivax* has significantly increased. In order to design more efficient elimination and control strategies in this region, it is critical to first understand the dynamics and organization of the *P. vivax* population. Here, we described a novel sequencing assay that enables robust genotyping of 130 single nucleotide polymorphisms (SNPs) in a high-throughput and cost-efficient manner. We applied this assay to 401 *P. vivax*-infected patients recruited from 9 study sites throughout Cambodia between 2004 and 2013. Our analysis provides a thorough perspective on the diversity, organization and dynamics of the Cambodian *P. vivax* population as well as a first assessment of the factors influencing complexity of infections. Overall, our study provides a foundation to design better control strategies against vivax malaria in Cambodia and to limit the spread of antimalarial drug resistance.

623

SPATIAL DISTRIBUTION OF THE MICRONEMAL PROTEIN CELTOS DURING MOSQUITO MIDGUT TRAVERSAL

Fiona Angrisano[1], Jake Baum[2]

[1]Walter and Eliza Hall Institute, Parkville, Australia, [2]Imperial College, London, United Kingdom

Malaria remains a major burden on the health of global populations. Transmission of the malaria parasite through the Anopheline vector has emerged as a major focus of interest with a view to developing strategies to block mosquito infection, or reinfection of the human host. To date, whilst greater detail is emerging, there are still very few molecular details of any of the stages of mosquito or host re-infection. One protein that functions in both of these stages is CelTOS (cell traversal in ookinetes and sporozoites protein) a secreted soluble micronemal antigen that plays a role in both the colonisation of the mosquito midgut by the ookinete (traversing the epithelium towards oocyst formation) and infection of the liver by the sporozoite (traversing liver cells towards hepatocyte infection). Whilst knockout of this protein has already definitively demonstrated its function in these two distinct processes we still do not know what CelTOS does or its spatial localisation during these key stages. Here we present an update on our work using a tagged line of CelTOS that we have developed along with a suit of imaging reagents and microscopy approaches to make steps towards the functional dissection of CelTOS during mosquito transmission. Given its biphasic role, further understanding of CelTOS is hoped to lay the foundations for developing this key transmission protein into a future multi-stage anti-malarial vaccine.

624

GENOTYPIC DIFFERENCES IN DENV NEUTRALIZATION ARE EXPLAINED BY A SINGLE AMINO ACID MUTATION THAT MODULATES VIRUS "BREATHING"

Kimberly A. Dowd, Swati Mukherjee, Christina R. DeMaso, Theodore C. Pierson

Viral Pathogenesis Section, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Flaviviruses explore multiple conformations via dynamic motion of virus structural proteins. This introduces complexity to the antigenic surface, as virus "breathing" varies epitopes available for antibody (Ab) binding. A recent study explored the structural basis for genotypic differences in the neutralization potency of a DENV-1 specific mAb (E111) that binds a poorly exposed domain III epitope on the envelope (E) protein, as reported previously. Studies with DENV strains WP and 16007 indicated that ~100-fold difference in neutralization sensitivity could not be explained by differences in the affinity of mAb E111 for each strain. Instead, we hypothesized that the ensemble of structures sampled by these two viruses was distinct. To investigate the basis for differences in the "breathing" of these two strains, we generated a panel of DENV RVP variants expressing E protein chimeras or single amino acid differences on both WP and 16007 backgrounds. By assessing E111 potency against the E protein chimera RVPs, the difference in neutralization was mapped to three consecutive residues in domain II that differed between 16007 and WP. Further use of single amino acid mutant RVPs demonstrated that residue 204, but not neighboring residues 202 and 203, was responsible for the difference in neutralization. That residue 204 is located at a distance from the E111 epitope suggested that this amino acid dictates changes in the conformational ensembles sampled by the virus. In support of this, differences in neutralization by a panel of mAbs representing epitopes distinct from E111 were all significantly modulated by the same residue. Our results demonstrate that neutralization susceptibility can be altered in an epitope-independent manner by subtle mutations that alter the overall structural ensemble. That different conformational ensembles of flaviviruses may affect the landscape available for Ab binding has important implications for vaccine development and antibody mapping studies.

625

CONSTRUCTION AND CHARACTERIZATION OF CHIMERIC DENGUE VIRUSES CONTAINING ANTIBODY EPITOPES FROM TWO SEROTYPES

William Messer[1], Boyd L. Yount[2], Ruklanthi de Alwis[3], Sarah Gabriel[1], Scott R. Royal[2], Doug Widman[2], Idia V. Rodriguez[4], Carlos A. Sariol[4], Aravinda M. de Silva[2], Ralph S. Baric[2]

[1]Oregon Health & Sciences University, Portland, OR, United States, [2]University of North Carolina Chapel Hill, Chapel Hill, NC, United States, [3]La Jolla Institute for Allergy and Immunology, La Jolla, CA, United States, [4]Caribbean Primate Research Center, San Juan, Puerto Rico

Dengue is the most prevalent mosquito-borne viral disease of humans and a major public health problem worldwide, with approximately one-half of the world's population at risk of infection by the four dengue virus (DENV) serotypes. Dengue vaccine development has been slow to date, as achieving a balanced and robust immune response against all four serotypes has proven elusive. The reasons for imbalanced immunity elicited by candidate vaccines are not fully understood, but are thought to include variable immunogenicity and competition between the live-attenuated viruses in the vaccine formulations. However, as the precise determinants of DENV immunity are incompletely understood, improving vaccine

formulations is difficult. Here we describe the results of investigations into the role of epitopes within the DENV envelope (E) domain I/II hinge region in DENV immunity. We transplanted DENV-3 EDI/II residues that constitute the epitope footprint of the DENV-3 specific monoclonal antibody (MAb) 5J7 into a DENV1 infectious clone, making the recombinant virus rDENV-1/3. We then transplanted the DENV-1 EDI/II residues that constitute the epitope footprint of the DENV-1 specific MAb 1F4 into a DENV-3 infectious clone, making the clone rDENV-3/1. Both rDENV-1/3 and rDENV-3/1 can be neutralized *in vitro* via the MAb corresponding to the transplanted residues, demonstrating that MAb epitopes can be transplanted between DENV serotypes. To test how epitope transplant affected rDENV-1/3 and rDENV-3/1 neutralization by human sera, these viruses were tested against a panel of DENV-1 and DENV-3 1° immune sera. Strikingly, both recombinant viruses are neutralized by 1° DENV-1 and 1° DENV-3 human immune sera, suggesting that the recombinant viruses displays epitopes recognized by both DENV-1 and DENV-3 neutralizing Abs in polyclonal immune sera, giving them "bivalent" qualities. To test the immunogenicity of these viruses *in vivo*, we have subsequently challenged groups of rhesus macaques with both recombinant viruses and here present the results of the macaque neutralizing antibody response following infection.

626

CHARACTERIZATION OF NEUTRALIZING ANTIBODY RESPONSES FOLLOWING NATURAL SECONDARY DENGUE VIRUS INFECTIONS

Bhumi Patel[1], Gopal Sapparapu[2], James B. Crowe[2], Ralph Baric[3], Aravinda M. de Silva[1]

[1]Department of Microbiology and Immunology, University of North Carolina School of Medicine, Chapel Hill, NC, United States, [2]Departments of Medicine, Pediatrics, Pathology, Microbiology and Immunology, and The Vanderbilt Vaccine Center, Vanderbilt University Medical Center, Vanderbilt University, Nashville, TN, United States, [3]Department of Microbiology and Immunology, University of North Carolina School of Medicine, Department of Epidemiology, University of North Carolina School of Public Health, Chapel Hill, NC, United States

Dengue Virus (DENV) is an arthropod-borne flavivirus and the causative agent of dengue fever and dengue hemorrhagic fever. The goal of this study is to characterize the human neutralizing antibody response that develops after secondary DENV exposure. As there are 4 serotypes of DENV, people can be infected multiple times, each time with a new serotype. Based on recent studies, the dengue field has learned a lot about the properties of neutralizing antibodies generated in people exposed to dengue for the first time (primary infections) but little is known concerning the response generated during a repeat infection with a new serotype (secondary infection). Following primary infections, people develop lifelong protective immunity against the serotype of infection. Primary infections stimulate serotype-cross reactive and serotype-specific (to the serotype of infection) antibodies. However, only a small fraction of serotype-specific antibodies that bind to quaternary structure epitopes at the hinge region between domains I and II of the viral envelope protein are responsible for DENV neutralization. In the present study we investigated the properties of neutralizing antibodies produced during a secondary infection. Following a secondary infection, people develop neutralizing/protective antibodies to multiple serotypes including serotypes that have not infected the individual. An antibody depletion technique using beads coated with purified virus was used to measure levels of serotype specific and cross reactive antibodies and their relative contribution to neutralization. We observed two types of responses: in some sera, dengue virus neutralization was dominated by cross reactive antibodies, whereas in other sera both type-specific and cross-reactive antibodies contributed to neutralization. Thus, unlike primary sera, secondary sera contain antibodies that cross neutralize and presumably cross-protect from DENVs. Ongoing studies with recombinant DENVs and human monoclonal antibodies indicate these secondary infection antibodies recognize novel conserved epitopes that are distinct from the E protein domain I/II hinge epitopes targeted after primary exposure.

627

TRANSPLANTATION OF A COMPLEX QUATERNARY SEROTYPE-SPECIFIC NEUTRALIZING ANTIBODY EPITOPE BETWEEN DENGUE 3 AND 4 REVEALS DETERMINANTS OF POLYCLONAL NEUTRALIZATION RESPONSES

Douglas G. Widman

University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Dengue virus (DENV) is the most significant human arboviral disease worldwide with upwards of 300 million infections annually; however the determinants of human immune responses to DENV infection remain largely unknown. Thus we set out to develop tools with which to characterize antibody (Ab) responses to DENV infection in humans. Using reverse genetics we developed infectious clones (IC) for all 4 DENV serotypes which allow us to study Ab-virus interactions. Characterization of a panel of monoclonal Abs (mAb) identified a strongly type-specific neutralizing Ab of DENV3. Using a structure-guided approach a 12Å region of the envelope (E) protein domain I/II (EDI/II) hinge region encompassing mutations that led to escape of neutralization was identified and transplanted from DENV4 into DENV3 (rDENV3/4) to assess the contribution of this epitope to the polyclonal immune response in humans. Interestingly, this rDENV3/4 gained full sensitivity to neutralization by human DENV4 immune sera while becoming resistant to DENV3 sera, indicating that this EDI/II hinge region contains major determinants of type-specific neutralization responses. When the reciprocal transplant was made into DENV4, mAb binding was not retained and there was not a significant shift in neutralization profiles, indicating that the adjacent residues in the recipient DENV serotype play a role in epitope presentation on the virion surface. The addition of 5 amino acid residues from DENV3 into DENV4 was able to restore mAb binding and neutralization, however polyclonal serum neutralization remained largely unchanged. Finally, we moved a complex quaternary epitope encompassing residues spanning multiple E dimers into DENV4 (rDENV4/3). This rDENV4/3 was viable and grew to high infectious titers and exhibited sensitivity to DENV3 immune sera, while neutralization responses to DENV4 remained largely unchanged. These results provide insights into the determinants of type specific neutralization responses that could guide future development of rationally designed DENV vaccine platforms.

628

GENERATION OF RECOMBINANT DENGUE VIRUSES ENCODING VARIANT ENVELOPE GENES FROM INTRASEROTYPIC GENOGROUPS REVEALS BREADTH OF TYPE-SPECIFIC NEUTRALIZATION RESPONSES

Scott R. Royal[1], Douglas G. Widman[1], Boyd Yount[1], Jesica A. Swanstrom[1], Carlos A. Sariol[2], Aravinda M. deSilva[1], William B. Messer[3], Ralph S. Baric[1]

[1]University of North Carolina, Chapel Hill, NC, United States, [2]University of Puerto Rico, San Juan, Puerto Rico, [3]Oregon Health Sciences University, Portland, OR, United States

It is estimated that over 300 million individuals are infected with dengue virus (DENV) each year, yet a detailed understanding of the humoral response to infection is poorly understood. Recent clinical trials of tetravalent DENV vaccine platforms have shown that eliciting a balanced and robust immune response against all 4 DENV serotypes has proven difficult. Within each DENV serotype exist multiple genogroups; however the breadth of serotype-specific immune responses across these genogroups is not well characterized. Using reverse genetics, we have developed infectious clone (IC) systems for all 4 DENV serotypes allowing for the generation of recombinant DENV (rDENV) for use as novel reagents for studying determinants of DENV-specific immune

responses. Using this technology we have created chimeric rDENV that express envelope (E) genes derived from variant genogroups within DENV serotypes 3 and 4 as well as a sylvatic DENV4 strain within the context of our DENV IC backbones . We examined immune responses from naturally infected persons and experimentally inoculated non-human primates (NHP) using these rDENV strains to characterize neutralization breadth across genogroups. Additionally, we have also identified critical epitopes localizing to the E domain I/II (EDI/II) hinge region that are critical components of serotype-specific neutralization responses. rDENV were generated by transplanting these epitopes between serotypes, and NHP were inoculated to examine immune responses elicited. These data showed NHPs had high serotype-specific antibody titers to the EDI/II hinge epitope; however the breadth of this response was unknown. Using our intraserotypic E chimeras, we characterized the extent to which these NHP sera neutralized other genogroups within each serotype. We show here these results detailing intergenotypic neutralization by naturally infected humans and rDENV vaccinated NHP. These results provide insight towards the rational design of DENV vaccine candidates capable of eliciting broad protection against multiple genotypes within each serotype.

629

LONG-TERM MAINTENANCE OF DENGUE VIRUS-SPECIFIC CELL MEDIATED IMMUNE RESPONSES

Alexandra Gurary[1], Melissa Nagata[1], Munkzhul Sukhbaatar[1], Janet Meeks[1], **Allison Imrie**[2]

[1]University of Hawaii at Manoa, Honolulu, HI, United States, [2]University of Western Australia, Perth, Australia

All 4 serotypes of dengue virus (DENV) can cause dengue fever (DF) and severe dengue (dengue hemorrhagic fever/dengue shock syndrome, DHF/DSS), mediated by cross-reactive T cells. Infection with any of the 4 DENV viruses is thought to confer life-long immunity to re-infection with that same serotype only, and heterotypic secondary infection is associated with development of severe disease. Our understanding of how memory T-cells may contribute to anti-DENV immunity is incomplete, and the duration of DENV-specific T-cell-mediated immune responses is not known. We enrolled volunteers in Hawaii who recalled experiencing a dengue-like illness in 1943, and following an outbreak of DENV-1 in 2001, the first known autochthonous transmission since 1943. Dengue is not endemic in Hawaii and in the absence of repeated exposures we were able to assess the duration, memory phenotype, and effector function of DENV-specific T cell-mediated immune responses in volunteers who experienced a single infection 3, 9, or 60+ years previously. DENV-specific proliferation was observed in all previously infected subjects, but not in any Control (seronegative) subject. Both CD4+ and CD8+ T-cells proliferated in response to stimulation with DENV-1 however there were marked differences between the two subsets with respect to longevity of responses. CD4+ responses were maintained at similar levels for the 3yr, 9yr and 60+yr groups, whereas CD8+ T-cell memory declined with time, with substantially diminshed responses apparent at 9yrs; in one subject this decline was already apparent 6 yrs after infection. B*3501/NS3500 tetramer analysis in age-matched 3yr and 60+yr subjects showed that the frequency of polyfunctional IFN-γ+/TNF-α + DENV-specific CD8+ T-cells declined significantly between 3yr and 6yrs after infection, reaching values similar to those seen at 60+yrs, and that IFN-γ production declined while TNF-α was maintained. We demonstrate the long-term persistence of dengue-specific T-cell immunity and show that the CD4+ and CD8+ memory pools are regulated independently.

630

INTERACTION OF A DENGUE-SPECIFIC CD8+ T CELL NS1 EPITOPE WITH KIR3DL1 ON NK CELLS REVEALS AN UNDERAPPRECIATED ROLE FOR NK CELLS IN IMPACTING DENGUE DISEASE SEVERITY

Elizabeth Townsley[1], Geraldine O'Connor[2], Cormac Cosgrove[3], Marcia Woda[1], Mary Co[1], Stephen J. Thomas[4], Siripen Kalayanarooj[5], In-Kyu Yoon[6], Ananda Nisalak[6], Anon Srikiatkhachorn[1], Sharone Green[1], Henry A. Stephens[7], Mary Carrington[2], Daniel McVicar[2], Galit Alter[3], Alan L. Rothman[8], **Anuja Mathew**[1]

[1]University of Massachusetts Medical School, Worcester, MA, United States, [2]National Cancer Institute, Frederick, MD, United States, [3]Ragon Institute, Boston, MA, United States, [4]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [5]Bangkok Children's Hospital, Bangkok, Thailand, [6]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [7]University College, London, United Kingdom, [8]University of Rhode Island, Providence, RI, United States

Killer immunoglobulin-like receptors (KIRs) interact with HLA class I ligands and play a central role in the regulation and activation of natural killer **(**NK**)** cells. During our investigation of CD8 T cell responses to a highly conserved HLA-B57 restricted DENV epitope, we observed substantial binding of a tetramer (B57-$NS1_{26-34}$ TET) to an NK enriched population. Since HLA-B57 is a known binding partner to KIR3DL1, we hypothesized that the B57-$NS1_{26-34}$ TET bound KIR3DL1 on NK cells. Staining of a KIR3DL1 transfectant cell line confirmed that B57-$NS1_{26-34}$ TET bound KIR3DL1. Consistent with the function of an inhibitory KIR, incubation of PBMC with HLA B57 expressing $NS1_{26-34}$ pulsed target cells suppressed the degranulation of only KIR3DL1+ NK cells. Furthermore, staining of PBMC from our clinical cohort revealed marked activation of NK enriched cells only in HLA B57+ patients who developed severe dengue disease DHF. These observations reveal a previously unappreciated role for a dengue T cell epitope in modulating NK cell function and have important implications for the pathogenesis of severe dengue disease.

631

A MARKED REDUCTION IN MORTALITY AMONG PARTICIPANTS IN A CLINICAL TRIAL THAT REMOVED BARRIERS TO CARE AND IMPLEMENTED NATIONAL CASE MANAGEMENT GUIDELINES

Mary J. Hamel[1], Martina Oneko[2], John Williamson[2], Maquis Sewe[2], Simon Kariuki[2], Frank Odhiambo[2], Laurence Slutsker[1], Kayla Laserson[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]KEMRI/Centers for Disease Control and Prevention, Kisumu, Kenya

KEMRI/CDC in Kisumu Kenya is an evaluation site for the RTS,S/AS01 vaccine trial to measure efficacy in children vaccinated at age 5-17 months or 6-12 weeks. Participants at this site appeared to have low mortality rates compared with non-enrolled children in the KEMRI/CDC demographic surveillance system (DSS) where the trial was conducted. Although clinical and severe malaria were prevented, RTS,S did not show protection against mortality at 18 months follow-up. The comparator vaccines, meningC and rabies, prevent rare diseases, and thus were also unlikely to reduce mortality. We conducted a case control study to quantify the reduction in mortality among children enrolled in the malaria vaccine trial (cases) vs. children not enrolled, but living in the KEMRI/CDC DSS (controls). Participants in the malaria vaccine trial were provided transport reimbursement of 150 KSh (1.80 USD) for clinic visits. Study clinicians followed national case management guidelines. Severely ill children were transported to the district hospital, where adequate staffing, supplies and equipment were made available through the trial to implement the Kenyan National Pediatric Protocol for inpatient care. Outpatient and hospital care were provided free of charge. Microbiology capacity (including blood culture) was instituted. Cases and controls were matched

1:3 by date of birth (within 14 days), proximity (control within 3 km of a case), and gender. Cox regression analysis was used to calculate hazard ratio (HR). In multivariable analysis, we controlled for distance from the clinic. In total, 1618 cases were matched to 3541 controls. Half of cases and controls were female and no difference in socio-economic status was detected (p=0.12). Mean distance to the clinic was 1.9 km for cases and 1.8 km for controls (p<0.01). In all, 229 deaths occurred, 31 among cases (1.9%) and 198 among controls (5.6%). Cases contributed 2719 person years (py) and controls 5335 py of observation. Mortality rates were 0.01 and 0.04 deaths/py among cases and controls, respectively; the adjusted HR was 0.30 (95% CI: 0.19, 0.47) corresponding to a 70% (95%CI: 53, 81) reduction in mortality. Children enrolled in the clinical trial at KEMRI/CDC experienced a marked reduction in all-cause mortality. These data suggest that considerable reduction in child mortality could be achieved by reducing barriers to health care and providing quality care according to national guidelines.

632

REACTIVE VACCINATION IN THE PRESENCE OF DISEASE HOTSPOTS

Andrew Azman, Justin Lessler

Johns Hopkins School of Public Health, Baltimore, MD, United States

Reactive vaccination has recently been adopted as an outbreak response tool for cholera and other infectious diseases. A global revolving stockpile of cholera vaccine was recently established for emergency use, but there is only enough vaccine globally - within and outside the stockpile - to vaccinate approximately one million people. When responding to outbreaks, public health officials must quickly decide who and where to distribute limited vaccine. Transmission hotspots have been discussed as one potential mechanism to efficiently allocate vaccine, however the effectiveness of this approach is likely to be context dependent. We compared strategies for allocating vaccine across multiple areas with heterogeneous transmission efficiency. We constructed meta-population models of a cholera-like disease and compared simulated epidemics where: vaccine is targeted at areas of high or low transmission efficiency, where vaccine is distributed equitably across the population, and where no vaccine is used. We find that connectivity between populations, transmission efficiency, vaccination timing, and the amount of vaccine available all shape the performance of different allocation strategies. In highly connected settings, like cities, when vaccinating proactively or early in the epidemic, targeting limited vaccine at transmission hotspots (i.e. areas with high transmission efficiency) is often optimal. However, once vaccination is delayed, targeting the hotspot is rarely optimal, and strategies that either spread vaccine between areas or those that focus on non-hotspots will avert more cases. Although transmission hotspots may seem like an intuitive target for outbreak control, we show that in many situations the hotspot epidemic may proceed so fast that hotspot-targeted vaccination will prevent relatively few cases, and vaccination shared across areas where transmission can be sustained is often the best approach. Our results suggest a general rule of thumb for vaccination in cities: reactive distribution of vaccine across areas that can independently maintain transmission is generally preferred to targeting any particular transmission hotspot, and hotspots should only be targeted when they are thought to be necessary drivers of transmission. These results provide new insights on how to efficiently vaccinate in response to an epidemic when vaccine is limited.

633

ASSESSMENT OF REASONS FOR POLIO VACCINE REFUSALS IN NORTHERN NIGERIA - OCTOBER, 2012

Charles A. Michael[1], Ikechukwu U. Ogbuanu[2], Chima Ohuabunwo[3], Oladayo Biya[1], Patrick Nguku[1]

[1]Nigeria Field Epidemiology and Laboratory Training Program, Abuja, Nigeria, [2]Centers for Disease Control and Prevention, Atlanta, Atlanta, GA, United States, [3]African Field Epidemiology Network, Abuja, Nigeria

Oral polio vaccine (OPV) refusals during supplemental immunization activities (SIAs) contribute to the spread of polio virus in Nigeria. In September 2012, the Expert Review Committee (ERC) on Polio Eradication and Routine Immunization in Nigeria recommended that social research be conducted to better understand the reasons for polio vaccination refusals among caregivers in Northern Nigeria.Following the OPV campaign in October,2012, we conducted a cross-sectional study using semi-structured questionnaires to assess polio risk perception, reasons for refusals and perception of OPV campaigns. We interviewed caregivers who refused OPV for their children in two purposively selected high refusal districts (one rural and one urban) in five north-western Nigeria states. Median age of the 148 study participants was 39.5years, 82.4% were male, all were Muslims and 28% had primary level education or higher. Polio risk perception was low (77%), 89% of participants believed that the polio vaccine was neither necessary nor helpful, and possibly harmful. Most participants (75%) had an unfavourable or indifferent view of the polio campaigns. Religious belief was reported as an important driver of the participants' understanding of health and disease (70%). Most (85%) study participants indicated they were more concerned about other health issues such as malaria. Caregivers refuse OPV largely due to poor polio risk perception, unmet felt-needs and religious beliefs. Partners have therefore adopted strategies including; communication schemes aimed at increasing awareness of polio as a health threat; educating communities about the safety of the vaccine; engagement of religious leaders, polio survivor groups and polio/pro-OPV Community Development Volunteers, comprehensive health camps and provision of basic amenities like water boreholes to address identified issues. The adoption and implementation of the recommendations of this study contributed to the 59% reduction in the incidence of polio in Nigeria between week 52 of 2012 and week 52 of 2013.

634

CHARACTERISTICS ASSOCIATED WITH THE REGULAR CONSUMPTION OF MILK OR SOLIDS IN BREASTFEEDING INFANTS IN MAL-ED, AN EIGHT SITE COHORT STUDY

Stephanie A. Richard[1], Ram K. Chandyo[2], Estomih R. Mduma[3], Cloupas Mahopo[4], Bruna Maciel[5], Anuradha Bose[6], Ali Turab[7], Mustafa Mahfuz[8], Laura Murray-Kolb[9], Tahmeed Ahmed[8], Laura E. Caulfield[10]

[1]Fogarty International Center, Bethesda, MD, United States, [2]Center for International Health, Bergen, Norway, [3]Haydom Lutheran Hospital, Haydom, United Republic of Tanzania, [4]University of Venda, Thohoyandou, South Africa, [5]Universidade Federal do Rio Grande do Norte, Natal, Brazil, [6]Christian Medical College, Vellore, India, [7]Aga Khan University, Karachi, Pakistan, [8]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [9]The Pennsylvania State University, University Park, PA, United States, [10]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

The WHO recommends exclusive breastfeeding for the first 6 months of life in order to maximize nutrient intake, increase immune function, and decrease exposure to pathogens. We expect that mothers choose to stop exclusive breastfeeding or introduce milks or solids based on multiple infant (size, illness, behavior), maternal (maternal age, education, number of live births) and environmental (food security, resources) factors. Using data from an 8-site birth cohort study, we explored the relationship between child, maternal and environmental characteristics and the timing

of regular consumption of milks or solids during the first six months of life. Overall, 1,967 children were included in this analysis, approximately 200 per study site. The median age of first regular consumption of milk or solids ranged from 41 days (Pakistan site) to 176 days (Bangladesh site). We used Cox proportional hazards models with time (in days) to regular consumption of milk or solid as the outcome, including both fixed (sex, maternal age, parity, etc.) and time varying (weight, height, illness, etc.) exposures. Higher weight-for-length (WLZ) and length-for-age Z-scores (LAZ) were associated with later introduction of milk or solids. Food insecurity, as well as illness (diarrhea, cough in the past 30 days), were also associated with later consumption of milks and solids. Factors related to breastfeeding initiation (colostrum, prelacteal feeding), maternal education, and parity were not strongly associated with timing of regular consumption of milks and solids. We included interactions between time and WLZ, LAZ, and cough and found that the protective effect of higher values of WLZ, LAZ, and cough decreased over time. These preliminary results indicate that the diet of the breastfed child may be altered according to the child's size or health status. These data will improve our understanding of the site-specific and overall timing of the regular consumption of milk and solids as it relates to child and maternal factors, and will allow us to make recommendations regarding how to incorporate breastfeeding in future analyses in order to avoid the potential for reverse causality. In addition, we can explore the factors associated with early regular consumption of milk and solids in these populations in order to understand and appropriately evaluate their impact on growth and overall health.

635

HEMOGLOBIN CONCENTRATION DURING PREGNANCY AND INFANT COGNITIVE AND MOTOR DEVELOPMENT: A PROSPECTIVE COHORT STUDY

Michael O. Mireku[1], Leslie L. Davidson[2], Ghislain K. Koura[3], Smaïla Ouédraogo[4], Michael J. Boivin[5], Xu Xiong[6], Manfred M. Accrombessi[7], Achille Massougbodji[8], Michel Cot[7], Florence Bodeau-Livinec[1]

[1]French School of Public Health (EHESP), Rennes, France, [2]Columbia University, New York, NY, United States, [3]Union Internationale Contre la Tuberculose et les Maladies Respiratoires, Paris, France, [4]Centre Hospitalier Universitaire Yalgado Ouédraogo, Ouagadougou, Burkina Faso, [5]Michigan State University, East Lansing, MI, United States, [6]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [7]Institut de Recherche pour le Développement, Paris, France, [8]Université d'Abomey-Calavi, Cotonou, Benin

There have been several studies on the consequences of anemia during pregnancy but to date, very little is known about the impact of prenatal anemia on infant neurocognitive function. The objective of this study was to assess the impact of anemia during pregnancy on the cognitive and motor functions of one-year-old children in Benin. Our prospective cohort study included one-year-old children born to women enrolled at their first antenatal care (ANC) visit, before 29 weeks of pregnancy, within the MiPPAD trial comparing sulfadoxine-pyrimethamine and mefloquine. Hemoglobin (Hb) concentrations of pregnant women were determined from venous blood samples collected at first and second ANC visits of at least, one-month interval and at delivery. Women were prescribed oral iron, folic acid and anthelminthics as part of the ANC package in Benin. A total of 635 children (76.7% of eligible children) were assessed for cognitive and motor functions, using the Mullen Scales of Early Learning (MSEL), at twelve months of age by trained research nurses. Prevalence of anemia decreased from 67.8% at first ANC visit to 40.1% at delivery. Children of mothers who were anemic at second ANC visit had better gross motor function (an estimated mean increase of 2.4 points, 95% Confidence Interval, CI, 0.1 to 4.7). We observed a significant negative quadratic relationship between infant gross motor function and Hb concentration at first ANC visit for women of gestational age greater 22 weeks (p=0.025). Thus, infant gross motor scores increased with increasing maternal Hb concentration until 88 g/L where it plateaued and began to decline at 110 g/l. We found no significant association between Early Learning Composite scores and maternal Hb concentration. There appears to be an optimal range Hb concentration (88-110 g/l) between 22 and 32 weeks of gestation that may be beneficial to infant gross motor function at age one year. These may reflect physiological hemodilution, which peaks between 22 and 32 weeks of gestation. Further studies are required to corroborate our findings.

636

FOOD INSECURITY AND INDIRECT COSTS OF MEDICALLY-ATTENDED GASTROENTERITIS IN CHILDREN YOUNGER THAN FIVE YEARS OF AGE IN A "POST-ROTAVIRUS" SETTING

Laura B. Rapoport[1], Fabiola D. Colquechagua Aliaga[2], Andrew J. Mirelman[1], Milagros G. Tejada Santiesteban[2], Brisaida M. Rafael Cordova[2], Dante A. Figueroa Quintanilla[3], Robert H. Gilman[4], **Sarah-Blythe Ballard**[1]

[1]Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Asociacion Benefica PRISMA, Lima, Peru, [3]Instituto Nacional de Salud del Nino, Lima, Peru, [4]Laboratorios de Investigacion y Desarrollo, Universidad Peruana Cayetano Heredia, Lima, Peru

Several vaccines are in development against norovirus (NV), the leading cause of pediatric diarrhea after rotavirus. In order to analyze the cost-effectiveness of potential vaccines, the direct and indirect costs of diarrhea must be ascertained. Since indirect costs have the potential to be high, accurate estimations of these costs are vital. In order to estimate the economic impacts of NV diarrhea, this study was designed to estimate the indirect and out-of pocket direct costs of a single diarrheal episode in a population with 90% rotavirus vaccine coverage. Children younger than five years of age presenting with diarrhea to the national children's hospital in Lima, Peru (Instituto Nacional de Salud del Niño) were screened for diarrhea according to the World Health Organization's case definition. Children meeting the case definition whose caregivers agreed to participate were actively followed with daily phone calls from the time of presentation until the conclusion of the diarrhea episode. The caregiver was then interviewed by a health care provider about the indirect costs of the diarrhea episode using a survey adapted from the WHO's indirect costs of diarrhea survey tool and the United States Department of Agriculture food security screening tool. Minimum wage was used to estimate the cost of lost productivity. Costs were converted to US dollars. The caregivers of 30 children younger than five years of age with diarrhea completed the interview. The median monthly household income was $306 (IQR: 252-360). The median estimated total indirect cost of the diarrheal episode was $75 (IQR: 54-117). The median number of hours of housework lost was 5 (IQR: 4-18). Thirty three percent of caregivers worked outside the house. Of those, the median number of workdays lost was 3 (IQR: 1.5-4.5). The median total out of pocket expense for one diarrheal episode was $85 (IQR: 65-126), including $22 (IQR: 14-41) for drugs and medical supplies. As a result of time and money lost due to the diarrheal episode, 70% of caregivers reported being worried about not having enough money to buy food, and 17% reported not having money to buy food. Significant indirect costs are incurred from a single episode of acute pediatric diarrhea, and these costs contributed to food insecurity in a segment of this population. Indirect costs should be considered in vaccine cost-effectiveness estimates and guide decisions about the introduction of new childhood vaccines.

637

CLINICAL ASSESSMENT OF CHILDREN WITH FEBRILE ILLNESS AT HEALTH FACILITIES IN THREE DISTRICTS IN SOUTHERN ZAMBIA

J. Joseph Lawrence[1], Toyin Ajayi[2], Kojo Yeboah-Antwi[3], William B. MacLeod[3], Godfrey Biemba[4], Karsten Lunze[5], Kebby Musokotwane[6], Simon Mutembo[6], Duncan Earle[7], Richard W. Steketee[8], Chilunga Puta[7], Davidson H. Hamer[9]

[1]Zambia Center for Applied Health Research and Development, Lusaka, Zambia, [2]Center for Global Health and Development, Boston University and Department of Family Medicine, Boston University Medical Center, Boston, MA, United States, [3]Department of Global Health, Center for Global Health and Development, Boston University, Boston, MA, United States, [4]Zambia Center for Applied Health Research and Development and Boston University Center for Global Health and Development, Lusaka, Zambia, [5]Boston University School of Medicine, Boston, MA, United States, [6]Southern Provincial Medical Office, Ministry of Health, Choma, Zambia, [7]Malaria Control and Evaluation Partnership in Africa (MACEPA), PATH, Lusaka, Zambia, [8]Malaria Control and Evaluation Partnership in Africa (MACEPA), PATH, Seattle, WA, United States, [9]Center for Global Health and Development, Boston University and Zambia Center for Applied Health Research and Development, Lusaka, Zambia

Given the decline in malaria prevalence in many areas in sub-Saharan Africa, fever in children can no longer be treated presumptively as malaria. The purpose of this evaluation was to examine health system capacity and clinicians' ability to accurately diagnose and treat pediatric febrile disease in three districts in Zambia with varying malaria endemicity. We conducted health facility surveys, health worker (HW) interviews, and observed patient-provider interactions for children under the age of 5 who presented with history of fever in the previous 48 hours. Quality of the clinical evaluation was assessed using the Zambian Ministry of Health IMCI guidelines. We observed 161 patient-provider interactions and interviewed 53 HWs at 24 health facilities with regard to their assessment of danger signs. Inability to feed was the most commonly assessed (53%) danger sign, followed by persistent vomiting (47%), convulsions (31%) and lethargy (29%). All four danger signs were assessed in just 8% (13/161) of children. None of the HWs conducted all, and less than 15% performed half of, the physical examination and history taking items as prescribed by IMCI guidelines. All facilities had capacity to test for malaria with either RDT or microscopy, but only 57% of children presenting with fever were tested. Clinical diagnosis of pneumonia was also incomplete. Less than one third of children with cough or difficulty breathing had their respiratory rate counted. Adherence to the malaria treatment protocol was high, as all patients who were RDT positive received ACT, while only 1 of 85 RDT-negative received ACT. However, none of the pneumonia-diagnosed children received the recommended treatment (amoxicillin), and 48% of those with fast breathing (WHO-defined, non-severe pneumonia) did not receive appropriate treatment. Identification of danger signs, diagnosis and treatment of febrile illness were not conducted in accordance with IMCI guidelines. There is an urgent need for interventions to improve management of pediatric febrile illness at the health facility level in Zambia.

638

THE TRANSCRIPTOMIC ANALYSIS OF EARLY ADULT *ECHINOCOCCUS GRANULOSUS*

Wenbao Zhang[1], Jun Li[1], Hui Wang[1], Zhuangzhi Zhang[1], Baoxin Shi[1], Huajun Zhen[2], Yan Zhou[2], Shengyue Wang[2], Donald P. McManus[3], Hao Wen[1], Wenbao Zhang[1]

[1]The First Affiliated Hospital of Xinjiang Medical University, Urumqi, China, [2]Shanghai Ministry of Science and Technology, Key Laboratory of Health and Disease Genomics, Chinese National Human Genome Center, Shanghai, China, [3]Molecular Parasitology Laboratory; QIMR Berghofer Institute of Medical Research, Queensland, Australia

Despite the substantial efforts have been made to control echinococcosis there is a clear need for new tools in its prevention. Dogs are pivotal in *Echinococcus granulosus* transmission and we contend that interruption of the parasite life cycle in the definitive host stage provides a very practical and cost-effective vaccination strategy. Specific or differential genes expressed in the scolex of adult worms are likely the vaccine targets for dogs against *E. granulosus*. To identify the genes, adult *E. granulosus* worms aged two weeks were collected from experimentally infected dogs. The worms were fixed and stored in ethanol. Each of the worms was cut into two parts, head and neck region. mRNA were extracted from the two parts respectively and RNA-seq technique was used to precisely quantify transcript levels in the two parts. A real time PCR was used to confirm the gene expression in the tissues. We identified 953 genes specifically or differentially expressed in the scolex of *E. granulosus*. Most of the up-regulated genes are novel, indicating these genes may compose a network in regulating specific function of worm head, and they may play an important role in settlement of *E. granulosus* in dog intestinal surface. Known genes include calcium-transport, dynein light chain, early growth response protein 2-like isoform 4, myosin heavy chain and transmembrane protein 144. Interestingly, neuropeptide f was highly expressed in the head of *E. granulosus*. The peptide acts as a neurotransmitter in the central nervous system(CNS), indicating the peptides may involve in the early CNS of the parasite. In addition, lanine aminotransferase was also up-regulated in the head, which may involve in nervous development in the early stage of adult worm. As scolex is an important worm part for attachment, the specific and differential genes are likely the targets for drug and vaccine development against adult *E. granulosus*.

639

A HIGH SENSITIVITY DOT-BLOT FOR THE DIAGNOSIS OF HEPATIC CYSTIC ECHINOCOCCOSIS USING WHOLE BLOOD SAMPLE

Mara Mariconti[1], Francesca Tamarozzi[2], Francesca Rinaldi[2], Jacopo Giovanni Nicoletti[2], Francesca Genco[3], Valeria Meroni[3], Enrico Brunetti[2]

[1]IRCCS Policlinico San Matteo, PAVIA, Italy, [2]Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy, [3]IRCCS Policlinico San Matteo, Pavia, Italy

The diagnosis of hepatic cystic echinococcosis (CE) is based on ultrasonography (US) and confirmed by serology. However, a high proportion of inactive cysts, as well as early CE1 cysts, remain seronegative with conventional serodiagnostic tests, which poses diagnostic problems where no pathognomonic signs of CE are present on US examination. We present preliminary results of the performance of a whole blood dot-blot with human hydatid cyst fluid (HCF) especially in patients with hepatic CE who are seronegative with routine diagnostic test. HCF was collected from a CE patient after percutaneous aspiration of an active CE1 liver cyst. Dot-blot membranes were prepared with 50ng of total proteins, and whole blood samples were diluted 1:2000. We tested a total of 34 whole blood samples, 24 of which from CE patients with active, transitional and inactive cysts and 10 from volunteers and patients with non-parasitic cysts (control group). All CE patients with different cyst stages recognized the proteins present in the HCF, while all samples in the control group did not

recognize any proteins.These preliminary results are encouraging and we plan to evaluate our test on a higher number of CE patients and patients with other helminthic infections. This diagnostic test could be useful for the development of a rapid finger prick test for the serodiagnosis of CE in remote endemic areas. Further identification of the different antigens recognized will support the development of diagnostic tools that could improve the sensitivity of CE diagnosis.

640

LONG-TERM FOLLOW-UP OF PATIENTS WITH ALVEOLAR ECHINOCOCCOSIS IN GERMANY

Beate Gruener[1], Petra Kern[2], Benjamin Mayer[2], Rainer Muche[2], Peter Kern[3]

[1]University Hospital Ulm, Ulm, Germany, [2]University Ulm, Department of Epidemiology and Medical Biometry, Ulm, Germany, [3]University Hospital Ulm, Department of Infectious Diseases and Clinical Immunology, Ulm, Germany

From 1992 to 2011, 312 patients with alveolar echinococcosis (AE) were diagnosed and treated at the specialized outpatient clinic of the Ulm University. Demographic and clinical data were assessed and updated from the patients' first visits. At that time, 74.7% of the patients were alive, 12.8% had died, and 12.5% were lost to follow-up. Patients were treated either by surgery with subsequent benzimidazole (BZM) prophylaxis for at least 2 years (n=133), or continuous BZM treatment in case of inoperability (n=157). At first diagnosis, 17 patients had inactive lesions. Imaging and treatment schemes changed during the 20 years of observation. AE was diagnosed more often by chance in patients from 2000 onwards (48.0%) than before 2000 (28.7%). Since 2000, the disease was detected more frequently with lesions at PNM stages I and II (27.0% vs. 15.8%) according to WHO classification; as a consequence, radical resections were feasible in more patients (57.7% vs. 20.0%). Surgical resections were less frequent since 2000 (38.2% vs. 50.5%). Since 1993, PET-CT-scans with 18F-FDG were used routinely to visualize larval activity at time of diagnosis. For follow-up, the rationale for performing a PET-CT every other year was to monitor the effect of continuous BZM treatment, and to detect relapses after surgery. At the end of follow-up, medical treatment had been interrupted for 25.3% of the patients. Of 56 patients with R0 resection, 42 had stopped medical treatment. At their last visit, the disease status of 73.1% of the patients was judged as stable, in 5.1% as progressive while under medical treatment. AE was considered as being cured in 15.7% of the patients.The 5- and 10-years' survival rates in this cohort were 96.8% and 90.5%. Data analysis of two decades' experience in the management of AE showed that best care can be provided to the patients when they present at an early stage of the disease. As the disease is rare, expertise is best acquired in a single specialized institution; a benefit for the patients results from strict adherence to the WHO treatment recommendations.

641

NEW CT-CLASSIFICATION OF HEPATIC ALVEOLAR ECHINOCOCCOSIS

Tilmann Graeter, Wolfgang Kratzer, Suemeyra Oeztuerk, Tanja Kaltenbach, Mark Martin Haenle, Peter Kern, Beate Gruener

University Hospital Ulm, Ulm, Germany

Computed tomography, mostly combined with PET, provides one of the most important diagnostic tools in suspected alveolar echinococcosis. Aim of the study was to establish a new CT-classification based on a large patient collective with confirmed hepatic alveolar echinococcosis. In 224 patients, CT-morphology of liver lesions due to an alveolar echinococcosis was retrospectively examined. The findings were grouped into the new classification scheme. Within the classification a lesion was dedicated to a "primary morphology" as well as to a "pattern of calcification". The primary morphology distinguishes following types: I. Diffuse infiltrating (with / without cystoid portion), II. Primarily circumscribed tumor-like (with / without cystoid portion and with / without offshoot at the edge), IIIa. Primarily cystoid, intermediate (with / without more solid portions at the edge), IIIb. Primarily cystoid widespread (with / without more solid portions at the edge), IV. Small-cystoid / metastatic* and V. Mainly calcified. Except for the "primary morphology" type V., following patterns of calcification were attributed additionally: without calcifications; with feathery calcifications; with focal (p.r.n. central - just possible with*) calcifications; with diffuse calcifications; with calcifications primarily at the edge. The various classification patterns are demonstrated by image examples. The proposed CT-morphological classification shall facilitate the interpretation of lesions due to a hepatic alveolar echinococcosis. This could help to interpret different clinical courses better and shall assist in the context of scientific studies to improve the comparability of CT findings.

642

COMPARING GROUP MEANS AND INDIVIDUAL RESPONSES TO TREATMENT OF SOIL-TRANSMITTED NEMATODES WITH BENZIMIDAZOLE DRUGS: A POOLED ANALYSIS OF INDIVIDUAL PATIENT DATA

Piero L. Olliaro[1], Aïssatou Diawara[2], Benjamin Speich[3], Jennifer Keiser[3], Carli M. Halpenny[4], Marco Albonico[5], Jimmy Kihara[6], Roger K. Prichard[4]

[1]World Health Organization, Geneva, Switzerland, [2]New York University Abu Dhabi, Abu Dhabi, United Arab Emirates, [3]Swiss Tropical and Public Health Institute, Basel, Switzerland, [4]Institute of Parasitology, McGill University, Sainte-Anne-de-Bellevue, QC, Canada, [5]Fondazione Ivo de Carneri, Torino, Italy, [6]Eastern and Southern Africa Center of International Parasite Control, Kenya Medical Research Institute, Nairobi, Kenya

Monitoring anthelmintic treatment efficacy is important to detect suboptimal response where drug pressure is high. The recommended method is egg reduction rate (ERR): percentage reduction in the mean number of eggs per gram (EPG) in excreta post- vs pre-treatment calculated using arithmetic rather than geometric mean (ERRam, ERRgm). We compared these group mean outcomes to individual patient outcomes: centile distribution of individual ERR (ERRic) from which we derived: proportion cured (cure rate, CR), or with reduced EPG (rEPG) or no change/ increased EPG (nEPG); and median ERR (mERR). We analysed trials which treated Ascaris lumbricoides (AL), Trichuris trichiura (TT) or hookworms (HW) with benzimidazoles (1832 subjects): four trials of albendazole (ABZ: n= 613; AL: n=121, TT: n=297, HW: n=195); two of mebendazole (MBZ: n=1219, AL: n=174, TT: n=513, HW: n=532). Both drugs were very effective on AL: CR were 90.9% with ABZ and 94.2% with MBZ. Treating TT with ABZ gave CR=15.4%, rEPG=48.8%, nEPG=35.7% and mERR=39%; and MBZ gave CR=22.4%, rEPG=58.1%, nEPG=19.4%; mERR=76%. Treating HW with ABZ gave CR=69.2%, rEPG=23.1%, nEPG=7.7, mERR=100%; MBZ gave CR=14.1%, rEPG=51.7%, nEPG=34.1%, and mERR=56%. Efficacy estimates expressed as ERRgm were systematically higher the ERRam for both drugs. As opposed to group mean estimates ERRam/gm, ERRic within one analysis, describes better the distribution of individual responses (centile distribution) and calculates proportions cured, with partial or no response, and median ERR; it should be tested on larger datasets to pinpoint changes in patterns of response and poor responders.

643

WATER AND SANITATION TARGET DIFFERENT SOIL TRANSMITTED HELMINTHES ACCORDING TO THEIR ROUTE OF INFECTION

Alejandro J. Krolewiecki[1], Adrana Echazu[2], Diego Cruz[3], Rosana Ruiz[4], Pamela Cajal[2], Marisa Juarez[2], Ruben Cimino[2], Paola Vargas[2], Nicolas Caro[2], Luis Arias[5], Daniela Bonanno[1], Viviana Heredia[5], Gladys Paredes[5]

[1]Ministerio de Salud de la Nacion, Buenos Aires, Argentina, [2]Universidad Nacional de Salta, Oran, Argentina, [3]Fundacion Mundo Sano, Tartagal, Argentina, [4]Fundacion Mundo Sano, Buenos Aires, Argentina, [5]Ministerio de Salud Publica de Salta, Tartagal, Argentina

Soil transmitted helminthes (STH) are a group of parasites of public health relevance due to its burden of disease and prevalence. The global strategy of the World Health Organization for the control of the morbidity related to STH includes preventive chemotherapy but also highlights the need for developmental improvements in the living conditions of affected populations. As part of a project for the development of strategies for the control of STH in northwestern Argentina, a survey was performed in randomly selected families of Wichii aboriginal communities of Tartagal with known high endemic prevalence of STH; socio-demographic information originating in the primary care routine sanitary data forms was incorporated into the database. The surveys were designed to include a representative randomized sample that included whole family groups. STH were surveyed through examination of single fresh stool specimens with 5 methods and an ELISA assay for Strongyloides stercoralis based on NIE antigen. In a community of 2914 individuals, 229 stool samples and 255 serum samples were evaluated and revealed an overall prevalence of STH of 54% with species specific prevalences of 49% for hookworms, 41% for S. stercoralis, 1% for Ascaris lumbricoides and 0.5% for Trichuris trichiura. The sanitary conditions of this communities revealed improved drinking water availability in 98% of the individuals which contrasts with a mere 2.3% with improved sanitation (unimproved pit latrines 94%, open defecation 1.4%, no data 1.9%). These results highlight the distinctly contrasting prevalence between STH with life cycles that infect through skin penetration of filariform larvae (hookworms and S. stercoralis) versus those that infect through the oral ingestion of embryonated eggs (A. lumbricoides and T. trichiura) according to environmental conditions. In the communities described in this report the lack of adequate sanitation favored skin penetrators but the availability of improved water blocked those transmitted trough oral ingestion. Life cycles determine risk factors and the interplay of these elements should guide control measures.

644

DETECTION AND CHARACTERIZATION OF AN IMMUNODOMINANT ANTIGEN PRESENT ON THE SURFACE OF *ASCARIS* L3 LARVAE

Johnny Vlaminck[1], Jozef Vercruysse[2], Peter Geldhof[2]

[1]Washington University, St. Louis, MO, United States, [2]Ghent University, Ghent, Belgium

The human roundworm *Ascaris lumbricoides* is estimated to infect over 800 million people and is a significant public health problem. The closely related pig parasite *A. suum* plays and important role in veterinary medicine and represents a suitable model for *A. lumbricoides*. A continued exposure to *Ascaris* induces immunity at the level of the gut in pigs, protecting the host against the migrating larvae. The objective of this project was to identify and characterize parasite antigens targeted by this immune response that may be crucial for parasite invasion and survival. Pigs were immunized by trickle infection (100 A. suum eggs 5 times per week) for 30 weeks, challenged with 1,000 eggs at week 32 and euthanized two weeks after challenge. At necropsy, there was a 100% reduction in L4s recovered from the intestine and a 97.2% reduction in white spots on the liver in comparison with challenged non-immune controls. Antibodies purified from the intestinal mucus of immune pigs were subsequently used to probe L3 larval extracts resulting in a strong specific recognition of a 12kDa antigen (As12). This antigen is present on the surface of infective L3 larvae and is shed actively. As12 appears to be a glycolipid that contains phosphorylcholine, and it cannot be visualized by protein staining. Furthermore, As12 is highly resistant to different enzymatic and chemical treatments. This molecule could be of significant importance to the survival of the parasite during the initial stages of infection. Further studies are needed to investigate its molecular nature and its role at the parasite-host interface.

645

FINE-SCALE SPATIAL AND TEMPORAL EVOLUTION OF WEST NILE VIRUS IN CALIFORNIA

Nisha K. Duggal[1], William K. Reisen[2], Greg D. Ebel[3], Aaron C. Brault[1]

[1]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [2]University of California Davis, CA, United States, [3]Colorado State University, Fort Collins, CO, United States

West Nile virus (WNV) is an arbovirus that was first reported in North America in 1999 and, by 2003, had spread nearly 3,000 miles from New York to southern California. The spread of WNV across the U.S. has been shown to be dependent on long-range movements, though sporadic sampling and an ascertainment bias from selecting isolates not associated with disease in humans may have affected these analyses. In addition, methods of viral overwintering and viral genetics associated with viral spread are not well understood. Herein, we report sequences for more than 125 WNV isolates collected from 15 counties of California. These isolates were made from mosquito pools collected as part of routine surveillance by the California Vectorborne Disease Surveillance System from 2003 - 2012. This unique dataset allows inference of fine-scale spatial and temporal movements of WNV. Using the Bayesian MCMC approach implemented in BEAST, we performed phylogeographic analyses and demonstrate that 3 independent introductions of WNV (2 WN02 strains and 1 SW03 strain) occurred in California between 2002 and 2003 via the Coachella and Sacramento Valleys. The two genotypes of WNV have remained in co-circulation in California from 2003 to 2012, though SW03 viruses were primarily restricted to southern California. An association between WNV genotype and WNV neuroinvasive disease (WNND) in humans has not been consistent, as both WNV genotypes have circulated in counties with high and low WNND incidence. Multiple examples of short-range movements of WNV across a few hundred miles, as well as geographic constraint of WNV strains within a single region for up to 8 years, suggest viral transmission has been driven by resident, rather than migratory, birds. In addition, while most mosquito pools containing infectious virus were collected in summer months, 2 viruses from mosquitoes collected during winter months were phylogenetically consistent with continued viral transmission as an overwintering mechanism for WNV. These data show that dense sampling across space and time can help to explain basic biological and ecological properties of WNV transmission.

646

AVIAN TROPISM-ASSOCIATED PATHOGENESIS OF WEST NILE VIRUS: CHARACTERIZING THE ROLE OF AVIAN LEUKOCYTE INFECTION

Elizabeth A. Dietrich[1], Angela Bosco-Lauth[2], Richard A. Bowen[2], Aaron C. Brault[1]

[1]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [2]Colorado State University, Fort Collins, CO, United States

West Nile virus (WNV) replicates in a wide variety of avian species, which serve as amplification hosts. In particular, WNV generates high titers and elicits severe pathology in American crows (AMCRs; *Corvus brachyrhynchos*), a species that has been used as a sentinel for WNV circulation. Based on preliminary time-course studies, as well as a

comparison with WNV tropism in mammals, we hypothesized that early WNV replication in AMCRs is driven specifically by replication in circulating monocytes. Therefore, peripheral blood mononuclear cells (PBMC) were isolated from AMCRs and infected with WNV in an *ex vivo* culture system. The degree of replication attained by various WNV strains and mutants in this *ex vivo* system recapitulated their relative viremia and pathogenicity *in vivo* in AMCRs. Flow cytometric analysis suggested that the cells infected in *ex vivo* PBMC culture were predominantly monocytes. A WNV virus engineered to express target sequences for miR223, a miRNA found specifically in myeloid lineage cells, was generated to assess the specific role of infectivity of these cells for modulating *in vivo* avian virulence phenotypes. This cell-restricted recombinant virus failed to replicate in AMCR *ex vivo* PBMC cultures, demonstrating the myeloid lineage as the source of viral replication in the leukocyte preparations. Furthermore, the myeloid-restricted virus exhibited peak viremias in AMCRs 200-fold lower than the parental WNV strain or a control virus expressing a mosquito miRNA target sequence. Additionally, mortality of the miR223 target sequence WNV was 50% with an average survival time of approximately ten days, while the wild type virus yielded 100% mortality with birds succumbing within approximately five days. Thus, several lines of evidence point to the importance of leukocytes, including monocytes, in WNV infection of AMCRs. The *ex vivo* PBMC culture system may be a useful model for pathologic assessment of WNV strains, and can be used to further elucidate the mechanism of action of viral mutations that affect WNV host competence in avians.

647

ROLE OF TNF-α RECEPTOR 2 IN WEST NILE VIRUS INFECTION

Moti L. Chapagain[1], Maile O'Connell[2], Douglas M. Watts[3], Vivek R. Nerurkar[2]

[1]Chitwan Medical College, Bharatpur, Chitwan, Nepal, [2]University of Hawaii, Honolulu, HI, United States, [3]University of Texas at El Paso, El Paso, TX, United States

West Nile virus (WNV) is the most common cause of arboviral encephalitis in the USA. Several lines of evidence suggest that production and release of TNF-α is one of the early cellular events in WNV infection. While the role of TNF-α receptor 1 (TNF-R1) in WNV infection is controversial, there are no studies that have evaluated the role of receptor 2 (TNF-R2) in WNV-associated neuropathogenesis. We *hypothesized* that TNF-α acting through TNF-R2 promotes survival of WNV-infected neurons and immune cells by counteracting WNV-induced apoptosis and enhancing regulatory T cells (Tregs) functions. Therefore, TNF-R2 knock out (KO) mice infected with WNV will have more severe disease and higher mortality than the wild type (WT) mice. The *objective* of this study was to examine the role of TNF-R2 signaling in protecting WNV-infected mice. Age- and gender-matched 8-12 weeks old, KO and WT (C57Bl/6) mice were inoculated with 100 or 10 plaque forming units (pfu) of WNV NY-99 strain and monitored daily for 3 weeks. Mice survival was analyzed using Log-rank (Mantel-Cox) tests and viremia was quantitated by quantitative reverse transcriptase real-time PCR (qRT-PCR). When infected with 100 pfu of WNV, 97% of KO mice succumbed to death whereas 31% WNV-infected WT mice survived (N=29 in each group, Chi-square 14.38, df 1, p= 0.0001). While WT mice had lower viremia ($7\pm3x10^3$ vs. $4\pm7x10^4$ pfu equ/mL) and slightly longer median survival time (11 days vs. 10 days), these differences were not significant. Interestingly, when infected with 10 pfu of WNV, there was no difference in the survival between WT and KO mice suggesting the role of TNF-R2 while is critical in heavy infection, it may not have any role in low dose WNV-infection. Therefore, because of limited distribution of TNF-R2 in immune, hematopoietic and neuronal cells that are primary targets of WNV, specifically augmenting the TNF-R2 could be a better and safer therapeutic strategy in heavy WNV infection.

648

RESTRICTION OF 2'-O METHYLATION DEFICIENT WEST NILE VIRUS IN INSECTS

James D. Brien[1], Sheri L. Hanna[2], Irma Sanchez-Vargas[3], Gregory D. Ebel[3], Michael S. Diamond[1], Sara Cherry[2]

[1]Washington University, St. Louis, MO, United States, [2]University of Pennsylvania, Philadelphia, PA, United States, [3]Colorado State University, Fort Collins, CO, United States

Interferon (IFN) induced proteins with tetratricopeptide repeats (IFITs) were recently identified as mammalian sensor molecules that recognize non-self viral RNA lacking 2'-O methylation at their 5' end and selectively block translation. Similar to mammals, the host mRNA of insects is methylated at both the N-7 and 2'-O positions yet insects lack any known IFN response pathway or apparent IFIT gene orthologs. Here, we show that a mutant West Nile virus (WNV) lacking 2'-O methyltransferase activity (WNV-NS5-E218A) is attenuated in mosquito cells and intact mosquitoes. Wild type WNV (WNV-WT) grows to approximately 2 logs higher titer than WNV-NS5-E218A in both C6/36 and AAG2 mosquito cells and WNV-WT had an increased infection rate and higher titer of infection in Culex tarsalis mosquitoes at day 3 and 5 post infection in comparison to WNV-NS5-E218A. These results suggest that WNV-NS5-E218A is restricted by a novel effector pathway that recognizes the loss of 2'-O methylation, analogous to our observations in mammals; alternatively, insects use 2'-O methylation for efficient translation and thus, restrict non-self RNA lacking this modification. To identify the genes and pathways responsible for restricting WNV-NS5-E218A, a genome-wide RNAi screen that targets 12,870 Drosophila genes was performed. We identified 140 genes within the primary screen that increased infection of WNV-NS5-E218A when silenced. Of these 140 genes we are currently pursuing four genes with mammalian homologs that increase WNV-NS5-E218A infection to a greater extent than WNV-WT. We will discuss how these genes preferentially restrict WNV lacking 2'-O methylation in insect cells.

649

SUBSTITUTION OF THE PREMEMBRANE AND ENVELOPE PROTEIN GENES OF MODOC VIRUS WITH THE HOMOLOGOUS SEQUENCES OF WEST NILE VIRUS GENERATES A CHIMERIC VIRUS THAT REPLICATES IN VERTEBRATE BUT NOT MOSQUITO CELLS

Bradley J. Blitvich[1], Rungrat Saiyasombat[1], W. Allen Miller[1], Peter J. Bredenbeek[2], Jimena Carrillo-Tripp[1]

[1]Iowa State University, Ames, IA, United States, [2]Leiden University Medical Center, Leiden, Netherlands

Most known flaviviruses, including West Nile virus (WNV), are maintained in natural transmission cycles between hematophagous arthropods and vertebrate hosts. Other flaviviruses such as Modoc virus (MODV) and Culex flavivirus (CxFV) have host ranges restricted to vertebrates and insects, respectively. The genetic elements that condition the differential host ranges and transmission cycles of these viruses have not been identified. In this study, fusion-PCR was used to replace the capsid (C), premembrane (prM) and envelope (E) genes and the prM-E genes of a full-length MODV infectious cDNA clone with the corresponding regions of WNV and CxFV. Fusion products were directly transfected into baby hamster kidney-derived cells that stably express T7 RNA polymerase. At 4 days post-transfection, aliquots of each supernatant were inoculated onto vertebrate (BHK-21 and Vero) and mosquito (C6/36) cells which were then assayed for evidence of viral infection by RT-PCR, Western blot and plaque assay. Chimeric virus was recovered in cells transfected with the fusion product containing the prM-E genes of WNV. The virus could infect vertebrate but not mosquito cells. The *in vitro* replication kinetics and yields of the chimeric virus were similar to MODV but the chimeric virus produced larger plaques. Chimeric virus was not recovered in cells transfected with any of the other fusion

products. In conclusion, our data indicate that genetic elements outside of the prM-E gene region of MODV condition its vertebrate-specific phenotype.

650

THE YELLOW FEVER ICEBERG: THE PROBABILITIES OF MILD, SEVERE AND FATAL DISEASE FOR PEOPLE INFECTED WITH YELLOW FEVER VIRUS

Michael Johansson[1], Pedro F. Vasconcelos[2], J. Erin Staples[3]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]Instituto Evandro Chagas, Ananindeua, Brazil, [3]Centers for Disease Control and Prevention, Fort Collins, CO, United States

Yellow fever virus, like other pathogens, only causes disease in a proportion of individuals it infects. Severe illness and death represent the tip of the iceberg relative to asymptomatic and mild infections, which is more critical for determining the overall disease burden and prevalence of infection. We compiled data on asymptomatic infections, mild disease, severe disease, and fatalities from eleven outbreaks affecting hundreds to thousands of people in Africa and South America between 1969 and 2011. We defined severe disease as fever with jaundice or hemorrhagic symptoms and mild disease as fever or other reported illness not meeting the severe disease criteria. Using a Bayesian model, we estimated the probabilities and 95% credible intervals (CI) for asymptomatic infection, mild disease, and severe disease in infected individuals. The average probability of being asymptomatic was 0.55 (95% CI: 0.37-0.74). The probability of infection resulting in mild disease was 0.33 (95% CI: 0.13-0.52) and severe disease was 0.12 (95% CI: 0.05-0.26). We also estimated the case fatality rate for individuals experiencing severe disease; the probability of fatality for severe cases was 0.47 (95% CI: 0.31-0.62). For unknown outbreaks, the uncertainty and variability between studies indicates that for every severe case of yellow fever, there may be 1 to 70 additional infections, representing asymptomatic or mild infections. The large range in all of these estimates reflects the scarcity of data, intrinsic variability, and variation between outbreaks, possibly due to study design or differences in environmental, host, vector, or virus characteristics. Nonetheless, the results are generally inline with previous estimates, which are derived only from individual studies. As it is generally only the most severe cases that are recognized and reported, these estimates will help improve the understanding of the burden of disease and the estimation of the potential risk of spread during YF outbreaks.

651

FLAVIVIRUS EXPOSURE IN NORTHEASTERN KENYA

A. Desiree LaBeaud[1], Tamara Banda[1], **Julie Brichard**[1], Samuel Muiruri[2], Saidi Dahir[2], Crystal Y. Teng[1], Ginny Gildengorin[1], Eva Harris[3], Eric M. Muchiri[4], James Kazura[5], Charles H. King[5]

[1]Children's Hospital Oakland Research Institute, Oakland, CA, United States, [2]Division of Vector Borne and Neglected Tropical Diseases, Nairobi, Kenya, [3]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, CA, United States, [4]Division of Vector-Borne and Neglected Tropical Diseases, Ministry of Health, Nairobi, Kenya, [5]Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH, United States

Flaviviruses are transmitted throughout the world, though their burden is still largely underestimated in Africa. The objective of this study was to assess the seroprevalence of flavivirus exposure in northeastern Kenya and to link previous exposure to risk factors and reported historical symptoms associated with prior febrile illness. Questionnaires on demographics, reported symptoms, and mosquito exposures were administered to 891 participants among 6 northeastern Kenyan villages in February and March of 2011. Visual acuity tests and physical exams were also performed. Sera were tested via standardized ELISA protocols for anti-dengue virus IgG. Risk factors associated with seropositivity were determined using Fisher's exact test and logistic regression models. Forty-three (4.9%; 95% CI 3.6-6.5%) participants were flavivirus seropositive (aged 3-85 years). Prevalence differed significantly among villages ($p<0.001$) with higher risk in Sabenale (19%), Golabele (10%) and Tumtish (9%), and lower risk in Gedilun (4%), Korahindi (2%) and Matarba (1%). Children (<16 years old) were less likely to be seropositive than adults ($p<0.001$). Women tended to be less at risk than men ($p=0.057$). Seropositivity was associated with poor measured visual acuity ($p=0.026$) and the following reported lifetime historical symptoms: red eyes ($p=0.005$), poor vision ($p=0.038$), eye pain ($p=0.005$), photophobia ($p<0.001$), backache ($p<0.001$), malaise ($p<0.001$), and sleepiness ($p=0.045$). Seropositivity was also associated with home flooding ($p=0.0215$). Flavivirus exposure is rare in northeastern Kenya, but more common among adults than children. Differences in seroprevalence between residents of nearby villages with similar ecology may be due to varied mosquito control practices or economic factors. Flavivirus exposure is associated with many historical visual symptoms and poor measured visual acuity. Despite low prevalence and no reported outbreaks, ongoing interepidemic transmission is demonstrated by documentation of seropositive children.

652

MALARIA IMPORTATION AND ELIMINATION IN SWAZILAND

Robert C. Reiner[1], Justin Cohen[2], David Smith[3]

[1]University of California Davis, Davis, CA, United States, [2]Clinton Health Access Initiative, Boston, MA, United States, [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Achieving country-wide elimination of malaria requires the cessation of local transmission caused by imported malaria. Swaziland, a sub-Saharan country that has worked towards malaria elimination for over a decade, has brought local transmission down to extremely low levels. As Swaziland makes further progress towards elimination, the methods and metrics typically used to assess the risk of local transmission initiated from imported malaria become inadequate. Additionally, independent of method or metric, accurate accounting of both local and imported cases becomes ever more critical as the relative weight carried by each individual case increases. To both assess transmission dynamics in a low-transmission setting such as Swaziland, as well as evaluate surveillance efforts, we developed algorithms to estimate malaria transmission chains. Using comprehensive case data from 2010 through 2013, these estimated chains - based on identifying likely causal links between successive cases through the use of spatio-temporal kernels - provided insight on the frequency and length of chains of local transmission. By identifying secondary cases that appear to have no likely primary case we catalogue likely gaps in surveillance. Our analysis suggests than most locally acquired cases were caused by a few imported cases and that most imported cases resulted in no extended local transmission. Further, we highlight regions in space where local transmission chains appear to be longest. Our results suggest these regions would benefit from more systematic control and surveillance efforts to identify and curtail extended local transmission in the future.

653

MAPPING SEASONAL INTERACTIONS BETWEEN POPULATION MOVEMENTS AND MALARIA TRANSMISSION FOR STRATEGIC MALARIA ELIMINATION PLANNING

Christopher Lourenco[1], Elisabeth zu Erbach-Schoenberg[2], Griffith Siloka[3], Victor Alegana[2], Peter Atkinson[2], Bonita Graupe[4], **Andrew Tatem**[2]

[1]Clinton Health Access Initiative, Boston, MA, United States, [2]University of Southampton, Southampton, United Kingdom, [3]Namibia National Vector-Borne Disease Control Programme, Windhoek, Namibia, [4]Mobile Telecommunications Limited, Windhoek, Namibia

In most countries that are planning for malaria elimination a strong seasonality in malaria transmission can be observed. This often drives the timing of intervention and surveillance efforts, and the seasonal patterns can potentially be exploited to optimally target resources

for achieving elimination. Factors that have received lesser attention in designing elimination strategies are population movements, their seasonal patterns and their demographic composition. As a country or area transitions towards malaria elimination, imported cases make up an increasingly larger proportion of those seen, and the importance of accounting for population movements rises. Throughout the world, the volumes and major routes of population movements tend to follow seasonal patterns, with certain times of year showing significantly greater amounts of movement than others, and this varying by demographic groupings. These movements can impact substantially on the dispersal of parasites in a region, depending on how the timings and routes of seasonal movements interact with the seasonality of malaria transmission. Here, we demonstrate how the simple combination of national malaria surveillance system data, case investigation information and population mobility metrics derived from mobile phone records can inform on these seasonal interactions, using Namibia as an example. Using anonymized cellphone call detail records to determine the mobility patterns of nearly 2 million residents, we show that the timing, duration of stay and magnitude of movements vary substantially across time and space, with significant movement peaks in December and January aligning with peak malaria transmission. Further, case investigation data from more than 100 malaria patients in the region with highest transmission (Zambezi) enables validation of these mobility patterns and provides valuable additional demographic and epidemiological insights into risk groups and contact patterns. The approaches presented can be updated rapidly and used to identify which regions would benefit from coordinating efforts at certain times of year and how spatially progressive elimination plans can be designed to account for the interacting seasonality in transmission and mobility.

654

A MONITORING AND EVALUATION TOOL FOR REACTIVE CASE DETECTION: PILOT EXPERIENCE IN ACEH PROVINCE, INDONESIA

Herdiana Hasan[1], Kelly Sanders[2], Chris Cotter[2], Roly D. Gosling[2]

[1]UNICEF, Banda Aceh Field Office, Aceh Province, Indonesia, [2]Global Health Group at the University of California San Francisco, San Francisco, CA, United States

Active case detection (ACD) strategies are recommended by the World Health Organization and are implemented in many low transmission settings worldwide as a critical part of malaria elimination programs. ACD strategies include determining the origin of infection, case investigation, and responding to locally acquired cases of malaria, known as reactive case detection (RACD). Effectively implementing RACD requires substantial programmatic and human resources. Aceh Province has a goal to eliminate malaria by 2015. Between June and September 2013, a pilot was conducted to evaluate RACD activities and identify best practices to inform RACD efforts in Aceh Province. Using a standardized monitoring and evaluation (M&E) tool, a sample of 5 districts including 34 health facilities were evaluated to measure RACD indicators, in addition to staff interviews regarding RACD procedures. To measure case investigation and RACD rates and timeliness, quantitative data was extracted from the district health offices and health facility registers and measured against defined indicators. Questionnaires were administered to 59 health facility staff involved in conducting RACD. From January to December 2012, a total of 289 cases (range 0 to 153) were reported at health facilities with a completeness of 92% when compared with the district health office records. Mean case investigation rates were 78% (range from 62 to 100). Screening by microscopy was conducted on a total of 931 community individuals (16.3 individuals per index case) with 3 new infections identified. Mean timeliness of RACD conducted within 7 days was 82% (range from 43 to 100). These findings indicate the need for improved patient data collection upon presentation at the health facility, and for staff to visit index cases after normal work hours to prevent loss due to follow up during case investigations. Rollout of the M&E tool to additional districts in Aceh Province and the monitoring of currently sampled districts will improve timeliness and reporting completeness of facilities and optimize RACD program effectiveness.

655

SUSTAINABLE MALARIA ELIMINATION ON ANEITYUM ISLAND, VANUATU, 1991 -2014

Akira Kaneko[1], Chim Chan[1], Rie Isozumi[2], Morris Kalkoa[3], Luis F. Chaves[4], Noriko Watanabe[2], Renu Wickremasinghe[5], Klara Unosson[1], Jackie Cook[1], Chris Drakeley[6], Masatsugu Kimura[2], Koji Lum[7], Marita Troye-Blomberg[8], Anders Björkman[1], Karl Rieckmann[9]

[1]Karolinska Institutet, Stockholm, Sweden, [2]Osaka City University, Osaka, Japan, [3]Ministry of Health, Port Vila, Vanuatu, [4]Nagasaki University, Nagasaki, Japan, [5]University of Sri Jayewardenepura, Gangidawila, Nugegoda, Sri Lanka, [6]London School of Hygiene & Tropical Medicine, London, United Kingdom, [7]Binghamton University, Binghamton, NY, United States, [8]Stockholm University, Stockholm, Sweden, [9]Australian Army Malaria Institute, Enoggera, Australia

Weekly mass drug administration (MDA) of chloroquine, pyrimethamine/sulfadoxine, and primaquine was carried out on the entire population of 718 inhabitants of Aneityum island for 9 weeks in 1991 before the onset of the rainy season. Simultaneously insecticide-treated bednets (ITNs) were distributed to the entire population. Microscopy showed the immediate disappearance of *Plasmodium falciparum*, whereas *P. vivax* disappeared from 1996 onwards until new cases were reported in January 2002. In July 2002 *P. vivax* infections were detected by microscopy in 22 of 759 individuals: 20/298 born after 1991, 2/126 born between 1991-82, and 0/335 born before 1982. Subsequent PCR increased the total to 77 (36, 21, and 20 in respective age groups). The age distribution was similar to those before elimination and on other islands. In November a similar age pattern was found but with fewer (39) infections. In December 2002, the 2nd MDA of weekly chloroquine for four weeks and daily primaquine for 14 days was carried out as a containment measure on the population born after 1982, in concert with re-strengthening of the community-based provision of ITNs. Population-wide mass blood surveys detected by PCR no cases in December 2002 (n=436, only those born after 1982), immediately after the MDA, but 26 in 2003 (730), 20 in 2004 (732), 34 in 2005 (836), and 15 in 2007 (719). No positive cases were detected in 2010 (950) and 2013 (1093). The age distribution of 2003-2007 positive cases was different from those before elimination and on other islands: i.e. a substantially lower prevalence was observed in the population born after 2002 (0.8%) than those between 2002-1992 (3.7%), between 1991-1982 (5.3%), and before 1982 (2.1%), suggesting that these submicroscopic infections mainly reflected relapses from liver stages. Sero-epidemiological monitoring suggested that the persistence of antibodies against *P. vivax* may partially explain the lower parasite prevalence in the oldest age group. On Aneityum, indigenous *P. falciparum* transmission has never re-established after the 1st MDA in 1991, despite surveillance by community microscopists that showed continuous parasite importation from other islands. A high degree of community engagement to prevent resurgence, in addition to high ITN coverage (1.05 net/person) and usage (95%, 2014), sustains malaria freedom on this island.

656

GENETIC CORRELATES OF DECLINING TRANSMISSION: *PLASMODIUM VIVAX* IN SRI LANKA

Sharmini Gunawardena[1], Marcelo U. Ferreira[2], G. M. Kapilananda[1], Dyann F. Wirth[3], **Nadira D. Karunaweera**[1]

[1]University of Colombo, Colombo, Sri Lanka, [2]University of São Paulo, São Paulo, Brazil, [3]Harvard School of Public Health, Boston, MA, United States

We examined whether the recent dramatic decline in malaria transmission in Sri Lanka led to a major bottleneck in the local *P. vivax* population, with a substantial decrease in the effective population size. To this end, we

typed 14 highly polymorphic microsatellite markers in 185 *P. vivax* patient isolates collected from 13 districts in Sri Lanka over a period of five years (2003-2007). Overall, we found a high degree of polymorphism, with 184 unique haplotypes (12-46 alleles per locus) and average genetic diversity (expected heterozygosity) of 0.8744. A marginal decline in Allelic diversity (15.4 to 10.9) as well as heterozygosity (0.87 to 0.82) was observed from the 2003-4 to 2006-7 period. Almost 69% (n=127) isolates had multiple-clone infections with no major changes observed over time. Significant spatial and temporal differentiation (F_{ST}=0.04 - 0.25; p≤0.0009) between populations was observed. The effective population size was relatively high but showed a decline from 2003-4 to 2006-7 periods (estimated as 45,661 to 22,896 or 10,513 to 7,057, depending on the underlying model used). We used three approaches - namely, mode-shift in allele frequency distribution, detection of heterozygote excess and the *M*-ratio statistics - to test for evidence of a recent population bottleneck but only the low values of *M*-ratio statistics (ranging between 0.15-0.33, mean 0.26) were suggestive of such a bottleneck. The persistence of high genetic diversity and high proportion of multiple-clone infections, with little change in effective population size, despite the collapse in demographic population size of *P. vivax* in Sri Lanka indicates the importance of maintaining stringent control and surveillance measures to prevent resurgence. It also appears that more samples (over decades) might be required to detect the genetic signatures of shrinking malaria transmission.

657

EVALUATION OF TOPICAL REPELLENTS AS ADDITIONAL VECTOR CONTROL MEASURES TO CONTROL RESIDUAL TRANSMISSION IN MALARIA PRE-ELIMINATION AREAS

Marc Coosemans[1], Vincent Sluydts[1], Sochantha Tho[2], Sovannaroth Siv[2], Heng Somony[2], Lydie Canier[3], Khim Nimol[3], Kim Soarin[3], Sokny Mao[2], Charlotte Gryseels[1], Uk Sambunny[2], Karel Van Roey[1], Karen Kerkhof[1], Koen Peeters Grietens[1], Didier Menard[3], Lies Durnez[1]

[1]Institute of Tropical Medicine Antwerp, Antwerp, Belgium, [2]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [3]Institut Pasteur du Cambodge, Phnom Penh, Cambodia

In Southeast Asia a substantial decrease of malaria has been achieved during the last decade and elimination is now becoming a realistic goal. However residual transmission due to outdoor and/ or early biting vectors is not tackled by wide coverage of insecticide treated nets (ITNs). This may compromise the elimination efforts. For this purpose we set up a study to evaluate the public health value of mass use of topical repellent in addition to ITNs. A randomized community based design has been adopted covering a population of 40,000 inhabitants in the province of Ratanakiri in Cambodia. The 98 clusters were randomly divided in two arms after a pre-trial survey: one intervention arm (ITN and repellent) and one control arm (only ITNs). Comparing to a randomized household trial, present design has the advantage to avoid the risk of the repellent aversion effect and the exchange of products between the households. The principal indicators of effectiveness is the prevalence of parasite carriers measured by PCR techniques using a mobile molecular lab in the field, and the measurement of malaria antibodies. While parasite-prevalence provides a snapshot of the exposure to malaria at a certain moment, serological indicators provide a picture of the "force of malaria infection" over a prolonged period. Moreover passive case detection provides a measurement of malaria disease incidence in both arms. We expect a community protection against residual transmission when a high adherence in the use of repellents is achieved. To address this working hypothesis of mass effect of repellents on the vector population entomological surveys are carried out in both arms. The effectiveness of the intervention is dependent on the efficacy of the repellents against vector bites and the effective use of repellents by the population and both are addressed in the study design. First results will be presented. The outcome of this study will be crucial in the development of new strategies to control not only the indoor transmission during sleeping time (ITN) but also the increasing proportion of residual transmission which occurs mainly outdoors, and before and after sleeping time.

658

A CLUSTER-RANDOMIZED TRIAL OF TARGETED CONTROL TO ELIMINATE MALARIA IN CENTRAL SENEGAL: STUDY DESIGN AND ACCEPTABILITY OF THE INTERVENTIONS

Abdoulaye Diallo[1], Badara Cissé[1], Fassiatou Tairou[1], El hadji Bâ[2], Ousmane Sy[3], Jules Gomis[1], Cheikh Sokhna[2], Jean Gaudart[4], Clare Flach[5], Oumar Gaye[1], Ousmane Faye[3], Paul Milligan[5]

[1]Laboratoire parasitologie, University Cheikh Anta Diop, Dakar, Senegal, [2]Institut de Recherche pour le Developpement, Dakar, Senegal, [3]Faculté des sciences, University Cheikh Anta Diop, Dakar, Senegal, [4]Aix-Marseille Université, Marseille, France, [5]London School of Hygiene & Tropical Medecine, London, United Kingdom

The purpose of this trial is to evaluate the extent to which a targetted malaria control strategy combining vector control with indoor residual spraying (IRS) and chemotherapy, delivered by district health staff to villages reporting clinical cases, can reduce the transmission of malaria, in a region where scaling up of control measures has been effective in reducing the incidence of malaria. The trial will also determine whether, as part of this strategy, chemotherapy should be delivered to all members of targetted communities (MDA, Mass Drug Administration) or only those who have been tested and are known to be infected (MSAT, Mass Screening and Treatment). Methods: 45 health posts, each serves about 10,000 people, were randomized in 40 clusters; 15 to receive IRS and MSAT, 15 to receive IRS and MDA, and 10 to serve as controls. In intervention clusters, villages with evidence of transmission (hotspots) were identified on the basis of confirmed malaria cases reported the previous year. Interventions are delivered over two years, the primary outcomes are the incidence of malaria and the prevalence of parasitaemia just after the main peak period of transmission in the second year. To assess effects on transmission, incidence in non-targetted areas in each cluster will be compared. In 30 clusters, all households in hotspot villages were targetted to receive IRS with Actellic 300CS in July, followed in 15 of the clusters by MDA with dihydroartemisinin-piperaquine (DHA-PQ) administered to all persons in the household in September and again in October, and in the other 15 clusters, instead of MDA, all persons in the household were screened using a malaria RDT and those who test positive treated with DHA-PQ. In all trial clusters, LLIN coverage was topped-up by providing persons diagnosed with malaria with a LLIN. Results and conclusion: Preliminary results after the first year of intervention will be presented, including description of the logistics of MDA and MSAT delivery, the coverage of the interventions, the sensitivity of RDTs for detecting infections, and the acceptability of both interventions by the community.

659

ZOONOTIC RISKS OF NON-TUBERCULOUS MYCOBACTERIA BETWEEN HUMANS AND SMALL MAMMALS (POTENTIAL TRANSMISSION OF BURULI ULCER) IN COTE D'IVOIRE AND GHANA

Lydia Mosi[1], Charles Quaye[2], Charles A. Narh[2], Christelle Dassi[1], Danielle Konan[1], Gilbert Fokou[1], Bertin Akpatou[1], Bassirou Bonfoh[1]

[1]Centre Suisse de Recherche Scientifique, Abidjan, Côte D'Ivoire, [2]Noguchi Memorial Institute for Medical Research, Legon, Ghana

The prevalence of Buruli ulcer, caused by the non-tuberculous Mycobacterium ulcerans (MU), ranges from 1-150/100,000 infected individuals in Ghana and Côte d'Ivoire, making it the second most important mycobacterial disease. The mode of transmission is unknown and compounding this is the fact that mycolactone, the major virulence factor, has been found in other environmental mycobacteria species (MPMs) which cause disease in some animals. Although there are no

reports of human disease caused by these novel species, they share similar ecological niches in the endemic aquatic environments. Our work is based on the One Health concept and suggests that overlapping environmental habitats of the pathogen, animals and humans directly influence transmission. Using (i) active case surveillance of human disease burden; (ii) molecular characterization of NTMs; (iii) and zoonotic risk analysis, we are conducting a study in 6 communities of Côte d'Ivoire and 4 communities in Ghana to understand transmission. Comparative analysis of our socio- anthropological data suggests that individuals in Ghana have a higher level of Buruli ulcer awareness than in Côte d'Ivoire. In both countries, association with dirty water is a risk factor and affected people are generally poor. Molecular analysis of our environmental samples indicate a high presence of mycobacterial DNA including MPMs. Almost all of our suspected human cases also type positive for MU DNA. Using Variable Nucleotide Tandem Repeat (VNTR) typing, we have been able to profile and identify one common genotype that is present in both humans and the environment within the same community in both countries. Interestingly we have identified mycobacterial DNA with MPM profiles in lesions and swellings from trapped small mammals within our study sites. Most of the animals were rats, with the majority from homes close to water bodies in both countries. We intend to develop a model of infection dynamics, and identify key risk factors that will ultimately improve control and surveillance strategies for National Control Program.

660

LINKED HUMAN AND LIVESTOCK STUDY ON SEROPREVALENCE AND RISK FACTORS FOR BRUCELLOSIS IN KENYA, 2012

Eric M. Osoro[1], Austine O. Bitek[2], Eric Ogola[3], Ian Njeru[1], Salome Wanyoike[2], Murithi R. Mbabu[2]

[1]Ministry of Health, Nairobi, Kenya, [2]Ministry of Agriculture, Livestock and Fisheries, Nairobi, Kenya, [3]Kenya Medical Research Institute, Nairobi, Kenya

Brucellosis is an endemic zoonotic disease in Kenya with dual burden with significant economic losses among livestock and illness and disability in humans. Brucellosis studies in Kenya have previously focused on either animals or humans. We conducted a cross-sectional survey that examined humans and livestock (cattle, sheep, bovine, camels) in the same households. We assessed the seroprevalence and risk factors for human brucellosis, animal brucellosis and their association. The study was conducted in three counties with different livestock production systems; Random households were selected with a two-stage cluster sampling method by sub-location and then household. Three persons were enrolled and livestock species proportional to the size of the herd were sampled per household. A questionnaire was administered to household heads and the persons sampled. Human sera were tested for Brucella IgG antibodies using competitive ELISA and animal sera were tested using an indirect ELISA. Risk factors were assessed using multivariate logistic regression. A total of 1,099 households, 2,811 persons and 11,039 livestock were enrolled. Overall, 14% (95%CI 12-16) of households had at least one Brucella seropositive person and 15% (95%CI 12-18) of herds had at least one seropositive animal. Among humans sampled, 6.7% (95%CI 5.6 -7.8) were seropositive. Risk factors for human seropositivity included taking unboiled milk (aOR=3.9, 95%CI 2.0-7.6), exposure to goats [herding, milking, feeding] (aOR=2.5, 95% CI 1.6-4.0) and handling hides (aOR=3.9, 95%CI 2.5-6.2). Animal seropositivity was associated with intermingling with wildlife (aOR=4.3, 95%CI 2.3-8.1) and keeping goats (aOR=2.7, 95%CI 1.4-5.2). The odds of human seropositivity given a seropositive animal in the same household was 5.3 (95% CI 3.2 -8.8). This linked survey shows that human and animal brucellosis seropositivity is associated with factors that increase exposure to seropositive animals. The survey contributes to understanding the burden of brucellosis in Kenya and targeting of health education programs.

661

Q FEVER OUTBREAK ON A LARGE U.S. GOAT AND CATTLE DAIRY: A ONE HEALTH INVESTIGATION

Holly M. Biggs[1], George Turabelidze[2], Suzanne R. Todd[1], Kara Jacobs Slifka[1], Naomi A. Drexler[1], Drew Pratt[2], Gail McCurdy[2], Jennifer K. Lloyd[2], Cecelia Y. Kato[1], David Sun[1], Minh L. Tang[1], Joseph Singleton[1], Gilbert J. Kersh[1], Alicia D. Anderson[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Missouri Department of Health and Senior Services, Jefferson City, MO, United States

Q fever, caused by *Coxiella burnetii*, is an under-recognized and under-reported zoonotic disease transmitted to humans primarily via infected livestock. Between June-November, 2013, 12 cases of Q fever linked to a Missouri community that operates a large goat and cattle dairy, Community A, were reported to the state health department. This compared to an average of 3 (range 1-5) cases reported annually in Missouri over the past 5 years. In December, 2013, we performed a coordinated human, animal and environmental investigation in Community A to determine the extent and epidemiology of *C. burnetii* infection. Community members were offered *C. burnetii* serologic testing and a standardized interview. A case was defined as a *C. burnetii* phase II IgG titer ≥1:128 in a person linked to Community A since June 1, 2013. Representative milk samples from the goat and cattle herds, vaginal swabs from peri-parturient animals, and environmental samples were tested by *C. burnetii* PCR. Of 135 persons interviewed and tested, 47 (35%) human Q fever cases were identified. Both cattle and goat samples were *C. burnetii* PCR positive, although goat samples were more frequently positive (17% vs. 2% of milk samples, and 26% vs. 7% vaginal swabs, respectively). Of environmental samples, *C. burnetii* was detected at highest levels in the goat birthing areas. The risk of Q fever was 2.7 times greater (95% CI: 1.3-5.3) for persons with livestock or manure contact compared to those without. Among persons without livestock exposure, having a household member with regular livestock contact was associated with 4.8 times greater Q fever risk (95% CI: 1.1-20.7). This is the largest human Q fever outbreak reported to date in the U.S. Contact with or proximity to goats and cattle was a significant risk factor for infection, and the possibility of fomite transmission to household members warrants further evaluation. A One Health approach incorporating education and modifications to husbandry practices was recommended to reduce potential morbidity and mortality and prevent future *C. burnetii* transmission.

662

RISK FACTORS ASSOCIATED WITH HUMAN MONKEYPOX IN THE DEMOCRATIC REPUBLIC OF CONGO

Nicole Hoff[1], Prime M. Mulembakani[2], Sara C. Johnston[3], Neville K. Kisalu[4], Jean-Jacques Muyembe[5], Lisa E. Hensley[6], Emile Okitolonda[7], Anne W. Rimoin[1]

[1]University of California Los Angeles, Fielding School of Public Health, Los Angeles, CA, United States, [2]Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo, [3]United States Army Medical Research Institute of Infectious Diseases, Frederick, MD, United States, [4]Vaccine Research Center, National Institute of Allergy and Infectious Diseases/ National Institutes of Health, Bethesda, MD, United States, [5]National Institute of Biomedical Research, Kinshasa, Democratic Republic of the Congo, [6]National Institutes of Health/National Institute of Allergy and Infectious Diseases/IRF, Bethesda, MD, United States, [7]Ministry of Health, Kinshasa, Democratic Republic of the Congo

Monkeypox (MPX), a zoonotic orthopoxvirus causes a serious, smallpox-like illness in humans. Much remains unknown about the risk factors associated with this disease; to-date, no large-scale case-control studies have been conducted. Between 2007 and 2009, two studies were conducted concurrently in the Sankuru District of the Democratic Republic of the Congo (DRC). The first study aimed to collect data through a population-based active disease surveillance program to improve disease

surveillance activities under the Ministry of Health and gain insight into transmission dynamics associated with MPX disease. The second study, a population-based serological survey, collected health behavior information from healthy participants in the same geographic region. Both studies collected information on demographic, health, and animal exposure data as well as tissue samples from all active cases and blood samples from healthy participants. We subsequently preformed a matched case-control analysis using these two similarly collected datasets to identify potential risk factors for human MPX in the DRC. Samples were collected from 595 suspected MPX patients identified through active surveillance and from 2,345 healthy persons enrolled in the population-based study. Suspected cases of human MPX were investigated based on the presence of monkeypox-like lesions and confirmed with real time Polymerase Chain Reaction (PCR) using vesicle fluid or scabs; controls were chosen based on the absence of both skin lesions and specific MPX antibodies in serum by laboratory diagnosis. 390 MPX-positive cases were included in the analysis, stratified by the presumed source of infection (animal (n=252) or human (n=138) source), and were matched to a total of 653 MPX-negative controls based on sex and age categories. Initial findings from univariate and multivariate analysis suggest individuals with evidence of smallpox vaccination are at a reduced risk for MPX infection (adjusted OR (aOR): 0.12, 95% C.I: 0.03, 0.56) while exposures to animals including Gambian rats (aOR: 2.58, 95% C.I: 1.63, 4.07), large terrestrial rodents (aOR: 1.80, 95% C.I: 1.08, 2.98), prosimiens (aOR: 1.85, 95% C.I: 1.24, 2.75), and non-human primates (aOR: 2.66, 95% C.I: 1.44, 4.90), may be associated with an increased risk of MPX infection, particularly in unvaccinated populations.

663

SOUTH AMERICAN EASTERN EQUINE ENCEPHALITIS: POSSIBLE ENZOOTIC HOSTS, HUMAN EPIDEMIOLOGY AND ASSOCIATIONS WITH LAND USE

Amy Y. Vittor[1], Blas Armien[2], Publio Gonzalez[2], Daniel Gonzalez[2], Claudia Dominguez[2], Mario Avila[3], Jean Paul Carrera[2], Julio Cisneros[2], Davis Beltran[2], Eryu Wang[1], Anayansi Valderrama[2], Doug Goodin[4], Jason Blackburn[1], Alex Castillo[2], Brechla Moreno[2], Nestor Sosa[2], Juan Miguel Pascale[2], Scott Weaver[5]

[1]University of Florida, Gainesville, FL, United States, [2]Instituto Comemorativo Gorgas, Panama, Panama, [3]Departamento de Control de Vectores, Panama, Panama, [4]Kansas State University, Manhattan, KS, United States, [5]University of Texas Medical Branch, Galveston, TX, United States

South American eastern equine encephalitis virus (SA EEEV, recently reclassified into the species *Madariaga virus*) had not been associated with significant human disease, unlike its North American counterpart, until a 2010 Panama outbreak. SA EEEV is an alphavirus (family *Togaviridae*) transmitted by *Culex* mosquitoes (subgenus *Melanoconion*). Since transmission and epidemiology of SA EEEV is not well understood, we conducted a serosurvey of small mammals and birds, coupled with a human serosurvey. We hypothesized that SA EEEV seroprevalence would be higher in agricultural areas compared to forested areas, mediated by habitat preferences of generalist species likely to serve as enzootic hosts. From January to December 2012, rodents and marsupials were trapped in 42 locations (village, cultivated fields, pasture, shrub, forest; 12,005 trap nights, n=556 rodents; n=20 marsupials). Mist nets were also placed at night for bat trapping (n=32), and bird samples obtained during a prior field effort were tested (n=162). During this period, 776 people from 3 population centers were surveyed. Sera were tested for SA EEEV and the closely related and endemic Venezuelan equine encephalitis virus (VEEV), by IgG ELISA and plaque reduction neutralization tests (PRNT), and mammal spleens were tested for virus by RT-PCR. We found that the short-tailed cane mouse (*Zygodontomys brevicauda*), a generalist species, had the highest overall EEEV seroprevalence (8.3%), and was the most numerous rodent species trapped (n=229). The abundance of this species was highly associated with cultivated fields and pasture. The abundance of EEEV-positive animals was greater for sites with high rodent diversity (using Shannon-Wiener and Simpson's indices). The overall human SA EEEV seroprevalence was 4.8%, with no age trend, suggesting that SA EEEV exposure may be recent, consistent with a lack of seroprevalence in past Panamanian surveys. The sites with highest abundance of EEE positive animals did not coincide, however, with highest seroprevalence in humans. Since human VEE seropositivity in these sites was as high as 78%, we hypothesize that this geographical discrepancy may be due to cross-protective immunity. Future studies to further clarify the impact of land use change on EEEV transmission are necessary to guide public health policy in Central and South American countries.

664

CANINE RABIES VACCINATION TO PREVENT HUMAN RABIES IN RURAL TANZANIA

Meagan C. Fitzpatrick[1], Katie Hampson[2], Sarah Cleaveland[2], Imam Mzimbiri[3], Felix Lankester[4], Tiziana Lembo[2], Lauren A. Meyers[5], A. David Paltiel[1], Alison P. Galvani[1]

[1]Yale School of Public Health, New Haven, CT, United States, [2]Boyd Orr Center for Population and Ecosystem Health, Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom, [3]Serengeti Health Initiative, Lincoln Park Zoo, Chicago, IL, United States, [4]Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA, United States, [5]Section of Integrative Biology, Austin, TX, United States

The annual mortality rate of human rabies in rural Africa is 3.6 deaths per 100,000 individuals. Rabies can be prevented by prompt post-exposure prophylaxis, but this is costly and often inaccessible in rural Africa. As 99% of human exposures occur through rabid dogs, canine vaccination also prevents transmission of rabies to humans. We evaluate the cost-effectiveness of rabies control through annual canine vaccination campaigns in two districts of rural Tanzania, Ngorongoro and Serengeti, using a dynamic model of transmission in both dogs and wildlife and incorporating empirical uncertainty in the biological parameters to make probability-based evaluations of cost-effectiveness. We find that annual canine vaccination campaigns are very cost-effective in both districts compared with no canine vaccination. In Serengeti, annual campaigns up to 70% coverage are cost-saving. Across a wide-range of parameter assumptions and levels of societal willingness-to-pay for life-years, vaccination campaigns are always cost-effective and life-saving, and therefore preferred.

665

CROSS-SPECIES TRANSMISSION OF TAXONOMICALLY DIVERSE PATHOGENS IN A COMMUNITY OF WILD PRIMATES

Tony L. Goldberg[1], Colin A. Chapman[2], David H. O'Connor[1], Thomas C. Friedrich[1], Michael Lauck[1], Samuel D. Sibley[1], Adam L. Bailey[1], David Hyeroba[3], Alex Tumukunde[3], Geoffrey Weny[3], Nelson Ting[4], Jens Kuhn[5], Ria R. Ghai[2], Noah D. Simons[4], Jorge Dinis[1], Mary I. Thurber[1]

[1]University of Wisconsin Madison, Madison, WI, United States, [2]McGill University, Montreal, QC, Canada, [3]Makerere University, Kampala, Uganda, [4]University of Oregon, Eugene, OR, United States, [5]Integrated Research Facility at Fort Detrick, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Frederick, MD, United States

Viruses are considered likely to "jump" species barriers due to their high genetic variability and adaptive potential, whereas more genomically complex pathogens are considered less likely to cross species barriers. We evaluated this hypothesis by examining a taxonomically diverse set of pathogens in a community of wild primates in Uganda. Metagenomic analyses of blood plasma identified approximately 12 novel RNA viruses of the families Retroviridae, Arteriviridae, and Flaviviridae. These viruses were highly diverse within primate species (up to approximately 13% sequence divergence) and displayed high levels of intra-host genetic variation (up

to approximately 2% nucleotide diversity), but all were restricted to hosts of a single species. Apicomplexan parasites of the genus *Hepatocystis*, related to *Plasmodium*, were detected using microscopy and PCR. "Deep sequencing" of the parasite cytochrome *b* gene showed one *Hepatocystis* lineage to be transmitted frequently between red colobus monkeys (*Procolobus rufomitratus*) and olive baboons (*Papio anubis*), whereas the remaining five lineages infected hosts of a single species. Analyses of fecal samples revealed helminth parasites of the genera *Oesophogostomum* (nodule worms) and *Trichuris* (whipworms), which were further characterized by PCR and sequencing of the internal transcribed spacer region of the ribosomal DNA complex. Both parasite genera contained multiple cryptic lineages, some representing putative novel taxa, and several infecting primates of multiple species, including humans. Collectively, these results suggest that pathogens with highly variable and mutable genomes (e.g. RNA viruses) are not necessarily more likely to cross species barriers than more genomically complex pathogens (e.g. protozoa and helminths), in certain ecological settings. Efforts to prevent zoonotic transmission in such settings should focus on common transmission pathways inferred from empirical studies of a range of pathogen taxa, rather than on specific classes of pathogens assumed to have high cross-species transmission potential.

666

SAFETY AND IMMUNOGENICITY OF RTS,S/AS01 MALARIA VACCINE CANDIDATE IN HIV INFECTED INFANTS AND CHILDREN: A PHASE III RANDOMIZED, DOUBLE-BLIND, CONTROLLED TRIAL

Lucas O. Tina[1], Martina Oneko[2], Walter Otieno[1], Abuodha Odunga[2], Emmanuel Owino[1], Chris Odero[2], Yolanda Guerra Mendoza[3], Ben Andagalu[1], Norbert Awino[2], Karen Ivinson[4], Dirk Heerwegh[3], Nekoye Otsyula[1], Maria Oziemkowska[2], Effua-Abigail Usuf[3], Allan Otieno[1], Kephas Otieno[2], Didier Leboulleux[4], Amanda Leach[3], Janet Oyieko[1], Laurence Slutsker[2], Marc Lievens[3], Jessica Cowden[1], Didier Lapierre[3], Simon Kariuki[2], Bernhards Ogutu[1], Johan Vekemans[3], Mary J. Hamel[2]

[1]KEMRI-Walter Reed Project, Kisumu, Kenya, [2]KEMRI/Centers for Disease Control and Prevention, Kisumu, Kenya, [3]GlaxoSmithKline Vaccines, Wavre, Belgium, [4]PATH Malaria Vaccine Initiative, Washington, DC, United States

Malaria and HIV remain important global health problems. The RTS,S/AS01 malaria vaccine candidate, which showed protection against malaria with a favorable safety and immunogenicity profile during trials in sub-Saharan Africa, had previously not been evaluated specifically in HIV-infected participants. We measured safety and immunogenicity in children with WHO stage 1 or 2 HIV disease at two centers in Kenya (NCT01148459). Children aged 6 weeks to 17 months were randomized 1:1 to receive 3 doses of RTS,S/AS01 or rabies vaccine, administered monthly. The primary objective was occurrence of serious adverse events (SAEs) from first vaccination until 14 months post-dose 1. A secondary objective, incident clinical malaria, was captured by passive case detection. Baseline characteristics were similar between study arms. Out of 200 children enrolled, 177 (89%) completed follow-up. The frequency of SAEs was 41.4% (95%CI:31.6-51.8) among RTS,S/AS01 recipients and 36.6% (95%CI:27.3-46.8) among rabies vaccine recipients. At least one SAE was reported within 30 days of vaccination in 20.2% (95%CI:12.8-29.5) of RTS,S/AS01 recipients and 11.9% (95%CI:6.3-19.8) of rabies vaccine recipients. At least one episode of pneumonia occurred in 13/99 (13.1%) RTS,S/AS01 recipients compared to 5/101 (5.0%) rabies vaccine recipients within 30 days post-vaccination. Fatal SAEs occurred in 5.1% (95%CI:1.7-11.4) of RTS,S/AS01 recipients and 4.0% (95%CI:1.1-9.8) of rabies vaccine recipients. One related SAE was reported: a febrile convulsion in an RTS,S/AS01 recipient. Solicited adverse event frequency 7 days post-vaccination was: injection site pain 18.1% and 6%, fever 41.7% and 18.8%, irritability 25.3% and 10.7%, and loss of appetite 17.7% and 8.7% in the RTS,S/AS01 and rabies vaccine group respectively. No evidence of differential HIV disease progression (CD4+, HIV viral load and WHO HIV Clinical Staging) was seen between study arms. In the RTS,S arm, anti-CS antibody geometric mean titer (EU/mL) was 0.3 (95%CI:0.3-0.4), 329.2 (95%CI:260.6-415.8) and 18.4 (95%CI:13.3-25.5) at baseline, one and 12 months post-dose 3. Vaccine efficacy against first episode of clinical malaria was 31% (95%CI:-19-60). RTS,S/AS01 showed no serious safety concerns and was immunogenic in HIV-infected children. When considered with efficacy data, HIV-infected children need not be excluded from potential future vaccination programs with RTS,S/AS01.

667

MATHEMATICAL MODELING OF THE SITE-SPECIFIC IMPACT OF THE RTS,S VACCINE UNDER MULTIPLE ROLL-OUT SCENARIOS

Caitlin A. Bever, Edward Wenger, Kevin A. McCarthy, Philip Eckhoff

Institute for Disease Modeling, Intellectual Ventures, Bellevue, WA, United States

Vaccines against malaria are one promising avenue for reducing the global burden of the disease beyond what has been achieved with current interventions. The pre-erythrocytic vaccine RTS,S is the candidate furthest along the development pipeline, and as such there is great interest in the potential impact of a large-scale roll-out. Here, we addressed this question by using the previously developed EMOD model of malaria to simulate the outcome of such a campaign. The central model is a mathematical description of the vector life cycle coupled to within-host parasite and immune dynamics. To study RTS,S, we incorporated a representation of the vaccine, characterized by an initial efficacy against infection and a half-life of protection. An iterative stochastic approach was used to optimize the modeled vaccine properties by sampling parameter space and evaluating a likelihood function that assesses the fit between the simulations and clinical trial data. Having obtained a calibrated model of RTS,S, we subsequently explored a variety of different roll-out scenarios to test the effects of policy choices (e.g., age of first administration, booster inclusion) and deployment setting characteristics (e.g., demographics, seasonality). Additionally, sensitivity analyses were performed on other axes, such as coverage level and treatment-seeking behavior, quantities that are at once difficult to measure and highly relevant to the success of a campaign. Simulated clinical and severe incidences were incorporated into a cost analysis in order to establish the incremental impact of the vaccine over other interventions, such as insecticide-treated nets (ITNs). We concluded that the cost effectiveness of population-wide infant vaccination is greatest in regions of moderate endemicity, though the specifics depend on a number of factors, including pre-existing ITN coverage and local anopheline feeding preferences.

668

COMPARISON OF ANTI-PFS25 ANTIBODY RESPONSES FOLLOWING VACCINATION WITH PFS25-EPA/ALHYDROGEL® IN A MALARIA NAÏVE AND MALARIA EXPOSED POPULATION

Sara A. Healy[1], Issaka Sagara[2], Kawsar Talaat[3], Mahamadoun H. Assadou[2], Merepen A. Guindo[1], Joan Aebig[1], Sarah Brockley[1], Charles Anderson[1], Kelly Rausch[1], David L. Narum[1], David S. Jones[1], Nicholas MacDonald[1], Daming Zhu[1], Olga Muratova[1], Erin Gabriel[4], Michael Fay[4], Amagana Dolo[2], Yimin Wu[1], Ogobara Doumbo[2], Patrick Duffy[1]

[1]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]Malaria Research and Training Center, Mali-NIAID ICER, University of Science, Techniques and Technologies of Bamako, Bamako, Mali, [3]Center for Immunization Research, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [4]Biostatistical Research Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

A vaccine to interrupt malaria transmission would be a valuable tool for local elimination or eradication of malaria. Phase 1 clinical trials to assess the safety and immunogenicity of the *Plasmodium falciparum* transmission blocking vaccine candidate Pfs25-EPA/Alhydrogel® have been conducted in malaria naïve adults in the US and in malaria-exposed adults in Mali. The US Phase 1 study was an open label dose escalating study in which five volunteers (safety dose of 8 µg Pfs25-EPA/Alhydrogel®) received 2 doses, five volunteers (low dose of 16 µg Pfs25-EPA/Alhydrogel®) received 2 doses (Days 0, 56) and 20 volunteers (high dose of 47 µg Pfs25-EPA/Alhydrogel®) received 4 doses (Days 0, 56, 120, 300). The Mali Phase 1 study enrolled 120 volunteers in the double blind randomized control study: 20 volunteers (low dose of 16µg Pfs25-EPA/Alhydrogel® or control) received 2 doses (Days 0, 56) and 100 volunteers (high dose of 47µg Pfs25-EPA/Alhydrogel® or control) have thus far received 3 doses (Days 0, 56, 112) with a scheduled fourth vaccination on Day 480. Vaccinations were well tolerated in both populations. Specific anti-Pfs25 antibodies were detected by ELISA in sera from subjects receiving two or three doses of Pfs25-EPA/Alhydrogel®. The anti-Pfs25 antibody results obtained following three vaccinations in the high dose (47 µg) Pfs25-EPA/Alhydrogel® group in the US are comparable to the results seen following three vaccinations in the high dose (47 µg) Pfs25-EPA/Alhydrogel® group in Mali. The antibody response at each vaccine dose increased with each subsequent dose of vaccine given but diminished quickly following vaccination. The percentage of responders with anti-Pfs25 antibody responses at levels ≥546 units, which was the anti-Pfs25 antibody level that had previously been seen to have functional activity in the US study, was the same between the two studies following the second and third vaccination in the high dose groups. Anti-Pfs25 antibody response results following the fourth vaccination in the Mali study and associated functional activity will be available in October 2014. Overall, Pfs25-EPA/Alhydrogel® transmission blocking vaccine has been well tolerated and produced significant antibody responses in a malaria naïve and malaria exposed adult populations.

669

ASSESSMENT OF SAFETY AND IMMUNOGENICITY OF INTRAVENOUS IMMUNIZATION WITH RADIATION ATTENUATED *PLASMODIUM FALCIPARUM* NF54 SPOROZOITES (PFSPZ VACCINE) IN HEALTHY AFRICAN ADULTS

Sara A. Healy[1], **Mahamadou S. Sissoko**[2], Freda Omaswa[3], Abdoulaye Katile[2], Bourama Kamate[2], Yacouba Samake[2], Amagana Dolo[2], Karamoko Niare[2], Amadou Niangaly[2], Merepen A. Guindo[2], Erin Gabriel[4], Michael Fay[4], Eric James[3], Anita Manoj[3], Anusha Gunasekera[3], B. Kim Lee Sim[3], Peter Billingsley[3], Irfan Zaidi[1], Stephen L. Hoffman[3], Ogobara Doumbo[2], Michael Walther[1], Patrick Duffy[1]

[1]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]Malaria Research and Training Center, Mali-NIAID ICER, University of Science, Techniques and Technologies of Bamako, Bamako, Mali, [3]Sanaria, Inc., Rockville, MD, United States, [4]Biostatistical Research Branch, National Institute of Allergy and Infectious Diseases, Rockville, MD, United States

For decades it has been known that humans can be protected against malaria by repeated immunization with radiation-attenuated *Plasmodium falciparum* (Pf) sporozoites (SPZ). Traditionally, those PfSPZ have been administered by exposing vaccinees to the bites of >1,000 PfSPZ-infected, irradiated mosquitoes. Recently, a process for manufacturing aseptic, purified, radiation attenuated cryopreserved PfSPZ has been developed. When administered by IV injection, this product, Sanaria® PfSPZ Vaccine, induced sterile protection against controlled human malaria infection in 6/6 malaria naïve adults who received the highest dosage. To begin the process of assessing the vaccine in Africa, we are conducting a double blind, randomized, controlled Phase 1 clinical trial to assess PfSPZ Vaccine's safety, immunogenicity, and protective efficacy against naturally occurring malaria infection in healthy, malaria exposed, 18-35 years old Malians. Among the 105 volunteers vaccinated by direct venous inoculation (DVI), 12 volunteers (pilot safety group) have received 2 doses of PfSPZ Vaccine (Day 0: $1.35x10^5$ and Day 14: $2.7x10^5$ PfSPZ) and 93 volunteers will have received 5 doses of $2.7x10^5$ PfSPZ or normal saline placebo (Day 0, 28, 56, 84, 140; total dosage: $13.5x10^5$ PfSPZ) by the end of July 2014. Nine subjects in the pilot group will join the larger group to receive an additional 3 vaccinations (total dosage: $12.15x10^5$ PfSPZ). The incidence and severity of local and systemic adverse events occurring within 7 days after dose are being recorded. During the malaria transmission season all volunteers will be examined every 14 days and as clinically indicated for blood-stage parasitemia by microscopy. All volunteers have been immunized twice. Immunizations have been well tolerated with no local reactogenicity, minimal mild to moderate systemic reactogenicity, no serious adverse events, and no patent parasitemia. These early results show PfSPZ Vaccine administered via DVI is safe and well tolerated in healthy malaria-exposed, African adults. Results of all immunizations will be presented.

670

ASSESSING EFFICACY OF THE PFSPZ VACCINE BY CONTROLLED HUMAN MALARIA INFECTION IN TANZANIA

Seif Shekalaghe[1], Said Jongo[1], Elena Moreno[1], Omar Lweno[1], Ali Hamad[1], Kamaka Kassim[1], Omar Juma[1], Conrad Gwandu[1], William Kato[1], Caroline Mavere[1], Ramla Rashid[1], Jackson Mollel[1], Maximillian Mpina[1], Anneth Tumbo[1], Catherine Mkindi[1], Tobias Rutishauser[2], Isabelle Zenklusen[2], Selina Joseph[1], Mohammed Mgeni[1], Claudia A. Daubenberger[2], Peter F. Billingsley[3], B. Kim Lee Sim[3], Thomas L. Richie[3], Marcel Tanner[2], Salim Abdullah[1], Stephen L. Hoffman[3]

[1]Bagamoyo Clinical Trial Unit, Ifakara Health Insitute, Bagamoyo, United Republic of Tanzania, [2]Swiss Tropical and Public Health Institute, Basel, Switzerland, [3]Sanaria Inc., Rockville, MD, United States

The rich potential for African scientists to spearhead phase 1 studies that have a controlled protection component has not been tapped because controlled human malaria infections (CHMI) have been available only at centers in the USA and Europe with access to infected mosquitoes. Sanaria has developed aseptic, purified, cryopreserved infectious *Plasmodium falciparum* (Pf) sporozoites (SPZ) (PfSPZ Challenge) manufactured in compliance with cGMPs, suitable for parenteral injection and shippable to any location. PfSPZ Challenge has been tested in the Netherlands, US, UK, Tanzania, Kenya, Germany and Spain using different dosages and inoculation routes. 3,200 PfSPZ administered by direct venous inoculation (DVI) reproduces the 100% infection rate and 11-11.5 day pre-patent period of 5 mosquito bite CHMI. In the present, first of its kind study, we will use PfSPZ Challenge to test the efficacy of Sanaria's radiation attenuated, non-replicating PfSPZ Vaccine against CHMI administered by DVI. PfSPZ Vaccine showed 100% efficacy in a recent study by the Vaccine Research Center (VRC), NIAID, NIH in the study group receiving the highest dose tested (5 doses of 1.35x10^5 PfSPZ by DVI). The Bagamoyo Clinical Trial Unit, Ifakara Health Institute, is now comparing the same regimen shown to be 100% protective at VRC, with a regimen that delivers twice the dose (2.7x10^5 PfSPZ) at each of the 5 time points, in minimally malaria-exposed Tanzanian adults. Both groups will be assessed for protection against CHMI using PfSPZ Challenge administered by DVI at 3 and 24 weeks after the 5th dose (40 immunized and 8 control subjects). An additional 6 volunteers will receive the higher dose and undergo CHMI only at 24 weeks. We will present safety, tolerability, and preliminary immunogenicity data for this unique study that is opening an exciting pathway for malaria vaccine and drug research and development in Africa and the world.

671

POTENT CELLULAR AND HUMORAL IMMUNOGENICITY OF CHAD63 MVA ME-TRAP IN AFRICAN INFANTS AND CHILDREN

Katie J. Ewer[1], Carly Bliss[1], Georgina Bowyer[1], Muhammed Afolabi[2], Alfred Tiono[3], Abdoulie Drammeh[2], Issa Nebie[3], Jankey Ya Jagne[2], Jane Adetifa[4], Momodou Cox[2], Nick Edwards[1], Jean Baptiste Yaro[5], Susanne Hodgson[1], Nick Anagnostou[6], Katharine Collins[1], Eleanor Berrie[7], Sarah Moyle[7], Sarah Gilbert[1], Nicola Viebig[8], Rachel Roberts[6], Alison Lawrie[6], Egeruan Imoukhuede[6], Katie Flanagan[2], Beate Kampmann[2], Philip Bejon[9], Adrian Hill[1], Sodiomon Sirima[3], Kalifa Bojang[2]

[1]The Jenner Institute, University of Oxford, Oxford, United Kingdom, [2]Medical Research Council Unit, Fajara, Gambia, [3]Centre National de Recherche et de Formation sur le Paludisme, Ougadougou, Burkina Faso, [4]Medical Research Council Unit, Faj, Gambia, [5]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [6]CCVTM, University of Oxford, Oxford, United Kingdom, [7]CBF, University of Oxford, Oxford, United Kingdom, [8]European Vaccine Initiative, Heidelberg, Germany, [9]Kenya Medical Research Institute, Kilifi, Kenya

Vaccination is one of the most cost-effective health care interventions available and an effective malaria vaccine against *Plasmodium falciparum* could save more than half a million lives each year. We developed a prime-boost immunisation approach employing the viral vectors ChAd63 and MVA, both encoding the pre-erythrocytic malaria antigen TRAP. Previous studies with ChAd63 MVA ME-TRAP have shown excellent immunogenicity and significant efficacy in adults with protection correlated with frequency of mono-functional $CD8^+$ T cells secreting IFNγ. We report here T cell phenotypes and antibody titres measured in 138 children vaccinated during three Phase I dose-finding and age de-escalation studies to assess safety and immunogenicity of the ChAd63 MVA ME-TRAP vaccine in malaria-exposed children in The Gambia and Burkina Faso. Age groups at first immunisation were 2-6 years, 5-12 months and 10 weeks in The Gambia and 5-17 months in Burkina Faso. IFNγ ELISPOT responses to TRAP were significantly lower in 2-6 year old and 5-12 month old children in The Gambia and 5-17 month old children in Burkina Faso than in malaria-naïve UK adults receiving the same vaccines. Immunogenicity in 10 week-old infants in The Gambia was high and comparable to that in adults. Flow cytometry revealed IFNγ secretion from both $CD4^+$ and $CD8^+$ T cells with IL-2 and TNFα also detected from $CD8^+$ T cells. Anti-TRAP IgG responses varied by age group and dose, with significantly higher titres detected after boosting in vaccinees primed with a higher dose of ChAd63 ME-TRAP. Titres were also significantly higher in 5-12 month and 10 week old children than 2-6 year-old children in The Gambia that received the same dose. IgG titres in 5-17 month-old children in Burkina Faso were comparable to those in 5-12 month old children in The Gambia. IgG responses were predominantly composed of IgG1 and IgG3 isotypes and we also detected IgA and IgM responses. We demonstrate excellent cellular and humoral immunogenicity of a pre-erythrocytic malaria vaccine in key target populations for vaccine deployment.

672

SAFETY AND IMMUNOGENICITY OF THE BLOOD-STAGE *PLASMODIUM VIVAX* VACCINE CHAD63-MVA PVDBP

Ruth O. Payne[1], Kathryn H. Milne[1], Sean C. Elias[1], David Llewellyn[1], Nick J. Edwards[1], Simone C. de Cassan[1], Anna L. Goodman[1], Chetan E. Chitnis[2], Alfredo Nicosia[3], Sarah Moyle[4], Eleanor Berrie[4], Alison M. Lawrie[1], Adrian V. Hill[1], Simon J. Draper[1]

[1]University of Oxford, Oxford, United Kingdom, [2]International Centre for Genetic Engineering and Biotechnology, New Delhi, India, [3]Okairos, Rome, Italy, [4]Clinical Biomanufacturing Facility, Oxford, United Kingdom

There has been comparatively little research into vaccines for *Plasmodium vivax* in the past, with only two antigens previously reaching Phase Ia

clinical trials. More recently, the importance of a vaccine for *P. vivax* has been recognised and included in the 2013 update to the Malaria Vaccine Technology Roadmap. Here we report on the first blood-stage *P. vivax* vaccine Phase Ia clinical trial. This has been carried out in Oxford using recombinant simian adenovirus ChAd63 and the poxvirus MVA encoding the *P. vivax* antigen PvDBP (Duffy-binding protein region II) in a heterologous prime-boost regimen. The *P. vivax* parasite requires an interaction between the Duffy-binding protein ligand and its host receptor, the Duffy antigen receptor for chemokines (DARC), in order to invade reticulocytes, making PvDBP a promising antigen for vaccine development. Twenty-three healthy volunteers have been vaccinated and followed up in this Phase Ia dose escalation study of ChAd63-MVA PvDBP. The ChAd63 PvDBP priming vaccine was initially given alone to 4 volunteers at a dose of 5×10^9 vp before being increased to 5×10^{10} vp following a planned safety review. MVA PvDBP has been given to 15 volunteers 8 weeks after ChAd63 PvDBP prime at doses of 1×10^8 pfu – 2×10^8 pfu. The primary objective of the study was safety, and the vaccines have been well tolerated with no serious adverse events. Follow-up of volunteers will be complete at the end of June 2014. The secondary objective was humoral and cellular immunogenicity which has been assessed using assays including PvDBP IFN-γ T cell ELISPOT, B cell ELISPOT, PvDBP IgG antibody ELISA and functional antibody analysis. The vaccine regimen is immunogenic, with increases in the cellular and humoral responses seen after MVA PvDBP boost (compared with ChAd63 PvDBP alone). This is the first vaccine against PvDBP to be assessed in humans, and has shown a favourable safety profile and promising levels of immunogenicity which support onward clinical development towards proof-of-concept efficacy testing.

673

ELQ-300 PRODRUGS FOR ENHANCED DELIVERY AND EFFICACY AGAINST MALARIA

Galen P. Miley[1], Sovitj Pou[1], Rolf Winter[1], Aaron Nilsen[1], Yuexin Li[1], Jane X. Kelly[1], Allison Stickles[2], Isaac P. Forquer[1], Michael W. Mather[3], April Pershing[3], Akhil B. Vaidya[3], Karen White[4], David Shackleford[4], Jessica Saunders[4], Gong Chen[4], Cassandra Noack[4], Susan A. Charman[4], Li-Min Ting[5], Kami Kim[5], Cristina Donini[6], Jeremy N. Burrows[6], Michael K. Riscoe[2]

[1]Veterans Affairs Medical Center, Portland, OR, United States, [2]Oregon Health & Science University, Portland, OR, United States, [3]Drexel University College of Medicine, Philadelphia, PA, United States, [4]Monash University, Parkville, Australia, [5]Albert Einstein College of Medicine, New York, NY, United States, [6]Medicines for Malaria Venture, Geneva, Switzerland

ELQ-300 is a preclinical candidate of the Medicines for Malaria Venture that targets the liver and blood stages of malaria, as well as the forms that are crucial to transmission of falciparum malaria: gametocytes, zygotes, and ookinetes. In mouse models of the disease, a single oral dose of 0.03 mg/kg prevented sporozoite induced infections while 4 daily doses of 1 mg/kg achieved complete cures of patent infections. A significant obstacle to the clinical development of ELQ-300 relates to its physical-chemical properties. Its relatively poor water solubility and high crystallinity limits absorption to the degree that only low bloodstream concentrations can be achieved by oral dosing. While these low bloodstream concentrations are sufficient for therapy the levels are too low to establish an acceptable safety margin required by regulatory agencies for clinical development. One way to address the challenging physical-chemical properties of ELQ-300 is through the development of prodrugs. In this presentation we will profile a series of ELQ-300 prodrugs, focusing primarily on the bioreversible nature of ELQ-337. At the equivalent dose of 3 mg/kg the delivery of ELQ-300 from ELQ-337 is enhanced by 4-5-fold, reaching a C_{max} of 6.9 micromolar by 4 hrs after oral administration. The superior *in vivo* efficacy of this compound will be discussed. Apart from highlighting the outstanding *in vivo* efficacy of ELQ-337, this data demonstrates that the prodrug strategy represents a viable approach to overcome the physical-chemical limitations of ELQ-300 to deliver the active drug to the bloodstream at high enough concentrations sufficient for safety and toxicology studies as well as achieving single dose cures.

674

UNDERSTANDING THE MECHANISM FOR EFFICACY AND TOXICITY OF 8-AMINOQUINOLINE ANTIMALARIALS: *IN VITRO* AND *IN VIVO* STUDIES WITH HYDROXYLATED METABOLITES OF PRIMAQUINE

Babu L. Tekwani, Rajnish Sahu, Narayan D. Chaurasiya, N.P. Dhammika Nanayakkara, Larry A. Walker

University of Mississippi, University, MS, United States

Hydroxylated metabolites are presumed to be responsible for toxicity and/or efficacy of primaquine and other 8-aminoquinoline antimalarials. However, no definitive data are available on pharmacokinetics, pharmacodynamics and *in vivo* plasma/tissue profiles of the hydroxylated metabolites. Recent *in vitro* studies with primary human hepatocytes and application of stable isotope (13C) labeled PQ provided evidences for generation of hydroxylated metabolites presumably through CYP mediated pathways. Previous studies by us have shown that 5-hydroxyprimaquine (5-HPQ) and 6-Methoxy 8-N-Hydroxy aminoquinoline (MHQ) generated robust methemoglobin formation and oxidative stress in normal and G6PD deficient human erythrocytes. Both 5-HPQ and MHQ produced selective depletion of GSH in G6PD deficient erythrocytes. The studies were extended to include 2-, 3-, 4- and 8-N-hydroxyprimaquine (8-NHPQ). Both 2-HPQ and 4-HPQ are relatively stable analogs. 2-HPQ and 4-HPQ did not generate significant methemoglobin and only marginal ROS in normal and G6PDD RBCs with & without microsomes. Neither metabolite showed activity *in vivo* in the blood stage *Plasmodium berghei* mouse malaria model (i.v. route of administration). Until recently, no definite evidences have been available regarding generation of 3-HPQ and/or 8-NHPQ *in vivo* & *in vitro*. 3-HPQ can be further metabolized with pooled human liver microsomes to generate more toxic/reactive species. 8-NHPQ is a reactive metabolite, which generates significant methemoglobin and oxidative stress. Among 2-, 3-, 4- HPQ and 8-NHPQ, none affected GSH levels in normal or G6PD-deficient human erythrocytes. Formation of 2- and 3-HPQ metabolites was confirmed *in vitro* on incubation of PQ with recombinant human CYP2D6; another HPQ, perhaps 4-HPQ is also formed. However, 2-HPQ, 3-HPQ and 4-HPQ metabolites appear not to be responsible for toxicity/efficacy of PQ.

675

A PHASE I/IB STUDY TO INVESTIGATE THE SAFETY, TOLERABILITY AND PHARMACOKINETIC PROFILE OF DSM265 IN HEALTHY SUBJECTS AND THEN ITS ANTIMALARIAL ACTIVITY IN INDUCED BLOOD STAGE *PLASMODIUM FALCIPARUM* INFECTION

James S. McCarthy[1], Julie Lotharius[2], Anthony Dayan[3], Margaret Phillips[4], Kennan Marsh[5], Don Walker[6], Mark Baker[2], Suzanne Elliott[7], Paul Griffin[7], Caroline Ng[8], David Fidock[8], Lidiya Bebrevska[2], Stephan Duparc[2], Joerg Moehrle[2], Thomas Rueckle[2]

[1]QIMR Berghofer Medical Research Institute, Herston, Australia, [2]Medicines for Malaria, Geneva, Switzerland, [3]University of London, London, United Kingdom, [4]University of Texas Southwestern, Dallas, TX, United States, [5]AbbVie, Lake Forest, IL, United States, [6]Pfizer Global Research and Development, Sandwich, United Kingdom, [7]QPharm Pty Ltd, Herston, Australia, [8]Columbia University, New York, NY, United States

DSM265 is a new antimalarial that targets the malaria parasite's pyrimidine biosynthetic enzyme dihydrootate dehydrogenase (DHODH). A Phase I/Ib study was designed to collect safety, PK and efficacy data. It was undertaken in two parts: the first comprised a single ascending dose (SAD) design to assess safety, pharmacokinetics, and the maximum tolerated dose. Embedded within this first part was an assessment of food effect. The second part comprised exploration of the antimalarial activity of

DSM265 in a single-dose cohort using the induced blood-stage malaria (IBSM) system at a dose guided by data from preclinical efficacy prediction and emerging PK data. To date, 5 cohorts comprising 49 volunteers have participated in the SAD up to 400mg, study of food effect, or the IBSM phase. To date, no significant drug-related clinical or laboratory toxicity have been observed. Fitting of a population PK model to the data indicates mono-exponential disposition, with a blood clearance/f of 0.45 L/hr (95%CI: 0.41-0.50), and an elimination t1/2 of 91 hrs. DSM265 shows dose proportionality, both in terms of Cmax and AUCinf. No food effect was observed. A dose of 150 mg was selected for the IBSM phase by combining the MPC predicted from preclinical data (0.5-1 µg/mL) with the human PK, aiming to observe parasite clearance kinetics, including recrudescence. When tested in the IBSM system in 7 healthy volunteers, the drug showed encouraging antimalarial activity with 99.9% parasite clearance by 130 hrs, with recrudescent infection observed in 5/7 subjects between 10 and 21 days after drug administration. No mutations in the DHODH gene were observed in recrudescent parasites. Fitting of a population PK/PD model to the parasitemia data led to an estimate of the MPC of 954 ng/mL (95%CI: 678-1267). Population estimates of PRR and parasitemia t1/2 were 2.2 (95%CI: 2.0-2.7) and 6.6 hrs (95%CI: 5.4 - 7.2) respectively. These results demonstrate that it is possible to obtain well characterised efficacy data early in the clinical development of an anti-malarial. The combination of early safety, PK, and efficacy data with the PK/PD model mean that future clinical trials of DSM265 will benefit by being smaller and focused towards confirming the observed efficacy. The observed duration of action of DSM265 suggests its promise as a component of a new combination single-dose treatment for malaria.

676

TARGETING DRUG RESISTANCE: HARNESSING EVOLUTIONARY FITNESS IN *PLASMODIUM FALCIPARUM* FOR DRUG DISCOVERY

Amanda K. Lukens[1], Pamela A. Magistrado[2], Jordan C. Canedy[3], Leila S. Ross[2], Ulf E. Ribacke[4], Jonathan D. Herman[5], Ralph Mazitschek[5], Roger C. Wiegand[1], Dyann F. Wirth[2]

[1]The Broad Institute, Cambridge, MA, United States, [2]Harvard School of Public Health, Boston, MA, United States, [3]Harvard University, Cambridge, MA, United States, [4]Uppsala University, Uppsala, Sweden, [5]Harvard Medical School, Boston, MA, United States

Management of drug resistance with combination therapies is key to prolonging the effectiveness of new antimalarials. Typical combination therapies pair drugs that are each effective as monotherapies and employ differing binding sites. Pairing drugs that target independent sites presents the opportunity for the parasite to individually develop resistance to each drug, rendering the combination ineffective. Here we explore new kinds of antimalarial combination therapies where the changes giving rise to resistance become the target for the second drug. This approach incorporates built-in protection for the partner drug from development of resistance. In the wild-type organism the partner drug has little or no effect and the organism is not subject to selective pressure to develop resistance. When resistant mutants to the first drug occur then, and only then, does the second drug act, greatly diminishing the population subject to selection. In the event that mutations occur conveying resistance to the second drug, those mutations will drive the organism back towards sensitivity to the first drug. We have taken a systematic approach in the study of resistant organisms and their unique susceptibilities. We screened the Malaria Box against a panel of mutant parasites with well-defined resistance mechanisms to identify compounds with differential activity between mutant and wild-type parasites. We show that a significant percentage of the Malaria Box targets one of the pathways tested. In particular, we find a number of compounds with negative cross-resistance, representing promising leads in pursuing inhibitor pairs as described above. Importantly, multiple chemotypes inhibit each target, indicating there are overlapping modes of action in the parasite despite broad chemotypic diversity in the compound set. This suggests the parasite has a limited number of chokepoints that can be exploited in drug development making it all the more critical to develop a means of protecting the long-term efficacy of compounds in the pipeline with combination strategies as discussed.

677

NON-INFERIORITY CLINICAL TRIAL COMPARING FIXED-DOSE COMBINATION AND CHLOROQUINE FOR *PLASMODIUM VIVAX* UNCOMPLICATED INFECTION IN THE BRAZILIAN AMAZON

André M. Siqueira[1], Aline Alencar[1], Belisa Magalhães[2], Gisely Melo[2], Kim Machado[1], Valérie Lameyre[3], Cláudio T. Daniel-Ribeiro[4], Marcus V. Lacerda[1]

[1]Fundacao de Medicina Tropical Heitor Vieira Dourado, Manaus - AM, Brazil, [2]Universidade do Estado do Amazonas, Manaus - AM, Brazil, [3]Access to Medicines, Sanofi Aventis, Paris, France, [4]Fundacao Oswaldo Cruz, Rio de Janeiro, Brazil

In Latin America, *Plasmodium vivax* is responsible for around 80% of malaria episodes. As Chloroquine (CQ) efficacy decreases, alternative therapies such as artemisinin-based combinations recommended by WHO must be evaluated. This phase III, noninferiority randomized trial, compared the antischizontocidal efficacy and safety of a 3 days supervised treatment of the fixed-dose artesunate-amodiaquine Winthrop® (ASAQ) versus CQ for the treatment of uncomplicated P.vivax infection in children above 6 months-old and adults. Primary endpoint was the efficacy rate at day 28. Patients were followed-up until day 42. 380 patients were included in Manaus, Brazil. Baseline characteristics and dropout rates were similar between arms. In Per-Protocol analysis, adequate clinical and parasitological response (ACPR) at day 28 was achieved in 100% and 92.4% of patients in the ASAQ and CQ arm (93.7% versus 89.5% in the Intention-To-Treat analysis). Non-inferiority was achieved in both populations and subsequent bilateral tests concluded in superiority of ASAQ (p=0.001% and < 0.001% in ITT and PP analysis respectively). Clearance of parasite from D1 to D3 was observed in significantly more patients (p<0.001) and significantly more patients were afebrile at D1 (p=0.001) in the ASAQ group. A significant reduction in the number of gametocyte carriers was also observed in the ASAQ group versus CQ group during follow-up. A significant difference in the proportion of recurrence during the 42 days of follow-up was also observed (26.5% versus 3.9% in the CQ versus ASAQ groups, p<0.001). 79% of recurrences in the CQ arm, and all in the ASAQ, occurred after D28. The occurrence of emergent adverse events (AEs) was similar in both arms (CQ=115; ASAQ=110), with no serious AE in the CQ and 5 (related to 3 patients) in the ASAQ arms. ASAQ is a safe and efficacious alternative to treat uncomplicated *P. vivax* infection. 42-days-follow-up enables better assessment of CQ efficacy. Microsatellite genotyping correction and CQ levels results will be presented.

678

QTC PROLONGATION AND DRUG SAFETY OF AN INCREASED DOSE OF DIHYDROARTEMISININ-PIPERAQUINE IN YOUNG CHILDREN 5-24 KG IN MALAWI

Eva Maria Hodel[1], Gertrude Chapotera-Kalanda[2], Tikhala Makhaza-Jere[3], Clyton A. Munthali[2], Mavuto Mukaka[3], Queen Dube[3], Cheryl Pace[1], Karen I. Barnes[4], David G. Lalloo[1], Dianne J. Terlouw[3]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]College of Medicine, Blantyre, Malawi, [3]Malawi-Liverpool-Wellcome Trust Clinical Programme, Blantyre, Malawi, [4]University of Cape Town, Cape Town, South Africa

Dihydroartemisinin-piperaquine (DHA-PPQ) is recommended for the treatment of uncomplicated *falciparum* malaria and is being explored as mass drug administration to reduce transmission. Recent pharmacokinetic (PK) studies and a pooled meta-analysis of individual patient efficacy data by the WorldWide Antimalarial Resistance Network have raised concerns

about potential under-dosing of young children with current weight-based dose recommendations and advocated higher dosing regimens in this vulnerable population. In contrast, the European Medicines Agency has called for more data to substantiate the cardiac safety of DHA-PPQ and its effect on QTc intervals. We conducted an open-label dose optimization study aimed at describing the population PK profile of PPQ and the tolerability of a higher dose regimen of DHA-PPQ in children of 5–24 kg with uncomplicated *falciparum* malaria [PACTR201303000506302]. In the first step of the study, 100 children received a dosing regimen based on supervised treatment with doses of 1.7–3.8 mg/kg DHA and 13.6–30.0 mg/kg PPQ given once daily over three days, using whole or half-tablets (20/160 mg and 40/320 mg DHA/PPQ tablets); children were followed-up for 63 days. In the second step 100 children received a more pragmatic regimen using whole tablets with wider ranging doses of 2.0–4.0 mg/kg DHA and 16.2–32.0 mg/kg PPQ. QTc was measured 4–6 h after the last DHA-PPQ dose and compared to baseline and Day 28 using digital 12-lead electrocardiograms and the vertical caliper on median overlapped template technique. We will present findings from the first step. Findings from the second step will be presented, dependent on study progress. Optimising the weight-based dose regimen for DHA-PPQ in the main high-risk group of young children ahead of its roll-out into control programmes in sub-Saharan Africa will help inform the translation of dosing recommendations into programmatically feasible, user-friendly, safe weight- and age-based dosing regimens.

679

SCHEDULED SCREENING VERSUS PREVENTIVE TREATMENT FOR THE CONTROL OF MALARIA IN PREGNANCY IN MALAWI: A RANDOMIZED CONTROLLED TRIAL

Mwayiwawo Madanitsa[1], Linda Kalilani-Phiri[1], Victor Mwapasa[1], Stephen Rogerson[2], Steve Taylor[3], Steve Meshnick[4], Annemieke van Eijk[5], Feiko ter Kuile[5]

[1]College of Medicine, University of Malawi, Blantyre, Malawi, [2]University of Melbourne, Melbourne, Australia, [3]Duke University Medical Center, Durham, NC, United States, [4]University of North Carolina, Wilmington, NC, United States, [5]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

The decline in effectiveness of intermittent preventive treatment in pregnancy with SP (IPTp-SP) due to high levels of sulphadoxine-pyrimethamine (SP) resistance by *Plasmodium falciparum* malaria parasites necessitates urgent alternative approaches for malaria control in pregnancy. The objectives of this study is to determine whether scheduled intermittent screening with a malaria rapid diagnostic test (mRDT) and treatment of mRDT-positive women with Dihydroartemesinin-Piperaquine (ISTp-DP) from the second trimesters is more efficacious than IPTp-SP in reducing adverse birth outcomes and malaria infection at term among HIV-sero-negative women protected by insecticide-treated bed nets in a malaria endemic setting. This two arm, open label, multicentre, randomized controlled superiority trial was designed to assess a 25% or greater reduction in adverse birth outcome in primi- and secundigravidae and a 25% or greater reduction in placental malaria in multigravidae. Between July, 2011 and March, 2013, a total of 1,155 and 717 women in their first and second pregnancy and third to fifth pregnancy respectively were recruited from 3 sites of perennial malaria transmission with high grade SP resistance and near saturation of the dihydropteroate synthase/dihydrofolate reductase quintuple haplotype. Of 3,214 women screened 1,872 (58.2%) were enrolled. 92% of participants were retained to delivery and 90.5% to study completion.

680

ECOLOGICAL GENOMICS AND PLASTICITY OF GENETIC REGULATION FOR SALTWATER TOLERANCE

Hilary A. Smith, Nora J. Besansky, Changde Cheng

University of Notre Dame, Notre Dame, IN, United States

Evolution of osmoregulatory systems is a key factor in the transition of species between fresh- and saltwater habitats, including anopheline mosquitoes known for their role as disease vectors. Here we use RNA-Seq to investigate gene expression differences between an obligate freshwater (*Anopheles coluzzii*) and euryhaline malaria vector (*An. merus*). After rearing in freshwater (FW), both young and old larval instars of each species were briefly (6 h) exposed to either saltwater (SW) or FW conditions to test the impact of water salinity on mRNA levels. We aimed to describe the transcriptomic response of anophelines to water salinity, and how the response differs between *An. merus* and *An. coluzzii*. We also tested how the transcriptomic response to water salinity differs with age, particularly for the tolerant species *An. merus*. Our results are congruent with the ability of gene induction to mediate SW tolerance, with the intolerant *An. coluzzii* exhibiting little difference in gene expression (<2% of the transcriptome), in contrast to greater plasticity by *An. merus.* In the latter, >16% of the 11,025 genes assayed responded to saltwater exposure, with similar levels of up- and down-regulation. The impact of age at exposure was less dramatic than species identity, with 567 genes significantly differentially expressed in response to water type between young and old *An. merus*. Besides effector genes with putative roles in ion transport (e.g., Na^+/K^+-ATPase), we also report differential expression in response to water salinity by genes involved in general stress responses such as heat shock proteins, and potential cross-talk between the immune response and osmoregulation. Additionally, we report on a network of 115 co-expressed genes associated with SW tolerance. Finally, we complement our investigation of gene expression with QTL mapping from a backcross of *An. merus* and *An. coluzzii*. We report regions of sequence divergence associated with SW tolerance, and discuss the presence of differentially expressed genes within these regions.

681

A COMPARATIVE APPROACH TO IDENTIFY PATHWAYS REGULATING FEMALE REPRODUCTIVE BIOLOGY IN ANOPHELINE MALARIA VECTORS

Sara N. Mitchell[1], Evdoxia Kakani[1], Paul Howell[2], Flaminia Catteruccia[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]MR4 at Centers of Disease Control and Prevention, Atlanta, GA, United States

Female *Anopheles gambiae* mosquitoes exhibit major behavioral and physiological changes in the first 24 hours after mating, including egg laying and refractoriness to further mating. These post-mating responses are in part mediated by transfer of seminal secretions contained in the mating plug, a complex of proteins, lipids and hormones, formed in the male, and deposited in the female atrium (uterus) during mating. However, not all Anophelines produce and transfer a plug, with this structure absent in the Nyssorhynchus mosquito series (*Anopheles albimanus*). This raises the question of how female post-mating changes are induced in absence of a plug? To address this question, we compared the molecular processes underpinning the mating responses of *An. gambiae* and *An. albimanus* females via RNAseq analysis of the atrium. A significantly larger response was observed in *An. gambiae* after mating (2235 genes, $p<0.05$) than in *An. albimanus* (212). Comparison of seminal secretions from the two species indicates that this >10-fold increase in *An. gambiae* response may be due to the transfer of potent transcriptional regulators. Enrichment analysis (DAVID) identified pathways of epithelial transport and molecule trafficking regulated in both species, suggesting the exchange of material across the atrium epithelium. Among the genes exclusively modulated in *An. gambiae,* were proteins associated with mating plug processing and energy production, suggestive of high-energy demands in mated females,

while in *An. albimanus* enriched processes included chitin metabolism, which may indicate structural changes to the uterus after mating, as well as carboxylesterase activity suggestive of xenobiotic metabolism. This comparison provides important insight into the different pathways shaping female post-mating behavior in *An. gambiae* and *An. albimanus*. Ultimately, a better understanding of Anopheline reproductive biology will aid vector control efforts aimed at reducing mosquito fertility, and may highlight conserved reproductive pathways that could be targeted in all malaria vectors worldwide.

682

PFS47 ROLE IN THE ADAPTATION OF *PLASMODIUM FALCIPARUM* TO NEW WORLD ANOPHELINES

Alvaro Molina-Cruz, Nitin Kamath, Emma Taylor-Salmon, Rebecca Greene, Gaspar Canepa, Ashley Haile, Jose Luis Ramirez, Urvashi Ramphul, Carolina Barillas-Mury

Laboratory of Malaria and Vector Research/National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States

The Pfs47 gene, a member of the 6-cys protein family, is required by *Plasmodium falciparum* to evade the *Anopheles gambiae* immune system. This led us to propose that Pfs47 may be important for the malaria parasite adaptation to different Anopheline mosquitos around the world. Pfs47 is a polymorphic gene with signatures of diversifying selection and a strong geographic genetic structure. The highest genetic diversity of Pfs47 was detected in Africa, and the lowest diversity was detected in the New World with one haplotype. Phylogenetic analysis of Pfs47 haplotype sequences present a geographic structure with most African haplotypes forming a clade separate from most of the Asian alleles. The haplotype found in the New World appears to be more closely related to the African clade. Infection of the New World malaria vector *A. albimanus* with *P. falciparum* was found to be highly dependent on the geographic origin of the parasite strain. The *A. albimanus* mosquito immune system was found to be responsible for the low infectivity found in African *P. falciparum* NF54 infections. Allele replacement showed that a Pfs47 haplotype from the New World can rescue the NF54 parasite infection of *A. albimanus*. This provides evidence that the mosquito immune system can be an important barrier for adaptation of *P. falciparum* to new vectors and that selection of particular Pfs47 haplotypes may be required to overcome this barrier.

683

DISCOVERY OF NOVEL LONG NON-CODING RNAS AND PROTEIN-CODING GENES IN *ANOPHELES GAMBIAE* USING DEEP RNA SEQUENCING

Adam M. Jenkins[1], Robert M. Waterhouse[2], Alan S. Kopin[3], Marc A. Muskavitch[4]

[1]Boston College, Chestnut Hill, MA, United States, [2]Massachusetts Institute of Technology, Cambridge, MA, United States, [3]Tufts Medical Center, Boston, MA, United States, [4]Biogen Idec, Cambridge, MA, United States

Recently, an expanding class of long non-coding RNAs (lncRNAs) that function in epigenetic regulation, the regulation of gene expression transcriptionally and post-transcriptionally, and the regulation of genomic stability have been described in mammals, Drosophila melanogaster and Caenorhabditis elegans. We have analyzed the transcriptome of the malaria vector *Anopheles gambiae*, based on deep-read RNA sequencing technology that generated over 500,000,000 reads from first and third instar larvae and adult females and males. Our de novo transcriptome assembly, and comparisons with gene models defined in VectorBase annotation Release 3.7, revealed over 1,100 novel lncRNAs and more than 200 previously unannotated putative protein-coding genes. The lncRNAs exhibit differential expression across life stages of the mosquito and display an increased rate of divergence over evolutionary time across the genus *Anopheles* when compared to the newly discovered protein-coding genes and previously annotated protein-coding genes. This initial description of lncRNAs in An. gambiae offers the first [large-scale] insights into non-coding RNAs in this mosquito and defines another potential set of targets for the development of vector-based interventions that may curb the malaria burden in disease-endemic countries.

684

THE EXPRESSION OF THE PIRNA PATHWAY GENES, *PIWI, AUBERGINE* AND *ARGONAUT 3*, DURING HEAT AND COLD STRESS RESPONSE IN THE MALARIA MOSQUITO, *ANOPHELES STEPHENSI*

Vanessa M. Macias, Kelcie Martin, Anthony A. James

University of California Irvine, Irvine, CA, United States

Stress-induced mobilization of transposons is well-documented in many organisms. An RNA-interference pathway, designated the piRNA pathway, is responsible for repressing transposon mobilization in germ-line tissues of the fruit fly, *Drosophila melanogaster*. The genes encoding the components of the piRNA pathway, *Piwi, Aubergine* (*Aub*) and *Argonaut 3* (*Ago3*), were identified and characterized in the malaria vector mosquito, *Anopheles stephensi*. Preliminary experiments show that they are induced in embryos following heat or cold stress. Current experiments in adult female mosquitoes are designed to assay the effects of heat and cold stress on the expression levels of the mosquito orthologs of the heat-shock protein genes, *hsp70* and *hsp90*, as well as *Piwi, Aub* and *Ago3* and endogenous transposase genes. Additionally, mosquitoes mutant for *Piwi, Aub* and *Ago3* are being generated and will be tested for a phenotype affecting the heat-shock response. The results of this work are expected to inform the development of transposon-based gene-drive systems for introgressing beneficial traits into vector mosquitoes.

685

THE ROLE OF INSECT INNATE IMMUNITY IN CONTROLLING *RICKETTSIA TYPHI* INFECTION

Kristen E. Rennoll-Bankert, Abdu F. Azad

University of Maryland, School of Medicine, Baltimore, MD, United States

Rickettsia typhi, the causative agent of murine typhus, is an obligate intracytosolic bacterium that is transmitted by the flea. Upon infection, *R. typhi* systemically infects fleas. However, infection level is kept manageable so that flea fitness is unaffected, presumably by the flea innate immune system. Two main arthropod innate immune signaling pathways, the Imd and Toll pathways, respond to Gram-negative bacteria and Gram-positive bacteria/fungi, respectively. Upon activation by the Imd or Toll pathway, Rel/NF-KB transcription factors Relish and Dorsal/DIF increase transcription of antimicrobial peptides. The immune pathways that control *R. typhi* infection in the flea have yet to be elucidated. We hypothesize that the Imd pathway is critical for controlling Gram-negative *R. typhi* burden within the flea. Dissecting the flea immune response to *R. typhi* has been hampered by the lack of a flea genome. Therefore, *R. typhi* infection was modeled in *Drosophila* S2R+ cells. *R. typhi* infected and replicated within S2R+ cells. To identify which innate immune signaling pathway(s) is critical for controlling *R. typhi* burden, S2R+ cells were treated with dsRNA to knockdown either the Imd or Toll pathway. Knockdown of negative regulators *caspar* and *cactus*, of the Imd and Toll pathways, decreased *R. typhi* burden suggesting both the Imd and Toll pathways control *R. typhi* burden. Knockdown of the NF-KB factor *Relish* but not *Dorsal* increased *R. typhi* burden, further confirming the role of the Imd pathway in control of *R. typhi* burden. More extensive screens will provide further evidence of which pathway(s) is controlling *R. typhi* burden. Additionally, a flea dorsal-like sequence has been identified and we are working to identify a flea relish-like sequence in hopes of parlaying the knowledge garnered from the *Drosophila* model into the flea. Identification of immune pathways in the flea and understanding the flea innate immune response to *R. typhi* could potentially lead to new treatment modalities that will decrease transmission and burden in the flea.

686

SIRNA NANOPARTICLE-MEDIATED TARGETING OF DOUBLESEX, A REGULATOR OF SEX-SPECIFIC DEVELOPMENT IN *AEDES AEGYPTI*

Keshava Mysore[1], Michael Tomchaney[2], Longhua Sun[2], Ping Li[1], Scott Emrich[2], David W. Severson[2], Molly Duman-Scheel[1]

[1]Indiana University School of Medicine at Notre Dame, South Bend, IN, United States, [2]University of Notre Dame, Notre Dame, IN, United States

Sexually dimorphic behaviors, including blood feeding and other sex-specific behaviors linked to reproduction, contribute to the global spread of mosquito-borne illnesses. Exploration of the developmental genetic basis for sexual dimorphism in mosquitoes has been hampered by a lack of methods to pursue functional developmental genetic studies. Although male and female splice variants of Doublesex (Dsx), a terminal transcription factor in the sex-determination pathway, have been detected in the dengue vector *Aedes aegypti,* the sex-specific expression patterns of these transcripts have not been detailed in developing tissues. Furthermore, although sex-specific *dsx* splice forms are believed to regulate sex-specific development in mosquitoes, this hypothesis has not been functionally tested. We have implemented a powerful methodological toolkit, including molecular markers for developing neural tissue subtypes and siRNA nanoparticle-mediated gene targeting, to characterize *dsx* function during *A. aegypti* development. These studies uncovered sex-specific *dsx* expression patterns in developing tissues, including the pupal brain mushroom body, antennal lobe, and optic lobe. 732 Dsx consensus binding sites were identified in the *A. aegypti* genome, including sites associated with 48 genes dimorphically expressed in the pupal head. *A. aegypti* genes associated with Dsx consensus sites group under a number of significant neural-related gene ontology terms, including neuron fate commitment, neuron differentiation, and neurological system processes, as well as numerous processes related to the sensory system and sensory development, particularly the compound eye and eye development. siRNA-mediated knockdown experiments confirmed that Dsx regulates sex-specific gene expression in the developing nervous system and uncovered adult phenotypes, including reproductive defects, associated with loss of *dsx* function. These studies are revealing the developmental genetic basis of mosquito sexual dimorphism and may one day be exploited in the development of novel control strategies.

687

THE SACRIFICED FLAGELLUM OF *TRYPANOSOMA CRUZI* PROVIDES VERY EARLY TARGETS OF PROTECTIVE CD8+ T CELLS

Samarchith P. Kurup, Rick Tarleton

University of Georgia, Athens, GA, United States

The CD8+ T cells crucial to the control of infection with *Trypanosoma cruzi*, the agent of human Chagas disease, are predominantly directed at members of large gene families, including trans-sialidase (ts)-like proteins that are diverse and variable within and among *T. cruzi* isolates. We hypothesized that CD8+ T cell responses directed against sub-dominant, invariant proteins may induce more potent cross-strain protection from *T. cruzi* infection and thus sought to identify such targets. Investigation of the early events in intracellular invasion by *T. cruzi* revealed that trypomastigotes sacrifice their flagella via an asymmetric division during amastigogenesis and thus release flagellar proteins into the host cell cytoplasm. Peptides derived from flagellar proteins appear to be among the earliest *T. cruzi* proteins to enter the class I MHC processing and presentation pathway. Overexpression of one of these proteins, the abundant paraflagellar rod protein (PAR) 4, by transgenic *T. cruzi* enhances the potency of the PAR4-specific CD8+ T cell response, resulting in significantly improved control of a challenge infection. This enhanced protection despite the relatively low abundance of PAR4-specific CD8+ T cells was associated with the ability of PAR4-specific CD8+ T cells to detect host cell infection by *T. cruzi* significantly earlier than the immunodominant ts-specific T cells. These results provide insights into previously unappreciated events in intracellular invasion by *T. cruzi* and suggest that the transgenic over-expression of appropriate endogenous proteins may significantly improve the protective capacity of viral vector based or live attenuated vaccines for *T. cruzi* and perhaps other pathogens.

688

UNTARGETED METABOLOMICS TO STUDY MULTIDRUG RESISTANCE IN *LEISHMANIA DONOVANI*

Maya Berg[1], Raquel Garcia-Hernandez[2], Bart Cuypers[1], Manu Vanaerschot[1], José Ignacio Manzano[2], Andris Jankevics[3], Rainer Breitling[3], Santiago Castanys[2], Jean-Claude Dujardin[1], Francisco Gamarro[2]

[1]Institute of Tropical Medicine, Antwerp, Belgium, [2]Instituto de Parasitología y Biomedicina 'López-Neyra', Granada, Spain, [3]Faculty of Life Sciences, Manchester Institute of Biotechnology, University of Manchester, Manchester, United Kingdom

Chemotherapy is the most important tool for the control of visceral leishmaniasis, but its efficacy is jeopardized by the growing resistance and treatment failure against first-line drugs. Antimonials have become obsolete in the Indian subcontinent because of resistance, the efficacy of miltefosine is decreasing, and isolated cases of amphotericin B failure are of concern for both the scientific and health community. To further delay the emergence of resistance, the WHO recommended combinations of anti-leishmanial drugs. Until today, the mode of action and drug resistance mechanisms of the four available drugs (antimonials, miltefosine, amphotericin B and paromomycin) are poorly understood. In this study, we performed untargeted LC-MS metabolomics to identify differentiating metabolites in *Leishmania donovani* promastigotes with induced resistance to single drugs and drug combinations. The most significant metabolic changes were found in the lines resistant (R) towards Sb^{III}, amphotericin B (AmB) and miltefosine, and the combinations Sb^{III} + AmB and Sb^{III} + paromomycin. A clear additive or synergistic effect of the single resistant lines in a combination-resistant line was not found, but significant overlap between differential metabolites in the single R lines and their combination-resistant line was observed. The detected metabolic changes upon drug exposure in wild-type and resistant lines were experimentally validated and showed that resistant parasites have (i) an increased capacity for protection against oxidative stress, and (ii) an altered fluidity of the plasma membrane. Our results elucidate the mechanisms underlying the ability of *Leishmania* to develop resistance to combinations of anti-leishmanial drugs: single and multidrug resistant parasite cell lines show distinct metabolic adaptations, but these all converge on the same defensive mechanisms.

689

DISSECTING THE PROMASTIGOTE TO AMASTIGOTE DIFFERENTIATION IN *LEISHMANIA AMAZONENSIS*

Catherine S. Nation[1], Ben Kelly[2], Juan C. Pizarro[1]

[1]Tulane University, New Orleans, LA, United States, [2]Louisiana State University Health Sciences Center, New Orleans, LA, United States

Leishmania is a genus of protozoan parasites transmitted by the phlebotomine sand fly that causes an array of disease ranging from cutaneous to visceral leishmaniasis resulting in significant morbidity and mortality. These parasites have a dimorphic life cycle, existing as a promastigote in the sand fly vector and an intracellular amastigote in the human host. While it is known that temperature and pH changes induce this change, the molecular basis of this differentiation is still unknown. To uncover the molecular mechanisms behind this transition, we used Hsp90 inhibitors, which are also capable of inducing the promastigote to amastigote change. In addition to the expected transition we observed a dose-dependent morphological change in response to the inhibitor, a situation not seen when changing the temperature or pH. We hypothesize that the morphological variability induced by the Hsp90 inhibitor is

associated with intermediate states in the promastigote to amastigote transition not seen under normal circumstances. We have employed different techniques to characterize these forms such as morphological analysis by microscopy and FACS, and gene expression analysis by qRT-PCR. We have also generated reporter plasmids expressing fluorescent proteins under the control of promastigote and amastigote specific promoters to follow the transition between the two forms in response to the typical heat and pH as well as in the presence of the Hsp90 inhibitor. Finally, proteomic analysis of promastigotes, amastigotes and intermediate forms has helped us proposed effector proteins responsible for the parasite transition. Ultimately, these signaling molecules will represent potential drug targets against leishmaniasis, a neglected disease that badly needs new therapeutic options.

690

A NOVEL FIELD-APPLICABLE MOLECULAR TEST FOR VISCERAL LEISHMANIASIS

Alejandro Castellanos-Gonzalez[1], Omar Saldarriaga[1], Lilian Tartaglino[2], Rosana Gacek[3], Ayesha Irani[1], Peter C Melby[1], Bruno Travi[1]

[1]University of Texas Medical Branch, Galveston, TX, United States, [2]Secretaria de Calidad de Vida, Posadas, Argentina, [3]Instituto Municipal de Sanidad Animal, Posadas, Argentina

In the Americas there are several thousand reported annual cases of full blown visceral leishmaniasis (VL) and mortality ranges between 7 and 10%. Furthermore, underreporting of VL is underestimating the actual disease burden. American VL is a zoonotic disease produced by Leishmania infantum and transmitted between dogs and humans by urbanized sand flies. Dogs are the principal reservoir hosts and, therefore, are critical targets for controlling urban transmission. The rK39® serological test is widely used to identify and remove infected dogs from the transmission cycle, but it has low sensitivity (<33%) to detect subclinically infected dogs. The lack of sensitivity of serological tests is considered as the main reason for the inefficacy of control programs. We have developed a sensitive and specific molecular test to detect L. infantum using a Recombinase Polymerase Amplification method coupled with Lateral flow reading (RPA-LF). This innovative isothermal amplification test is as sensitive as real time PCR (gold standard) but it does not require sophisticated equipment, is fast, and the result is read with the naked eye. This makes it ideal for point of care diagnosis and field epidemiology studies. In the lab RAP-LF detected <2 parasites in blood samples. Furthermore, evaluation of dog blood samples from an endemic area showed that RPA-LF detected more subclinically infected dogs than rK39 (51.9% vs. 14.3% positivity, respectively; p= 0.01). The RPA-LF fills the need for an effective diagnostic tool that will play a critical role in control interventions to reduce urban transmission of visceral leishmaniasis to humans.

691

IDENTIFYING VACCINE CANDIDATES FOR VISCERAL LEISHMANIASIS

Toolika Singh[1], Michaela Fakiola[2], Joyce Oommen[3], Jaya Chakravarty[1], Shyam Sundar[1], Jenefer Blackwell[3]

[1]Institute of Medical Sciences, Varanasi, India, [2]Cambridge Institute for Medical Research, University of Cambridge, Cambridge, United Kingdom, [3]Telethon Institute for Child Health Research, Perth, Australia

Leishmaniasis, a neglected tropical disease, prevalent in developing countries with 90% of them in Asia (Bangladesh, India, and Nepal), Sudan, Ethiopia and Brazil. The technical challenges and the complexity in the immunity against the parasites clearly contribute to the absence of vaccines. A major challenge in human vaccine design is to overcome variation in immune response in a genetically heterogeneous population. This is largely determined by genetic heterogeneity in processing and presentation of Ag to T cells, the outcome of which is dependent on binding of T cell epitopes to HLA class I and class II molecules that drive CD8 and CD4 T cell responses, respectively. In silico screening for putative epitopes binding to DRB1 molecules can identify multiple epitopes per single parasite antigen. Our quest here is to determine what actually occurs during the course of a complex infection *in vivo*. We are using in silico prediction tools in an effort to understand more about the processes that direct antigen selection and binding to different DRB1 molecules during natural leishmanial infection. This will be done in concert with analysis of naturally processed leishmanial peptides. A previously conducted GWAS and further sequence based haplotyping on an Indian population has indicated HLA class II as a major genetic risk factor for visceral leishmaniasis(VL) and revealed DRB1*13/14 and DRB1*15/16 as risk and protective alleles, respectively in VL. In a preliminary study, we have obtained data on leishmanial epitopes predicted to bind to DRB1*13/*14 risk vs DRB1*15/*16 protective class II molecules using the in-silico predictive tool NetMHCIIpan v2.1. Data for overlapping 9-mer epitopes has been generated for 43 known *Leishmania* antigens (antigens of diagnostic value, vaccine candidates) and we have found peptides exclusively binding to risk as well as protective group and also some differentially binding peptides. Functional validation of these peptides will be done by measuring immune response against these antigens in individuals carrying different allele group from endemic region in India. This will pave the way for appropriate vaccine candidates which can drive the immune response to protective response in genetically susceptible individuals.

692

USE OF CLINICAL PREDICTORS AND MOLECULAR DIAGNOSIS TO IDENTIFY THE SPECIES RESPONSIBLE FOR SNAKEBITE IN RURAL NEPAL

Sanjib K. Sharma[1], Ulrich Kuch[2], Patrick Höde[2], Laura Bruhse[2], Deb Pandey[2], **François Chappuis**[3], Emilie Alirol[3]

[1]B.P. Koirala Institute of Health Sciences, Dharan, Nepal, [2]Forschungszentrum Biodiversität und Klima (Bio+C), Frankfurt am Main, Germany, [3]Geneva University Hospital, Geneva, Switzerland

Snakebite is an important medical emergency in rural Nepal. Correct identification of the biting species is crucial for clinicians to choose appropriate treatment and anticipate complications. This is particularly important for neurotoxic envenomation which, depending on the responsible species, may not respond to antivenoms. Adequate species identification tools are lacking. This study used a combination of morphological and molecular approaches (PCR-aided DNA sequencing from swabs of bite sites) to determine the relative contribution of venomous and non-venomous species to the snakebite burden in southern Nepal. The study also investigated the performance of baseline patient history and clinical characteristics to distinguish between cobra (*Naja* spp.) and krait (*Bungarus* spp.) bites. Out of 749 patients admitted to one of the 3 study centres with a history of snakebite, the biting species could be identified in 194 (25.9%). Out of these, 87 had been bitten by a venomous species, most commonly *Naja naja* (n=42) and *Bungarus caeruleus* (n= 22). When both morphological identification and PCR/sequencing results were available, a 100% agreement was noted. Among patients bitten by venomous snakes, 71 (81.6%) presented with signs of envenomation including neurotoxic signs in 55 (77.4%). Being bitten at night (OR= ∞), while sleeping (OR= 56 [95%CI= 9.9-318.2]), indoors (OR= 9.4 [95% CI= 1.9-46.9]), and presenting with abdominal pain (OR= 23.4 [95% CI=3.8-142.5]) was associated with krait bite, and local signs of envenomation (e.g., edema) with bites by cobras and pit vipers. This study is the first to report the use of forensic genetics methods for snake species identification in a prospective clinical study, and to identify epidemiological and clinical features associated with krait and cobra envenomation in Nepal, thereby providing decisive guidance to improve patient care.

693

MULTIPLEX PCR DETECTION OF ENTEROPATHOGENS CAUSING TRAVELERS' DIARRHEA FROM SPIKED STOOL SMEARS ON WHATMAN FTA CARDS USING MULTIPLEX PCR

Michele Tisdale[1], David Tribble[2], Chansuda Wongsrichanalai[3], Jason Maguire[1], **Tahaniyat Lalani**[1]

[1]IDCRP, Portsmouth, VA, United States, [2]IDCRP, Bethesda, MD, United States, [3]United States Agency for International Development Regional Development Mission for Asia, Bangkok, Thailand

Molecular diagnostics offer significant advantages over conventional testing for enteropathogen detection in travelers' diarrhea. There is limited data on the performance of PCR assays using stool samples and smeared stool cards. We sought to determine the limits of detection (LoD) for diarrheal pathogens using a multiplex PCR assay on spiked stool samples, and spiked stool smeared onto Whatman FTA® Elute cards. The multiplex PCR panel used a combination of Luminex xTAG analyte-specific reagents (ASRs) and in-house primers to detect: enterotoxigenic *Escherichia coli* (ETEC), enteroaggregative *E coli* (EAEC), enterohemorrhagic *E coli* (EHEC), *Shigella spp.*, *Salmonella enteritidis, S typhimurium*, *Campylobacter spp.*, norovirus [GI, GII], *Giardia lamblia* and *Cryptosporidium spp.* LoD analysis was carried out by performing serial 1:10 dilutions, with the 4th and 5th dilution performed in triplicate. We evaluated the impact of prolonged storage of stool cards (1 and 3 months) on the LoD. The mean fluorescence intensity value for detection was ≥300. LoDs in spiked stool ranged from 10^2 to 10^4 cfu/g for bacterial pathogens, 10^2 to 10^3 pfu/g for Norovirus GI and GII respectively and 10^3 cysts/g for Giardia. Cryptosporidium could not be reliably detected in the stool or stool card. The LoD for ETEC, EHEC and *S enteritidis* were similar in the stool card and spiked stool, while the LoD for EAEC, *S typhimurium*, Shigella, Campylobacter and norovirus GII were 1 log higher in smeared stool cards compared to stool. Giardia had a lower LoD in the stool card (1 cyst/g). Significant variability (2-4 logs) in the LoDs was observed with prolonged storage of stool cards. LoDs for *S enteritidis* and ETEC increased from 10^1 cfu/g at 1 month to 10^3 cfu/g at 3 months, while EHEC decreased from 10^5 cfu/g to 10^1 cfu/g during the same time-points. *Campylobacter* increased from 10^4 cfu/g at baseline to 10^5 cfu/g at 1 month and could not be detected at 3 months. Further evaluation of the multiplex PCR assay using spiked specimens and diarrheal samples is needed before deployment in field studies.

694

A HOSPITAL-BASED SURVEY ON HUMAN BRUCELLOSIS ITS ASSOCIATED FACTORS IN KANO METROPOLIS, KANO STATE-NIGERIA 2011

Aisha N. Isa[1], Emmanuel C. Okolocha[2], Junaid Kabir[2]

[1]Federal Ministry of Agriculture and Natural Resources, Abuja, Nigeria, [2]Ahmadu Bello University, Zaria, Kaduna State, Nigeria

The global burden of human brucellosis remains enormous; it causes more than 500,000 infections per year. It is one of the most widespread zoonotic diseases globally. Brucellosis is a multi-systemic, acute to chronic, disease characterized by fever, headache, joint pains, musculo-skeletal pains, sweating, malaise and body wasting. It is a severe debilitating disease that requires prolonged treatment, if untreated can result in permanent disability and loss of productivity. Human brucellosis presents a great variety of clinical manifestations making it difficult to diagnose clinically. In some endemic areas every case of human fever of unknown origin is assumed to be due to brucellosis. Therefore, the diagnosis must be confirmed by laboratory tests. A hospital-based survey on human brucellosis was undertaken to determine its prevalence, associated factors and the extent of missed diagnosis. A descriptive study and laboratory analysis were conducted. A suspected case was defined as any patient $\geq$ 5years with fever (>37.5°C) plus any two of the clinical signs suggestive of human brucellosis. Socio-demographic features, clinical information and assessment of potential risk factors were obtained through questionnaires. Their sera were screened with Rose Bengal plate test (RBPT) and positive cases were subjected to Enzyme Linked Immunosorbent Assay (ELISA) to confirm and detect evolution of the infection. A total of 250 suspected cases were enrolled and 50(20%) were confirmed positive. There were 131 males and 119 females. Mean age was 24years$\pm$16. Sensitivity and specificity were 90% and 85%. Of the 50 cases, 31(62%) were males; clinical signs consistent were recurrent fever (p 0.04), cough/sneeze (p 0.02) and osteomyelitis (p 0.00). Risk factors for human brucellosis were consumption of fresh milk (p 0.02) and local yoghurt kindirmo (p 0.00), keeping goats (p 0.00), assisting animal parturition (p 0.01), processing and eating raw meat (p 0.03 and p 0.00). Thirty six (72%) of the 50 cases were positive by IgG thus chronic infection. Diagnosis of human brucellosis is missed in hospitals in Kano. Risk factors identified were processing and consumption of unprocessed milk and meat; keeping goats and assisting parturition. Authorities should educate the populace on the risks of this disease and its prevention; physicians should raise the index for diagnosis of human brucellosis in patients that present with signs suggestive of it.

695

REAL-TIME QUANTITATIVE PCR SURVEILLANCE OF GASTROINTESTINAL PARASITES IN A SYMPTOMATIC RURAL ARGENTINIAN POPULATION: INITIAL RESULTS OF THE LATIN AMERICAN MULTICENTER PARASITE STUDY (LAMPS)

Rojelio Mejia[1], Paola Vargas[2], Rebecca Jeun[1], Alejandro Krolewiecki[2], Marisa Juarez[2], Pamela Cajal[2], Julio Nasser[2], Peter Hotez[1], Rubén Cimino[2]

[1]National School of Tropical Medicine, Houston, TX, United States, [2]Universidad Nacional de Salta, Salta, Argentina

There are over 2 billion people infected with gastrointestinal (GI) parasites. Diagnosis of GI parasites relies on stool microscopy that has low diagnostic sensitivity and specificity. To better understand the parasitic etiology of abdominal symptoms, we implemented our rapid, high throughput, multi-parallel quantitative real-time PCR (qPCR) for the 8 common GI parasites including the helminths, *Ascaris lumbricoides (Al), Ancylostoma duodenale (Ad), Necator americanus (Na), Strongyloides stercoralis (Ss), Trichuris trichiura (Tt)* and protozoa, *Cryptosporidium parvum (Cp), Entamoeba histolytica (Eh)* and *Giardia lamblia (Gl).* This assay was used to analyze stool samples collected from 99 patients seen in a rural Argentinian clinic. For *Al*, qPCR identified (56.6%) positives whereas McMaster's microscopy technique identified (47.5%) with 91.3% sensitivity and 90.5% negative predictive value (NPV). For hookworm, there was 37.4% detected by qPCR compared to 22.2% by microscopy ($p < 0.05$), with a 95.5% sensitivity and 98.4% NPV. Hookworm ova are indistinguishable by microscopy, but qPCR is species specific. While *Na* was the predominate hookworm detected, *Ad* DNA was detected in higher concentrations (0.61 versus 119.6 fg/µL, $p < 0.0001$). This has important implications, since *Ad* is more aggressive in causing anemia. The difference between qPCR and microscopy was dramatically seen for *Gl* (63.6% versus 8.1%) with 55 additional positives for *Gl* ($p = 0.001$). For *Ss*, qPCR identified (21.2%) positives whereas microscopy identified (3.0%)($p < 0.05$) with 100% sensitivity and negative predictive value. qPCR was also able to detect polyparasitism by a factor of 7 compared to microscopy ($p < 0.05$). We have deployed a quantitative molecular based system that has improved diagnostic accuracy than stool microscopy. This is the first time this assay has been used in Argentina and has shown the prevalence of GI parasite infections in symptomatic patients. The results will help refine treatment options on a public health scale and lead to better health outcomes in endemic settings.

696

UTILITY OF PROVISIONAL DIAGNOSES OF DENGUE FROM A SENTINEL-ENHANCED SURVEILLANCE SYSTEM IN PONCE, PUERTO RICO

David Noyd[1], **Tyler M. Sharp**[1], Leslie Camacho[2], Olga Lorenzi[1], Janice Perez-Padilla[1], Aidsa Rivera[1], Luisa Alvarado[2], Kay M. Tomashek[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]San Lucas Episcopal Hospital, Ponce, PR, United States

Dengue and a number of other acute febrile illnesses (AFI) present with similar signs and symptoms. The unavailability of sensitive and specific rapid diagnostic tests poses a significant challenge to early and accurate diagnosis of dengue because early identification is important for management of patients to reduce mortality. We used data from the first year of a Sentinel Enhanced Dengue Surveillance System (SEDSS) in Ponce, Puerto Rico between May 7, 2012 and May 6, 2013 to evaluate the accuracy of clinicians' diagnoses of patients presenting with an AFI (N = 2,027) in a dengue-endemic area. A classification system was developed, and diagnoses were grouped into syndromes defined as dengue, influenza, viral infection, gastrointestinal, respiratory, genitourinary, and other. Laboratory diagnostic testing was performed for 23 pathogens associated with AFI, including the four dengue viruses (DENV-1-4). Of 1,152 cases in which an infecting pathogen was identified, 579 (50.3%) were DENV-positive, 301 (26.1%) were positive for influenza, and 272 (23.6%) were positive for another pathogen. Among the DENV-positive cases, 243 (42%) received a provisional clinical diagnosis of dengue, while 183 (32%) and 60 (10%) were diagnosed as viral infection and respiratory illness, respectively. At initial clinical presentation, the DENV-positive cases with a provisional diagnosis of dengue had a higher proportion with rash (53% vs. 0.38%; p = 0.001) and diarrhea (44% vs. 32%; p = 0.005), more pronounced leukopenia (3,100 vs. 4,600; p < 0.001), thrombocytopenia (93,203 vs. 155,937; p < 0.001), and elevated aspartate transaminase (175 vs. 112; p = 0.001). Multiple logistic regression modeling and stratification analysis by age group will be performed to determine the clinical and laboratory factors most associated with a clinical and laboratory diagnosis of dengue. This will both inform physicians in their diagnostic approach to dengue and facilitate the use of provisional diagnoses as an early detection tool for increases in dengue in Puerto Rico.

697

CARDIAC INVOLVEMENT IN PEDIATRIC DENGUE; A SERIAL ECHOCARDIAGRAPHIC STUDY

Anon Srikiatkhachorn[1], Tawatchai Kirawittaya[2], In-Kyu Yoon[3], Sharone Green[1], Francis A. Ennis[1], Robert V. Gibbons[4], Stephen J. Thomas[5], Alan L. Rothman[6], Siripen Kalayanarooj[2]

[1]University of Massachusetts Medical School, Worcester, MA, United States, [2]Queen Sirikit National Institute for Child Health, Bangkok, Thailand, [3]Armed Forces Research Institute of Medical Sciences-Virology, Bangkok, Thailand, [4]United States Army Institute of Surgical Research, San Antonio, TX, United States, [5]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [6]University of Rhode Island, Providence, RI, United States

Dengue is the most prevalent vector-borne viral infection of humans. There have been case reports of severe cardiac involvement including myocarditis in dengue, although the true incidence is not well defined. To better characterize cardiac involvement we performed daily echocardiographic studies in children with suspected dengue from 2010 to 2012 at a tertiary care center in Bangkok, Thailand. Plasma levels of cardiac troponin-T were measured daily. We analyzed 180 confirmed dengue cases classified as dengue fever (DF) and dengue hemorrhagic fever (DHF) according to the 1997 WHO case definitions. There were 119 cases of DF, and 12, 27, 21, and 1 cases of DHF grade 1, 2, 3, and 4, respectively. On the day of presentation (approximately 4.5 days of illness), DHF cases had significantly lower stroke volume, left ventricular end-diastolic volume, ejection fraction, and cardiac index, and higher systemic vascular resistance compared to cases with DF (p< 0.001 to < 0.05). Flow and tissue Doppler studies demonstrated a decreased early diastolic component of flow at the mitral valves as well as decreased tissue plane excursion of the mitral valves in DHF cases. These early differences were attributed to findings from DHF cases with ultrasound-documented plasma leakage at this time point. Serial studies showed that differences in hemodynamic and cardiac functional indices between DF and DHF were most pronounced around the time of deferevescence. There were no clinically evident cases of cardiac failure in this study. An abnormal ejection fraction (EF <55%) was observed in 6%, 9%, 18%, and 32% of cases of DF, DHF grade 1, DHF grade 2, and DHF grades 3/4, respectively (p<0.001). However, the abnormal EF was transient and usually coincided with the development of plasma leakage. No cases with abnormally elevated troponin-T levels were identified. We conclude that cardiac involvement is uncommon in pediatric dengue and is not a major contributing factor for dengue shock syndrome. Subtle changes in cardiac function are common but appear to be transient and may reflect plasma leakage and volume status.

698

CHAGAS DISEASE TRANSMISSION AND CARDIAC MANIFESTATIONS AMONG TEXAS BLOOD DONORS

Melissa N. Garcia, Kristy O. Murray, Laila Woc-Colburn, Maria Elena Botazzi, Rodion Gorchakov, Christie Ballantyne, Peter J. Hotez, David Aguilar

Baylor College of Medicine, Houston, TX, United States

Although well established as an important cause of morbidity and mortality in Latin America, Chagas disease is increasing in recognition as a potential cause of heart disease in the United States. Screening of blood donors in the greater Houston area for Chagas disease (*Trypanosoma cruzi* infection) began in 2007. The transmission mechanisms and potential cardiovascular manifestations of these *T. cruzi* positive individuals have not been previously studied. An one-time assessment of Houston area blood donors that screened positive from 2008-2012 for *T. cruzi* infection (n=30) included 1) blood draw for confirmatory *T. cruzi* diagnostic testing, 2) a questionnaire to evaluate source of infection, cardiac symptoms and health co-morbidities, 3) high-sensitivity troponin T biomarker evaluation, 4) an electrocardiogram, and 5) an echocardiogram if electrocardiogram was abnormal. We found 57% (17/30) of blood donors had two or more positive tests confirming infection. Of those with confirmed infection, 41% (7/17) had an electrocardiographic abnormality consistent with Chagas cardiomyopathy. In addition, 36% (6/17) were suspected to be locally acquired cases. High-sensitivity troponin serum levels were increased in a linear manner with cardiac severity. Cardiologists should consider the changing transmission dynamics associated with Chagas disease in the southern United States and should consider Chagas disease in patients who may have clinically-compatible electrocardiogram or cardiomyopathy, even if the patient has no history of residing in a Chagas-endemic country.

699

EXAMINATION OF 2006-2013 MALARIA INCIDENCES IN RELATION TO THE SCALING OF PREVENTATIVE CONTROL INTERVENTIONS IN MULEBA DISTRICT IN NORTHWEST TANZANIA

Shabbir Lalji[1], Osiah Mwaipape[1], Rose Lusinde[1], Anold Mutafungwa[1], Rajeev Colaco[1], Renata Mandike[2], Jeremiah Ngondi[1]

[1]RTI International, Dar-es-Salaam, United Republic of Tanzania, [2]National Malaria Control Programme, Dar-es-Salaam, United Republic of Tanzania

Following outbreak of malaria in Muleba district in 2006, intensive malaria control interventions, including Indoor Residual Spraying (IRS), and distribution of LLINs were introduced. IRS was introduced as an outbreak preemptive measure in selected areas of the district in 2007 and 2008, with gradual scale-up of one round of IRS per year covering the entire

district until 2011. About 80,000 LLINs were distributed to under-fives in 2009 and 170,000 nets distributed in early 2011 to the remaining population. However, due to universal attainment of LLINs in 2011, as well as limited resources, IRS was scaled down in 2012-2013, with the last round targeting only 25% (17,000 house structures) of the district. After 2011, no significant efforts have also been made to keep-up LLIN coverage. We compared health facility based malaria incidence per 1000 population of respective village catchment areas from Jan 2006-May 2013, a period in which a total of 352,488 out-patient malaria cases were recorded. District incidence in 2006 (pre-control period) was 118 per 1000, with the rate declining to its lowest level of 37 per 1000 in 2010. This peak decrease of 67% in malaria incidence at 2010 compared to 2006 was most likely due to intensification of effective multiple control interventions over that period. However from 2011 onward, scale down and/or low maintenance of control interventions, coupled with reported stock-outs of ACTs, and low net use (68%-THMIS 2011-12) likely contributed to a rise in malaria incidence; by 2013, increasing to 181 per 1000 (adjusted for 12 month period). The 2013 rate was 35% higher than the pre control era of 2006. Muleba's case suggests that scaling-down malaria control efforts has resulted in loss of initial gains in controlling malaria. This could have serious implications with possible rebound of malaria to pre-intervention levels as well as frequent malaria outbreaks. Once the control of malaria has reached to manageable levels, it is important to advocate for effective monitoring and response so as to sustain the fight against malaria.

700

FOCAL SCREENING AND TREATMENT (FSAT) CAMPAIGN IN FOCI OF MALARIA TRANSMISSION: IMPACT ON MALARIA PREVALENCE AND COMPLEXITY

Amrish Y. Baidjoe[1], Gillian Stresman[2], Jennifer Stevenson[2], Wycliffe Odongo[3], Chrispin Owaga[3], Elizabeth Marube[3], Victor Osoti[3], Euniah Makori[3], Shehu Shagari[3], Lynn Grignard[2], Chris Drakeley[2], Jonathan Cox[2], Teun Bousema[1]

[1]Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Kenya Medical Research Institute, Centre for Global Health Research, Kisumu, Kenya

Mass screening and treatment campaigns have had limited success in curbing malaria transmission, most likely due to the prevalence of subpatent infections missed using the field based diagnostic tools. It has been shown that subpatent malaria infections are more likely to occur in households where patent infections are identified. Therefore it is possible that a more focal approach to treatment campaigns using patent infections as a marker for the presence of a subpatent reservoir may be more effective at reducing the infectious reservoir in humans. To test this strategy, a focal screening and treatment (FSAT) intervention in foci of malaria transmission (prevalence 18,6%, 95% C.I. 10.8-26.5%) was conducted in the western Kenyan highlands as part of a larger cluster randomized trial. All consenting individuals under 15 years old or febrile adults residing within the foci were tested for malaria with a rapid diagnostic test (RDT) and if found positive all individuals residing in the compound received a curative dose of artemisinin combination therapy. Blood spots on filter paper were collected from all household members (N=2083), regardless of RDT result. To assess the impact of FSAT, parasite prevalence and complexity using nested polymerase chain reaction and merozoite surface protein-2 genotyping was determined. The impact of the FSAT approach on parasite prevalence and allelic diversity was assessed with two follow-up cross-sectional surveys at 2-month intervals post intervention. Of the compounds within the intervention foci, 168 of 406 (41.4%) households sampled received treatment. Twenty-seven households declined or had no individuals present during time of sampling. Initial results indicate that, at baseline, PCR prevalence in compounds with a patent infection and therefore targeted for treatment was 34.0% compared to 11.1% in those that had no patent infections ($p<0.0001$) with the FSAT approach successfully identifying 78.3% of parasite carriers. This strategy could provide a useful and operationally attractive alternative to detecting subpatent infections in foci of infection.

701

SPATIAL PATTERNS OF MALARIA TRANSMISSION OVER ONE YEAR IN A CLINIC CATCHMENT AREA OF CHONGWE DISTRICT, ZAMBIA

German Henostroza[1], Jessie Pinchoff[2], Jessie Pinchoff[3], Sisa Hatwiinda[4], Busiku Hamainza[5], Hawela B. Moonga[5], Frank C. Curriero[3]

[1]University of Alabama at Birmingham, Birmingham, AL, United States, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [4]Centre for Infectious Disease Research in Zambia, Lusaka, Zambia, [5]National Malaria Control Center, Lusaka, Zambia

As malaria transmission declines, it may be more cost-effective and efficient to focus control efforts on high-risk areas. Describing spatial patterns of malaria transmission may identify opportunities to develop and improve current interventions. Individuals testing positive for malaria at a single clinic in Chongwe District, Zambia, were approached for enrollment in the study. These index cases were administered a questionnaire and a blood sample was taken for a rapid diagnostic test (RDT) and microscopy. Upon enrollment their homes were visited, all household contacts were enrolled, administered a questionnaire, and gave a blood sample for RDT and microscopy. GPS coordinates were recorded at each household. If an individual from a previously enrolled household sought care, these were considered re-infection households. Spatial patterns of malaria in this clinics catchment area were analyzed using SaTScan for cluster detection and programs from R statistical software to describe patterns of spatial clustering. From June 2012 to June 2013 a total of 472 index cases and 1,901 household contacts (43% RDT positive) were enrolled. Two statistically significant space-time clusters of index case households were identified controlling for age and gender; one in December 2012 and one in January 2013 (the peak transmission season). No clustering of RDT positive household contacts was identified; factors associated with being an RDT positive household contact included distance to the clinic, socioeconomic status, and bednet ownership ($p<0.05$ for all comparisons). In conclusion, transmission in this area appears to be high with a seasonal peak in incidence during the rainy season. Two space-time clusters of index case households were detected during the peak transmission season in close proximity to the clinic. No clustering of malaria comparing household contacts was detected likely because of high transmission in the entire catchment area.

702

CESSATION OF COTRIMOXAZOLE PROPHYLAXIS IN HIV EXPOSED CHILDREN DOES NOT INCREASE THE INCIDENCE OF MALARIA AND OTHER MORBIDITIES

Nyanyiwe M. Mbeye[1], Feiko O. ter Kuile[2], Kamija Phiri[1]

[1]Malawi College of Medicine, Blantyre, Malawi, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

HIV-exposed infants born to mothers with HIV should receive cotrimoxazole prophylaxis (CPT) until HIV infection can be excluded and the child is no longer exposed through breastfeeding. As daily CPT provides prophylaxis against malaria, it could modulate the development of malaria-specific immunity and increase the incidence of malaria after it is stopped (a rebound effect). To determine if this is the case, we investigated the incidence of malaria and other morbidities during and after CPT in the first two years of life in HIV exposed children in southern Malawi. A cohort of 500 HIV exposed children on CPT for 12 months from the age of 6 weeks to one year and 500 non-HIV exposed children not on CPT were followed until their second birthday. HIV exposed children were recruited at the Prevention of Mother to Child Transmission (PMTCT)

HIV clinic while location and age-matched non-HIV exposed children were recruited from the same population. The incidence of malaria, all-cause morbidity, admissions and mortality was compared during the first and second year of life in the two study groups using multivariate, negative binomial regression analyses. In year-1, the incidence of uncomplicated malaria in HIV exposed children was 65% lower compared to the non-HIV exposed group (IRR = 0.35, 95% CI 0.25, 0.49, p<0.001). In year-2 the incidence of malaria in the HIV exposed group was similar to that in non-HIV exposed group (IRR 0.94, 95% CI: 0.53, 1.68, p=0.839) among the first 315 children that completed the follow-up period. The same pattern was observed for all-cause morbidity and hospital admissions. CPT was associated with marked reductions in the incidence of uncomplicated malaria, all-cause morbidity and hospital admissions during the period in which it was given. The follow-up in year-2 is on-going but preliminary results suggest that the incidence of malaria does not increase after cessation of CPT at around 14 months of age.

703

STANDARDIZED MULTIDISCIPLINARY METHOD FOR THE EVALUATION OF THE EFFECTIVENESS OF MALARIA CONTROL INTERVENTIONS

Thomas Kesteman[1], Franck Remoue[2], Milijaona Randrianarivelojosia[1], Cédric Pennetier[3], Martine Audibert[4], Michel Etchepare[5], Marc Egrot[3], Christophe Rogier[1], Palevalut Scientific Committee[6]

[1]Institut Pasteur de Madagascar, Antananarivo, Madagascar, [2]Institut de Recherche pour le Développement, Montpellier, France, [3]Institut de Recherche pour le Développement, Cotonou, Benin, [4]Centre d'Etudes et de Recherches pour le Développement International, Clermont-Ferrand, France, [5]Independent, Paris, France, [6]Madagascar

In a global context of international funding plateauing, control programs are looking for innovative options to better perform with a constant budget. In absence of groundbreaking new control tools, the only option is to make the best use of the control measures that are already available. In order to guide policy making, we propose to carry out a broad and comprehensive evaluation of interventions deployed in a given setting, including an evaluation of their effectiveness and the identification of the key determinants affecting their effectiveness. We wrote and selected 20 Standard Operating Procedures (SOP) through a multi-country multidisciplinary workshop, including experts from Belgium, Benin, Cameroon, Côte d'Ivoire, France, Madagascar and Niger. These SOP cover the following fields: analysis of health systems (1), anthropology (1), biological diagnosis (4), drugs resistance (2), entomology (3), epidemiology (4), health economics (2), and immunology (3). All 20 SOP are combined in a single toolbox that is being implemented in Benin and Madagascar in 2014. Preliminary results will be presented. For each malaria control intervention the following indicators are evaluated: coverage, protective effectiveness against infection, protective effectiveness against morbidity, cost-effectiveness, socio-anthropological determinants of effectiveness, entomological determinants of effectiveness (vector behavior, insecticide resistance) if applicable, and *in vivo* and *in vitro* measure of antimalarial drug resistance. Results also include an analysis of health systems and management of malaria control in general. The toolbox -named PALEVALUT- will be further reviewed before and after implementation in Cameroon, Côte d'Ivoire and Niger in 2015. The whole tool will be soon available with free access on the internet.

704

CHANGING HEALTH CONDITIONS AND THE DECLINE OF ALL-CAUSE UNDER-FIVE MORTALITY IN RWANDA 2000-2010: A DECOMPOSITION ANALYSIS

Lia S. Florey[1], Rebecca Winter[1], Thomas Pullum[1], Cameron Taylor[1], Corine Karema[2], J. Eric Tongren[3], Carrie Nielsen[4], S. Rene Salgado[5]

[1]ICF International - The DHS Program, Rockville, MD, United States, [2]National Malaria Control Program, Kigali, Rwanda, [3]Centers for Disease Control and Prevention - President's Malaria Initiative, Kigali, Rwanda, [4]Centers for Disease Control and Prevention - President's Malaria Initiative, Atlanta, GA, United States, [5]United States Agency for International Development - President's Malaria Initiative, Washington, DC, United States

All-cause child mortality (ACCM) has declined rapidly over the past decade in Rwanda, from 196 deaths per 1,000 live births during 1996-2000 to 76 deaths per 1,000 live births during 2006-2010. Rapid development and improvements in many socioeconomic and health indicators between 2000 and 2010 are likely to have played a role in determining these declines. For example, the percentage of households with improved toilets increased from 9% to 72%, the percentage of households with mosquito nets rose from 8% to 94%, and the percent of children whose mothers used ITNs increased from 5% to 77%. In this analysis, decomposition models were used to describe the decline in ACCM between two Demographic and Health Surveys (DHS) (Rwanda DHS 2000 and Rwanda DHS 2010). The relative importance of changes in the distribution of key socioeconomic and health-related variables (coverage) and changes in the magnitude of association between these variables and ACCM (coefficients) across these surveys was examined. The model explains a decline of 83 deaths per 1,000 live births from 2000 to 2010, or 69% of the reduction observed from the surveys. Results show that the combined effect of all of the changes in intervention coverage explained almost all (99%) of the modeled reduction in ACCM. Holding other factors constant, the observed increase in household bednet ownership could have explained as much as 45% of the modeled decline in ACCM between 2000 and 2010, and 31% of the observed decline in ACCM from survey data. The increasing percentage of children whose mothers used ITN between 2000 and 2010 could have explained an additional 4.2% of the modeled reduction in ACCM, presumably through reductions in neonatal mortality. Improvements in coverage of antenatal care and increasing birth intervals could have explained an additional 13.4% and 3.1% of total modeled ACCM decline, respectively. Changes in the distribution of household wealth and of multiple births between surveys would have led to small but significant increases in ACCM, holding other variables constant. These results clearly show the important role of malaria control interventions in the reduction of child mortality in Rwanda.

705

VARIATIONS IN MALARIA EPIDEMIOLOGY IN RELATION TO VECTOR CONTROL COVERAGE IN NINE DISTRICTS OF UGANDA

Tarekegn A. Abeku, Michelle Helinski, James Ssekitooleko, Matthew J. Kirby, Irene Kyomuhangi, Godfrey Magumba, Sylvia R. Meek

Malaria Consortium, London, United Kingdom

Malaria has been a major public health problem in Uganda. The disease is highly endemic in 95% of the country where 90% of the population live. A scale up of malaria control interventions in the past decade coupled with environmental, social and economic factors appears to have contributed to a changing pattern of the epidemiology of the disease in the country. This study was part of a larger study on the distribution of insecticide resistance and resistance management. The aim of this component of the study was to understand the impact of varying coverage of vector control interventions on the observed epidemiological pattern of malaria

in different groups of districts. Three groups of nine districts were selected among all highly endemic districts that were in existence since 2001, using criteria based on coverage with long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS): 1) Districts that had undergone several rounds of IRS (Apac, Gulu, Pader), 2) Districts where LLINs had been distributed but no IRS had taken place (Kayunga, Kiboga, Mbale), and 3) Districts that had not received IRS or LLINs as part of large campaigns at the time of selection (Bugiri, Mayuge, Soroti). Forty-five cluster were sampled in the nine districts in which a cross-sectional survey was conducted during September-October 2012 transmission season. A total of 528 interviews were conducted in the 45 clusters, an average of 12 households per cluster. Blood samples were gathered from all household members and mosquitoes were collected using the pyrethrum spray catch method. *Anopheles gambiae* s.l. and A. funestus s.l. were the main vectors collected. Prevalence of *Plasmodium falciparum* and density of malaria vectors varied between groups of districts with different levels of vector control coverage. The highest prevalence was observed in the unsprayed group with historically low coverage with LLINs. Pyrethroid resistance was widespread in nearly all districts. The role of vector control interventions is discussed in the light of observed variations in malaria epidemiology.

706

PERCEPTIONS OF COMMUNITY-LED TOTAL SANITATION ON SANITATION BEHAVIORS IN RURAL ZAMBIA: A QUALITATIVE STUDY

J. Joseph Lawrence[1], Kojo Yeboah-Antwi[2], Godfrey Biemba[3], Pavani K. Ram[4], Nicolas Osbert[5], Davidson H. Hamer[3]

[1]Zambia Center for Applied Health Research and Development, Lusaka, Zambia, [2]Center for Global Health and Development, Boston University and Department of Global Health, Boston University School of Public Health, Boston, MA, United States, [3]Zambia Center for Applied Health Research and Development and Center for Global Health and Development, Boston University, Lusaka, Zambia, [4]University at Buffalo, Buffalo, NY, United States, [5]UNICEF, Lusaka, Zambia

Despite emerging initiatives in the sanitation sector, inadequate sanitation remains a leading global contributor to morbidity and mortality in children. The government of Zambia, UNICEF, and partners are engaged in the rollout of an ambitious hygiene and sanitation program aimed at reaching 3 million new users of improved sanitation hand washing practices. One of the key approaches used to reach this objective is Community Led Total Sanitation (CLTS), a grassroots, subsidy-free strategy with the goal of changing sanitation practices to create open defecation free (ODF) villages. During CLTS, community members participate in activities that lead them to realize and declare that they are "eating each other's stool". This qualitative study explores community members' and stakeholders' sanitation behaviors, knowledge and perceptions during early CLTS implementation in Zambia. We conducted 67 key informant interviews and 24 focus group discussions with community members and other WaSH stakeholders in 6 districts in Zambia who were selected using purposive sampling based on recommendations from project staff. The study was conducted in July and December of 2013, 12 to 18 months after initiation of CLTS implementation. According to key informants, triggering activities elicit a strong emotional response involving shame, disgust and peer pressure which persuades individuals to build and use latrines. Pride and dignity were also reported as important influential factors, as individuals and communities become empowered. Traditional leaders and community Sanitation Action Groups have strong hierarchical influence that is also persuasive in changing behaviors. Respondents frequently mentioned that children help to influence their families to improve sanitation behaviors. Overall, participants reported an increase in latrine construction and usage after triggering; however, poor (e.g. rocky or sandy) soil conditions and taboos prohibiting certain family members from sharing the same toilet act as barriers in many areas. CLTS results in powerful individual and community emotional responses that serve to encourage construction and use of latrines and adoption of improved hygiene practices. This formative research suggests that CLTS has potential to be an effective approach for improving sanitation beliefs and behaviors in Zambia, which in turn may result in improved child health.

707

FACTORS ASSOCIATED WITH PUPIL LATRINE USE IN KENYAN PRIMARY SCHOOLS

Joshua V. Garn, Carey D. Drews-Botsch, Matthew C. Freeman

Emory University, Atlanta, GA, United States

Little empirical data exist on how the type, design, and maintenance of school latrines affect pupil latrine use. The purpose of this study was to characterize how school sanitation conditions are associated with latrine use patterns in 60 primary schools in Nyanza Province, Kenya. We conducted a longitudinal assessment, using structured observations to measure both latrine conditions and latrine use during the 30 minute morning break. We modeled the association between pupil to latrine ratio and latrine use (pupil used a latrine vs. not), using multivariable logistic regression. Lower pupil to latrine ratios were associated with increased latrine use, with the odds of use more than doubling when comparing schools with the lowest pupil to latrine ratios (<15:1) to schools with the highest ratios (>75:1; odds ratio=2.18, 95% CI: 1.69-2.80). We also modeled the association between different latrine characteristics and the number of uses at specific facilities, using multivariable negative binomial regression. Pupils preferred to use newer latrines over older latrines (incidence rate ratio (IRR)=1.13, 95% CI: 1.01-1.26), and also preferred ventilate improved pit latrines (IRR=1.15, 95% CI: 1.00-1.31) and urinals (IRR=1.96, 95% CI: 1.57-2.44) over prefabricated plastic latrines (IRR=0.68, 95% CI: 0.54-0.85) and traditional pit latrines (referent). An increased number of latrines in a block (a group of conjoined latrines) was associated with increased use at that block, although the increase in use was not proportional to the block's added capacity (IRR for two doors compared to one=1.13, 95% CI: 0.96-1.33; IRR for four doors=1.71, 95% CI: 1.39-2.10; IRR for six or more doors=2.26, 95% CI: 1.73-2.93). We found some evidence suggesting latrine dirtiness was a deterrent to pupil latrine use, although the 95% CI included one (IRR=0.91, 95% CI: 0.82-1.02). Our study provides insights into factors affecting latrine use, potentially leading to a better allocation of resources for school sanitation, with the end goal to improve pupil's health and educational outcomes.

708

THE IMPACT OF HOUSEHOLD IMPROVED SANITATION ON HUMAN FECAL EXPOSURE IN RURAL INDIA: APPLICATION OF MICROBIAL SOURCE TRACKING TECHNIQUES

Mitsunori Odagiri[1], Alexander Schriewer[1], Pravas R. Misra[2], Pinaki Panigrahi[3], Stefan Weurtz[1], Tom Clasen[4], **Marion W. Jenkins**[1]

[1]University of California Davis, Davis, CA, United States, [2]Asian Institute of Public Health, Bhubaneswar, India, [3]University of Nebraska Medical Center, Omaha, NE, United States, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom

In India, where 626 million mostly rural people practice open defecation and 535,000 children under age five die each year from diarrhea, improved sanitation is clearly necessary to reduce fecal exposure. As part of a large cluster randomized controlled sanitation trial in Odisha, India, to measure the impact of household latrines on diarrhea diseases, human fecal contamination and exposure in intervention and control communities was measured and compared using microbial (fecal) source tracking (MST) methods based on host specific genetic markers of the gut organism Bacteroidales. MST is an emerging approach to discriminate and quantify human and other animal sources of fecal contamination. The goals of this study were (1) to measure levels of total, human and animal fecal contamination in the public domain (improved and unimproved water sources) and the domestic household domain (household stored drinking water, mother and child hand rinses) of fecal-oral disease transmission, and (2) to compare observed levels of total and human

fecal contamination in intervention and control villages for a better understanding of pathways by which latrines reduce exposure in rural India. In intervention (n = 30) and control (n = 30) villages, 20-L samples were collected from improved (public deep and private shallow tube wells for drinking, n = 209) and unimproved (community ponds for bathing and hygiene activities, n = 94) water sources along with samples from 5 to 6 households per village, comprising 300-mL of household stored drinking water (n = 348), and hand rinses of mothers (n =349) and children (n = 346), during the monsoon seasons of 2012 and 2013, after the intervention had ended. After concentration by filtration, total-, human-, and ruminant-associated markers were measured using quantitative PCR assays validated in India. Our results show that despite relatively high detection rates of total Bacteroidales in the public domain (55 to 100%), human-associated markers were rarely detected (1 to 5%). In contrast, detection rates of human-associated markers were significantly higher in the domestic domain (18 to 21%) while total Bacteroidales detection rates were lower (53 to 70%). We present and discuss results of in-depth analyses of observed levels of human fecal exposure, including effects of household latrines on the different pathways tested in the public and domestic domains of disease transmission in study communities.

709

IMPACT OF A COMMUNITY-LED TOTAL SANITATION INTERVENTION ON CHILD HEALTH IN RURAL MALI: EVIDENCE FROM A CLUSTER RANDOMIZED CONTROLLED TRIAL

Amy J. Pickering[1], Maria Laura Alzua[2], Habiba Djebbari[3]

[1]Stanford University, Stanford, CA, United States, [2]University of La Plata, Argentina, La Plata, Argentina, [3]Aix-Marseille University, Marseille, France

Globally 2.5 billion people lack access to an improved sanitation facility; only 15% of rural households use improved sanitation in Mali (JMP 2014). Community-led total sanitation (CLTS) uses participatory approaches to mobilize communities to build their own toilets, facilitate sustained behavior change, and eliminate open defecation. Although CLTS has been implemented in over 50 countries, there is a lack of rigorous and objective data on its impacts on sanitation behaviors and child health. This cluster-randomized trial evaluated a CLTS program implemented by the government of Mali with support from UNICEF among 121 villages from the Koulikoro district of Mali. Household survey data (N=4299) collected 1.5 years post intervention delivery revealed that CLTS almost doubled access to private latrines (65% *vs.* 35%), as well as reduced open defecation rates by over 70% among adults and by 50% among children (p<0.001). CLTS households were half as likely to have human feces observed in courtyards (p<0.001). Latrines in CLTS communities were 3 times more likely to have soap and have a cover over the pit, as well as 20% less likely to have flies visible inside (p<0.001). Among children under five, CLTS did not reduce the case definition of diarrhea (relative risk [RR] 0.96, 95% CI 0.80-1.16), although risk of loose stool as measured by an image chart was reduced by 24% for those children not exclusively breastfeeding (RR 0.76, 95% CI 0.59-0.98). When accounting for baseline height, children under five in CLTS villages were taller (+0.16 height-for-age Z-score, 95% CI 0.0-0.32) and less likely to be stunted (RR 0.87, 95% CI 0.75-1.0). Improvements in child weight (+0.09 weight-for-age Z-score, 95% CI -0.04 -0.21) and a reduction in the proportion of children underweight (RR 0.86, 95% CI 0.71-1.04) were observed but were not statistically significant. This study provides evidence that a pure behavioral intervention with no monetary subsidies substantially increased access to sanitation facilities in rural Mali. CLTS may have improved child growth through pathways other than preventing diarrhea, such as lessening the subclinical condition of environmental enteropathy through potential reduced exposure to environmental fecal contamination.

710

THE EFFECTIVENESS OF A RURAL SANITATION INTERVENTION ON HEALTH AND ORISSA, INDIA: A CLUSTER-RANDOMIZED, CONTROLLED TRIAL

Thomas Clasen[1], Sophie Boisson[1], Parimita Routray[1], Belen Torondel[1], Marion Jenkins[2], Matthew Freeman[3]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]University of California Davis, Davis, CA, United States, [3]Rollins School of Public Health, Emory University, Atlanta, GA, United States

We undertook a cluster-randomized, controlled trial to assess the effectiveness of a rural sanitation intervention under the Government of India's Total Sanitation Campaign (TSC) to prevent diarrhoea, child malnutrition and soil transmitted helminth infection. 100 rural villages in Orissa, India were selected for participation in the study. We enrolled households with a child <4 years or a pregnant woman at the time of enrolment. Following a baseline survey, 50 villages were randomized to the intervention arm and underwent latrine promotion and construction in accordance with the TSC; control villages received no intervention. Following the implementation period, we collected and assayed stool samples for soil-transmitted helminths (STH) and provided deworming tablets to assess the rate and intensity of reinfection; we also measured the height of children <2 to assess HAZ. Thereafter, we visited study participants 7 times over 18 months, collecting self-reported diarrhoea prevalence for children <5 (primary outcome) and all members of the household as well as weights for children <5 to assess WAZ. At endline, stools were again assessed for STH eggs and heights measured for children <2. In sub-samples of households, we also assessed faecal contamination of household water supplies, hands of child caretakers and sentinel toys given to children and monitored the density of synanthropic flies that can serve as mechanical vectors of faeces. We assessed latrine coverage and use throughout the study villages with spot checks at mid-line and end-line of the surveillance period. The intervention increased mean village-level latrine coverage from 9% to 63% in intervention villages compared to an increase of 8% to 12% in control villages. 63% of households with any latrine reported using them. Health surveillance data was collected from 1437 households with children under 5 in the intervention arm (1919 <5s, 10014 individuals overall), and 1465 (1916 <5s, 10269) in the control arm. The intervention had no effect on diarrhoeal disease among children <5 (period prevalence ratio 0.97, 95%CI: 0.83-1.12) or all ages (1.02, 95%CI: 0.88-1.18). Neither did it impact HAZ for children under 2, WAZ for children <5, or the prevalence or egg count of STHs. There was no evidence that the intervention impacted contamination of household drinking water, hands or sentinel toys, or impacted density of flies in food preparation areas.

711

CURRENT STRATEGIES AND CHALLENGES TO IMPLEMENTING HANDWASHING HARDWARE IN HUMANITARIAN EMERGENCIES

Jelena Vujcic[1], Lauren S. Blum[2], Pavani K. Ram[1]

[1]University at Buffalo, Buffalo, NY, United States, [2]Independent Consultant, Dakar, Senegal

Diarrhea and respiratory infections are prevalent in humanitarian emergencies but can be prevented by handwashing with soap. The challenges to handwashing promotion in emergencies have not been systematically documented. Our objective was to examine current strategies and barriers to implementation of hardware to facilitate handwashing among emergency-affected populations. We conducted key informant interviews with representatives at the global, regional or country level of agencies supporting handwashing promotion in emergencies. We identified key informants at an emergency environmental health forum and used snowball sampling to identify additional respondents with similar expertise. We coded themes based on key concepts and used content analysis to identify data trends. Our 12 respondents were

staff of United Nations agencies, non-governmental and government organizations. They reported that communal handwashing stations are common in the acute phase of an emergency but maintenance of soap and water are problematic due to lack of ownership. Organizations aim to distribute soap according to SPHERE standards but SPHERE does not recommend quantities needed for handwashing. Consistency and frequency of distribution of water dispensers and soap is highly variable in the post-acute phase due to funding constraints, prioritization by response agencies, and local market availability. There is a tradeoff between using local materials which are lower in cost and readily available and improved materials that are typically costly but more desirable. Sanitizer was not deemed a viable option for community-wide use due to acceptability, cost and sustainability. Evaluations of hardware uptake and acceptability are rare despite perceived utility of such data. Respondents indicated a strong interest in identifying hardware that is most acceptable among what is already available to emergency-affected populations. Assessing the type of soap most attractive to populations and how hardware choices affect handwashing behavior could provide useful guidance to improve handwashing promotion during emergencies.

712

HANDWASHING BEHAVIOR CHANGE STRATEGIES IN HUMANITARIAN EMERGENCY SITUATIONS: THE PERSPECTIVES OF EXPERTS

Lauren S. Blum, Jelena Vujcic, Pavani K. Ram

University of Buffalo, Buffalo, NY, United States

Handwashing can prevent diarrhea and acute respiratory infections, but is practiced infrequently in long-standing refugee camps. Little information is available on handwashing behavior change strategies targeting displaced persons during emergencies. We conducted key informant interviews between February and April 2013 with professionals supporting water, sanitation and hygiene (WASH) services for emergency-affected populations to understand behavior change strategies and to identify research gaps. We used purposive and snowball sampling to identify experts involved in handwashing promotion. Twelve respondents representing United Nations agencies, non-governmental organizations, and a government agency were interviewed. Respondents reported that technical and infrastructure aspects of WASH are not well coordinated with behavioral approaches; behavior change strategies are less of a priority. Typically, implementing organizations set no specific goals related to improving handwashing practices. Respondents described a dearth of behavior change expertise at the global, regional and local levels. Information on pre-existing knowledge and practices related to hand hygiene is generally not collected prior to implementation of handwashing promotion. Messages focusing on health benefits are given over prolonged timeframes, with little effort to understand motivators to handwashing or assess effectiveness and modify messages. A relatively unskilled workforce is expected to deliver often complex, participatory methods to improve behavior. Effectiveness of behavior change strategies is rarely assessed. Our findings underscore the need to strengthen behavior change expertise at all levels. The current reliance on anecdotal evidence hampers promotion of appropriate handwashing behavior. Lack of understanding of pre-existing behaviors and motivators for handwashing restricts adaptations to the local context and likely undermines behavior change efforts. Establishing specific behavior change objectives and developing contextually specific approaches could improve the effectiveness of handwashing promotion in emergency settings.

713

DETERMINANTS OF MORTALITY AMONG HUMAN IMMUNODEFICIENCY VIRUS AND TUBERCULOSIS (HIV/TB) CO-INFECTED PATIENTS IN AMINU KANO TEACHING HOSPITAL, KANO, NIGERIA

Mahmood M. Dalhat[1], Sadiq S. Wali[2], Musa B. Maiyaki[2], Ahmad M. Yakasai[3], Musa M. Borodo[2], Abdulhamid I. Dutse[2], Abdulrazaq G. Habib[1]

[1]Infectious and Tropical Diseases Unit, Department of Medicine, Kano, Nigeria, [2]Department of Medicine, Bayero University, Kano, Nigeria, [3]Department of Medicine, Kano, Nigeria

Management of HIV and tuberculosis co-infection remains a major challenge for clinicians and public health authorities. We conducted a prospective cohort study to determine risk factors of death among HIV/TB co-infected patients in Aminu Kano Teaching Hospital, Kano, Nigeria. We recruited 160 consenting, newly diagnosed HIV/TB co-infected patients 18 year or older between June and December 2012. We diagnosed TB by clinical features and any of positive sputum acid fast bacilli (AFB), chest radiographic features of tuberculosis or biopsy proven TB adenitis. We excluded patients with previous exposure to anti-TB or anti-retroviral drugs. We administered structured questionnaires to collect socio-demographic, clinical and laboratory information. Patients started on highly active antiretroviral therapy (HAART) within 8 weeks of starting anti-TB were considered as commencing early treatment; delayed commencement defined as starting HAART after 8 weeks of starting anti-TB. Patients who reported ever missing a dose of either or both of anti-TB and HAART drugs were considered as having sub-optimal adherence. All patients were followed up for 6 months. We conducted bivariate and multivariate analyses to determine independent risk factors of death during the study. A total of 71 (44.4%) patients were females. The median (IQR) age was 33 (28 – 401) years). The mean (range) hospital stay for admitted patients was 20 (5 – 37) days. The mean CD4 counts for patients who commenced early and those who delayed treatment were 144 cells/mm^{3}and 112 cells/mm^{3}respectively. On bivariate analysis, sputum AFB positivity HR (P-value): 3.1 (0.01); Hepatitis C co-infection HR (P-value): 9.8 (0.03) and sub-optimal adherence HR (P-value): 4.3 (0.001) to increase risk of dying among HIV/TB co-infected patients. In contrast, early commencement of HAART HR (P-value): 0.2 (<0.001) was found to decrease risk of dying. On multivariate analysis, risk of dying was reduced by early commencement of HAART HR (P-value): 0.2 (0.005), while hepatitis C co-infection HR (P-value): 12.3 (0.03) and sub-optimal adherence HR (P-value): 2.8 (0.04) remained independent risk factors of death. Early commencement of HAART among HIV/TB co-infected patients improves survival. Clinicians should adhere to universally accepted guidelines on timing of commencement of HAART. Sub-optimal adherence should be addressed by strengthening adherence units in HIV programs.

714

RECOVERY OF CD4+ T CELL COUNTS IN HIV-ASSOCIATED TUBERCULOSIS ACCORDING TO AGE, NUTRITIONAL STATUS AND ANTIRETROVIRAL THERAPY

Amara E. Ezeamama[1], Ezikiel Mupere[2], James Oloya[1], Robert Kakaire[1], Xiaoping Yin[1], Juliet Sekandi[1], Christopher C. Whalen[1]

[1]The University of Georgia, Athens, GA, United States, [2]Makerere University School of Public Health, Kampala, Uganda

We quantified associations between age and the monthly change in absolute CD4+ T cell count among HIV-infected tuberculosis (TB) patients during six months of TB therapy with or without concurrent antiretroviral therapy (ART). We determined whether this association is modified by sex and baseline nutritional status. The parent study for this secondary analysis was a randomized clinical trial of concurrent versus delayed ART during TB treatment. The study population included 208 non-pregnant, HIV-seropositive TB patients who had CD4+ T cell counts >350 cells/μL and were naïve to anti-retroviral therapy. Age at enrollment was

defined in years within categories as ≤24, 25-29, 30-34, 35-39 and ≥40. Nutritional status was classified as normal (BMI > 18.5 kg/m2) or low (BMI ≤18.5 kg/m2). Multivariate random effects linear regression models were used to estimate mean differences in absolute CD4-cell count in relation to concurrent ART status and baseline age. Concurrent ART during TB therapy was associated with a monthly rise of 15.7 CD4+ T cells/μL (P<0.0001). There was no overall difference in CD4+ T cell response by age during TB therapy (P=0.6550). However, among patients who received ART with TB treatment, greater gains in CD4+ T cell counts were seen among younger patients (age*time*ART, p-value=0.0443), whereas the same effect was not seen among patients who delayed ART. This inverse association between age at ART initiation and CD4+ T cell increase during concurrent ART and TB therapy was strongest in females (p-value: age*time=0.0059) and in patients with BMI ≥18.5kg/m2 at enrollment (p-value: age*time=0.0061). Our findings suggest that older HIV-seropositive patients on ART might experience a slower rate of immune restoration, especially if female or BMI ≥18.5kg/m2 at initiation. Older HIV-positive patients may benefit from closer monitoring of immune status and nutritional support during ART.

715

FOOD INSECURITY, DIET DIVERSITY AND BMI OF HIV INFECTED INDIVIDUALS ON ANTIRETROVIRAL THERAPY IN RURAL HAITI

Gabriel W. Rebick[1], Molly F. Franke[2], Jessica E. Teng[3], J. Gregory Jerome[4], Louise C. Ivers[3]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Department of Global Health and Social Medicine, Harvard Medical School, Boston, MA, United States, [3]Division of Global Health Equity, Brigham and Women's Hospital, Boston, MA, United States, [4]Zanmi Lasante, St. Marc, Haiti

Food insecurity and malnutrition are both important risk factors for poor outcomes in the treatment of people living with HIV. Food supplementation is increasingly offered in conjunction with HIV programs. Current programs focus on those with acute or chronic malnutrition. The optimal target population is unknown. We evaluated 490 HIV-infected individuals receiving antiretroviral therapy (ART) in rural Haiti. Baseline data including body mass index (BMI), household food security (using the Household Hunger Scale), socioeconomic status (SES), medication adherence and dietary diversity were collected via structured interview. We analyzed factors associated with low BMI and severe food insecurity using logistic regression. Moderate to severe food insecurity was present in 89% of individuals. Amongst severely food insecure subjects, 49% had a normal or above normal BMI. After adjusting for age, sex, BMI and presence of a garden in the home, severe food insecurity was associated with relative poverty [odds ratio (OR) 2.37, 95% confidence interval (CI) 1.58 - 3.54, p<0.001], illiteracy (OR 1.79, 95% CI 1.19 - 2.71, p< 0.01), and having no source of income generation (OR 1.63, 95% CI 1.04 - 2.56, p< 0.05). Individuals with severe food insecurity had a less diverse diet, with less frequent consumption of proteins. Food insecurity was highly prevalent in patients with HIV infection receiving ART. Normal or high BMI did not rule out severe food insecurity. Current guidelines regarding the use food support to supplement HIV treatment that are based on BMI will miss a significant proportion of patients who may benefit from this intervention.

716

HIV AND CHAGAS DISEASE: AN EVALUATION OF THE CLINICAL PRESENTATION AND THE USE OF REAL-TIME QUANTITATIVE PCR TO MEASURE LEVELS OF *TRYPANOSOMA CRUZI* PARASITEMIA IN HIV PATIENTS

Melissa Reimer-McAtee[1], Carolina Mejia[2], Caryn Bern[3], Monica Pajuelo[4], Daniel Lozano Beltran[2], Daniel Lozano Beltran[2], Faustino Torrico[2], Robert Gilman[5]

[1]Tulane University, New Orleans, LA, United States, [2]Universidad San Simon, Cochabamba, Plurinational State of Bolivia, [3]University of San Francisco California, San Francisco, CA, United States, [4]Universidad Peruana Cayetano Heredia, Lima, Peru, [5]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

With the persistence of Chagas disease in Latin America and with increased migration from rural to urban settings- where rates of HIV/AIDS are increasing- there has been an increase in the number of persons co-infected with HIV and Chagas disease in recent years. This study evaluated the clinical presentation and laboratory results of patients co-infected with HIV and Chagas in Cochabamba, Bolivia, with a focus on the levels of *Trypanosoma cruzi* parasitemia measured by real-time quantitative PCR (qRT-PCR) of blood clot. Clinical evaluation, electrocardiogram, chest radiograph, CD4 count, viral load, serology for *T. Cruzi*, direct microscopy of blood, and real-time PCR of blood clot was performed on each patient. Some patients also have a sample for qRT-PCR from whole blood with EDTA. 38 of the 133 HIV patients evaluated were co-infected with Chagas disease (28.6%). Four of the 38 (10.3%) were positive by direct microscopy, thus meeting the criteria for reactivation. Two of the patients with reactivation had very high levels of parasitemia (>1000 parasites/ml) by qRT-PCR of clot and by whole blood, however two patients with reactivation had no parasitemia detected by qRT-PCR of clot and only low levels by whole blood (<40 parasites/ml). Our results demonstrated that high levels of parasitemia are associated with high HIV viral loads and low CD4 counts; however, quantifiable levels of parasitemia did not show a strong correlation with symptoms of Chagas disease or reactivation. Although the levels of *T. cruzi* parasitemia detected by qRT-PCR do not show direct correlation with reactivation of Chagas disease or with clinical symptomatology in all patients, the level of parasitemia in HIV patients may be an indication of those at risk for progression of disease. Further studies are needed to determine the significance of parasitemia detected by PCR, and whether asymptomatic patients with detectable *T. cruzi* parasitemia should be treated with antiprotozoal agents.

717

RISK OF ANTIRETROVIRAL TREATMENT FAILURE AMONG CLINICALLY STABLE ADULTS IN BLANTYRE, MALAWI

Randy G. Mungwira[1], Matthew B. Laurens[2], Titus H. Divala[1], Osward M. Nyirenda[1], Maxwell C. Kanjala[1], Francis Muwalo[1], Felix A. Mkandawire[1], Nettie Dzabala[3], Terrie E. Taylor[1], Jane Mallewa[4], Joep V. Ousterhout[5], Miriam K. Laufer[6]

[1]Blantyre Malaria Project, Blantyre, Malawi, [2]Howard Hughes Medical Institute/Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, [3]College of Medicine, Blantyre, Malawi, [4]Department of Medicine, College of Medicine, Blantyre, Malawi, [5]Dignitas International, Zomba, Malawi, [6]University of Maryland, Baltimore, MD, United States

Millions of people living with HIV infection are receiving antiretroviral therapy (ART) in sub-Saharan Africa without access to routine viral load monitoring due to its expense and associated logistical challenges. If those likely to have detectable HIV viral load could be identified by readily identifiable risk factors, such patients could be prioritized for targeted viral load monitoring. Targeted monitoring in patients who are clinically well will allow for interventions before they develop severe illness or advanced ART resistance and will likely be a more affordable and feasible strategy than universal testing. As part of an on-going clinical trial at Ndirande

Health Centre in urban Blantyre, we conducted viral load and CD4 count assessments for 945 healthy adult outpatients who had been on ART for a minimum of six months. Ninety (10%) had HIV viral load >1000 copies/mL and 64 (7%) >5000 copies/mL. Among patients with a detectable viral load of greater than 40 copies/mL, the geometric mean was 3157 copies/mL (95% CI 2083 - 4786 copies/mL). The median CD4 cell count was 454 cells/mm3 (95% CI 433 - 475), 147 (16%) had a CD4 cell count <250 cells/mm3. We will use logistic regression to investigate factors associated with elevated viral load. The covariates in the modelling will include age, sex, BMI, CD4 cell count, reason for ART initiation, current ART regimen, duration of ART and self-reported adherence. We will also explore the association between infections including TB and malaria and viral load. Our goal is to identify patient characteristics that are associated with increased risk of virological failure on ART that can be identified prior to overt ART clinical failure. Targeted viral load testing among high risk individuals may be a cost-effective strategy to improve patient survival and limit the spread of ART resistance in resource-limited settings.

718

TRYPANOSOMA CRUZI PARASITEMIA IN IMMUNOSUPRESSED PATIENTS WITH HIV INFECTION OR ORGAN TRANSPLANT RECIPIENTS

Maria A. Shikanai-Yasuda[1], Noemia Barbosa Carvalho[1], Christina Novaes Gallafrio[2], Caroline Medeji[1], Sheila Cristina Silva[1], Rita Bezerra[1], Federico Dulley[3], Luiz Sérgio Azevedo[4], Vicente Amato Neto[1], Vera Lúcia Freitas[5]

[1]Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil, [2]HIV Clinics, Divisão de Clínica de Moléstias Infecciosas e Parasitárias, São Paulo, Brazil, [3]Bone Marrow Transplant Unit, Hospital das Clínicas da Faculdade de Medicina da USP, São Paulo, Brazil, [4]Kidney Transplant Unit, São Paulo, Brazil, [5]Departamento de Doenças Infecciosas e Parasitárias, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil

Chagas disease is now an active and emergent disease in urban centers of endemic and non endemic areas and affects around 7 million inhabitants in Latin America and a contingent of infected immigrants in other continents. Considering the high morbimortality of chronic Chagas disease reactivation associated with immunosupression, we analyzed the levels of parasitemia in immunosupressed patients under solid and stem cells transplantation (N=12) and in patients with HIV/*Trypanosoma cruzi* co-infection (N= 43 patients). Blood samples from organ transplants recipients were analyzed between the 4-6 months post transplant. A control group of 91 patients with Chagas disease was also included. Inclusion criteria for Chagas disease and HIV infection were the presence of anti-*Trypanosoma cruzi* antibodies by ELISA or immunofluorescence and presence of antibodies for HIV antigens by ELISA confirmed by imunoblot, respectively. For diagnosis of Chagas disease reactivation, the parasite was identified by direct microscopy on peripheral blood or other biological fluid. Quantitative PCR was performed with genomic sequencies as previously described (Freitas *et al,* 2011). Higher parasitemia was observed in the co-infected group in comparison to the control group with Chagas disease, and is related to the presence of reactivation of Chagas disease in seven HIV infected patients in this group. Increased parasitemia was detected in two of the seven kidney transplant patients: one patient with reactivation of Chagas disease, and one patient without reactivation but increased number of parasite DNA copies patient (higher than 200x the pre transplant levels). Increased parasitemia is also observed in co-infected HIV/*T. cruzi* patients without reactivation in association with high parasitemia showed in xenodiagnosis test (higher than 20% of nymphs positive in each exam). Monitoring parasitemia by quantitative PCR should be considered as an useful tool for the management of chronic Chagas diseases in patients under immunosupression.

719

COPING WITH CHRONIC DISEASE AND DISABILITY IN ISOLATED COMMUNITIES OF THE PERUVIAN AMAZON: A CASE SERIES

Jonathan Williamson[1], Ronald Ramirez[2], **Tom Wingfield**[3]

[1]Bristol Medical School, Bristol, United Kingdom, [2]The Vine Trust and Union Bíblica, Iquitos, Peru, [3]Universidad Peruana Cayetano Heredia, Lima, Peru and Imperial College London, United Kingdom, Lima, Peru

Healthcare, physiotherapy and social services are limited in the Peruvian Amazon and visits from boat-clinics infrequent. Qualitative research in Peruvian Amazonian communities concerning the impact of chronic disease and disability on households is scarce. We aimed to explore real-life situations faced by families in which a family member had chronic disease or disability. Whilst conducting a descriptive pilot project of patients accessing healthcare from a mobile boat-clinic serving 13 Peruvian Amazon communities, we performed four qualitative, extended case studies with consenting families. Interviews were performed in patient households and explored disease and disability burden, healthcare access, social support, and use of traditional and modern medicine. All interviews were reviewed with a translator and transcribed in Spanish and English. Case 1: Mrs B, a 52 year old school teacher, experiences chronic, inflammatory small joint pains for which she takes ibuprofen and a self-prepared natural home remedy. Case 2: Mr C, a 49 year old subsistence farmer, has hypertension managed quarterly by a visiting medical boat and lives with his bedbound 85 year old mother without social support. Case 3: Mrs P, a 56 year old subsistence farmer, consulted the mobile medical boat-clinic due to problems with her 6 year old daughter's speech and mobility due to which she has been denied local schooling. During the consultation her daughter was diagnosed for the first time with cerebral palsy. Case 4: Miss R, a 29 year old female, was diagnosed with epilepsy when she was 13 years old and after initial local treatment with traditional medicine has been intermittently treated by a distant clinic (3-4 hours by boat) and visiting medical boats. Miss R was also more recently diagnosed with pseudoseizures. Her seizure activity remains uncontrolled. In conclusion, these cases exemplify the difficulties of coping with chronic disease and disability in the Peruvian Amazon with constrained healthcare infrastructure and minimal formal social support. The cases reveal ongoing use of traditional medicine in addition to modern medicine potentially relating to local health beliefs and reduced access to modern healthcare. Whilst mobile medical boats may be an adjunct to existing local healthcare services, treating chronic diseases such as hypertension or epilepsy, is limited by infrequent and sporadic consultations.

720

DEVELOPMENT AND EVALUATION OF MULTIMEDIA INFORMED CONSENT TOOL FOR A LOW LITERACY AFRICAN RESEARCH POPULATION

Muhammed O. Afolabi[1], Kalifa A. Bojang[1], Umberto D'Alessandro[1], Egeruan B. Imoukhuede[2], Raffaella M. Ravinetto[3], Heidi J. Larson[4], Nuala McGrath[5], Daniel Chandramohan[4]

[1]Medical Research Council, Banjul, Gambia, [2]University of Oxford, Oxford, United Kingdom, [3]Institute of Tropical Medicine, Antwerp, Belgium, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom, [5]University of Southampton, Southampton, United Kingdom

International guidelines recommend the use of appropriate informed consent procedures in low literacy research settings because written information is not known to guarantee comprehension of study information. This study developed and evaluated a multimedia informed consent tool for people with low literacy in an area where a malaria treatment trial was being planned in The Gambia. Methods: We developed the informed consent document of the malaria treatment trial into a multimedia tool integrating video, animations and audio narrations in three major Gambian languages. Acceptability and ease of use of the multimedia tool were assessed using quantitative and qualitative

methods. In two separate visits, the participants' comprehension of the study information was measured by using a validated digitised audio questionnaire. The majority of participants (70%) reported that the multimedia tool was clear and easy to understand. Participants had high scores on the domains of adverse events/risk, voluntary participation, study procedures while lowest scores were recorded on the question items on randomisation. The differences in mean scores for participants' 'recall' and 'understanding' between first and second visits were statistically significant ($F(1, 41) = 25.38$, $p<0.00001$ and $F(1, 41) = 31.61$, $p<0.00001$ respectively. In conclusion, our locally developed multimedia tool was acceptable and easy to administer among low literacy participants in The Gambia. It also proved to be effective in delivering and sustaining comprehension of study information across a diverse group of participants. Additional research is needed to compare the tool to the traditional consent interview, both in The Gambia and in other sub-Saharan settings.

721

PANDEMIC INFLUENZA PREPAREDNESS IN CAMBODIA: AN ECONOMIC EPIDEMIOLOGICAL DECISION ANALYSIS

Tom Drake[1], James Rudge[2], Aronrag Meeyai[2], Khieu Borin[3], Sok Touch[4], Richard Coker[2]

[1]University of Oxford, Oxford, United Kingdom, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Centre for Livestock and Agriculture, Phnom Penh, Cambodia, [4]Ministry of Health, Phnom Penh, Cambodia

Pandemic burden is predicted to be high in low income countries, relative wealthy well-resourced countries. Yet evaluations of cost effective pandemic preparedness investments focus almost exclusively on high income countries. We undertake an economic evaluation of two pandemic preparedness investment options in Cambodia. We developed a dynamic pandemic simulation model including age structure, health system capacity and cost of illness. The model was used to perform cost utility analysis (CUA) on three pandemic preparedness investment options i) antiviral stockpiling and ii) additional mechanical ventilators and iii) a 1:1 mixed investment strategy. The analysis was repeated at three investment levels US$250k, US$4 million and US$20 million. Due to the inherent uncertainty in pandemic modelling we use sampling distributions in place of single point estimates for most parameter inputs. We also present a cost-consequence analysis (CCA) to place model results within the context of non-quantified costs and consequences. At investment levels of US$250k and US$4 million stockpiling is most cost effective, investment level of US$20 million a mixed investment is preferable, reflective diminishing marginal returns from increased stockpile size. However there is substantially more uncertainty in incremental cost effectiveness ratio (ICER) estimates for antiviral stockpiling. Also, the CCA highlights that investing in ventilators would have considerable utility between pandemics. It is likely that both antiviral stockpiling and investment in mechanical ventilators are cost effective pandemic preparedness options. The caveats to this are the considerable uncertainty inherent in these estimates and that depending on the payer, they may or may not be affordable or the most urgent public health investment for Cambodia. Note: In light of the recent Cochrane publication on antiviral effectiveness (10th April 2014) we will be updating our analysis within the coming weeks.

722

RELATIONSHIP OF ANGIOGENIC AND INFLAMMATORY BIOMARKERS AT MID-PREGNANCY TO SMALL-FOR-GESTATIONAL AGE OUTCOMES IN A PROSPECTIVE COHORT OF TANZANIAN WOMEN

Chloe E. McDonald[1], Anne Marie Darling[2], Andrea L. Conroy[1], Kyla T. Hayford[1], Molin Wang[2], Said Aboud[3], Willy S. Urassa[3], Wafaie W. Fawzi[2], Kevin C. Kain[1]

[1]University of Toronto, Toronto, ON, Canada, [2]Harvard University, Boston, MA, United States, [3]Muhimbili University of Health and Allied Sciences, Dar es Salaam, United Republic of Tanzania

Intrauterine growth restriction (IUGR) is a major public health problem that affects an estimated 27% of pregnancies in low and middle-income countries. Inadequate fetal growth is associated with increased risk of neonatal morbidity and mortality as well as developmental delay and cardiometabolic disorders in adulthood. We examined the relationship between a panel of angiogenic and inflammatory biomarkers measured in mid-pregnancy and small-for-gestational age (SGA) outcomes in Tanzanian women. Plasma samples were collected from a prospective cohort of 432 primigravid women at enrolment (12-27 weeks gestation). Levels of 18 biomarkers (Ang-1, Ang-2, Ang-L3, VEGF, sFLT-1, sTNFR2, PlGF, MIP-1β, MCP-1, Leptin, IL-1β, IL-18 BP, sICAM-1, FAC-D, sEndoglin, CRP, CHI3L1, C5a) were analyzed by ELISA. Infants falling below the 10th percentile of birth weight for gestational age relative to the applied growth standards were considered SGA. Multivariate binomial regression models were used to determine the relative risk of SGA associated with levels of each biomarker. Receiver operating curves obtained from multivariate logistic regression models were used to assess the discriminatory capability of selected biomarkers. A total of 60 participants (13.9%) gave birth to SGA infants. Compared to those in the first quartile, the risk of SGA was reduced among those in the fourth quartiles of VEGF-A (adjusted risk ratio (RR) 0.38, 95% Confidence Interval (CI), 0.19-0.74), PGF (adjusted RR 0.28, 95% CI, 0.12-0.61), sFlt-1 (adjusted RR 0.48, 95% CI, 0.23-1.01), MCP-1 (adjusted RR 0.48, 95% CI, 0.25-0.92), and Leptin (adjusted RR 0.46, 95% CI, 0.22-0.96). Our findings provide evidence of altered angiogenic and inflammatory mediators, at mid-pregnancy, in women who went on to deliver small for gestational age infants. Studies are currently under way to validate these findings in both internal and external cohorts.

723

MULTINATIONAL DISEASE SURVEILLANCE PROGRAMS FOR CROSS-BORDER EPIDEMIOLOGIC INFORMATION EXCHANGE

Aiden K. Varan, Robson Bruniera-Oliveira, Christopher R. Peter, Maureen Fonseca-Ford, Stephen H. Waterman

Centers for Disease Control and Prevention, Division of Global Migration and Quarantine, San Diego, CA, United States

Cross-border disease surveillance and response efforts in regions sharing borders depend on effective international collaboration. The Border Infectious Disease Surveillance (BIDS) program facilitates epidemiologic information exchange between the United States and Mexico. To understand the global context for BIDS, we conducted a survey of multinational disease surveillance programs (MNDSPs) operating worldwide. Our survey assessed program organization, goals, operations, decision-making processes, and evaluation. From January 2013 to March 2014, we identified MNDSP representatives through internet search and querying domestic and international colleagues. We contacted 12 MNDSPs and obtained responses from ten programs spanning all continents except Antarctica. In general, responding programs aim to enhance epidemiologic surveillance capabilities, strengthen cooperation for infectious disease monitoring and prevention, and increase consistency among countries. Reportable conditions are jointly selected by participating countries based on common importance. Most programs have specific algorithms for disease surveillance and laboratory testing. Eight (80%) of the ten programs have a central database that obtains information through

manual data entry or electronic linkage. E-mail is the primary mode of communication. Eighty percent of the programs have multinational emergency notification contact lists. All ten programs meet at least annually in person or via video or teleconference. Fewer than half (40%) of the programs share specimens or laboratory testing reagents among countries. Local and national public health laboratories are the primary infrastructure for diagnostic testing. Seven of the ten programs have dedicated funding allocated to MNDSP operation. Few programs have implemented a routine quality assurance program. Despite variation in health priorities, geography, and socioeconomic context, our survey identified key operational commonalities among MNDSPs and provides an important perspective on global disease surveillance efforts.

724

KNOWLEDGE, ATTITUDES AND BEHAVIOR TOWARD PARASITE INFECTIONS AMONG HIGH SCHOOL STUDENTS IN SOLOMON ISLANDS

Tzu-Ping Weng[1], Shih-Huai Hsiao[1], Lin-Kun Wu[2], Tenneth Dalipanda[3], Wen-Ter Lai[2]

[1]Kaohsiung Medical University, Kaohsiung City, Taiwan, [2]Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan, [3]Ministry of Health and Medical Service, Honiara, Solomon Islands

Pacific region is an epidemic area of different parasite infections. The countries have provided examinations and medical supports to their citizens. However, few statistic data and information on attitudes and behavior toward parasite infections are available. The survey was trying to investigate the knowledge, attitudes and behavior toward parasite infections among high school students in Solomon Islands. The survey was conducted in August, 2012. Total of 679 participants, aged from 12 to 18 years old, were randomly selected from St. Nicholas and St. Joseph high schools. Approximately 95% of participants indicated that they had clean water for food preparation or cooking, however nearly 72% showed they didn't have drinking water boiled. 67% and 13% of the students said the main source of their drinking water was from rain and bottled water, respectively. 90% of the students had toilets at home, and 82% indicated they had the habit of hand washing. However, up to 53% of participants didn't wear shoes, while 40% with excessively long fingernails. Nearly 28% of them had been diagnosed for parasite infections. 40% claimed they had never learned about parasite prevention. A screening program executed by Taiwan Health Center in Solomon Islands showed the prevalence of parasite infection among local students was up to 34%. The understanding of parasite prevention among high school students in Solomon Islands is still deficient, and it is not taught as part of the curriculums in schools. Appropriate medical and educational resources need to be prompted to make significant changes in parasite prevention.

725

A LOW-COST ICT TOOL KIT FOR IMPROVED DENGUE SURVEILLANCE, LABORATORY MANAGEMENT AND CLINICAL DECISION SUPPORT IN NICARAGUA

Heather Zornetzer[1], Josefina Coloma[2], Eva Harris[2], William Avilés[1]

[1]Sustainable Sciences Institute, Managua, Nicaragua, [2]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States

Dengue is a mosquito-borne viral disease of major medical and public health importance worldwide. We have developed and tested three low-cost informatics tools in Nicaragua to help improve laboratory, surveillance and clinical management practices to reduce dengue-related morbidity and mortality. As part of the Dengue FIRST initiative (Fighting Infections through Research, Science and Technology), these tools were developed collaboratively between the NGO the Sustainable Sciences Institute and the Ministry of Health of Nicaragua. Input from stakeholders at the national, state and local levels was incorporated at multiple phases. "DENGUE-ALERT" is an innovative automated early warning system for outbreak detection and response that provides a customizable "dashboard" with information from traditional epidemiological disease reports and entomological, climatic and crowd-sourced data. It targets a wide range of end-users including public health and community-based actors and is designed to enable earlier detection and reporting of virus circulation or outbreak indicators to increase the efficiency of response and resource use. "DENGUE-SPECIALIST" is a web-based mobile application designed to improve the efficiency and accuracy of clinical data access and analysis in hospitalized dengue cases. End-users are clinicians and nurses in public sector hospitals. Simulations of SPECIALIST were tested with data from multiple years of a clinical study in a Nicaraguan public hospital. "DENGUE-LAB" is a web-based platform that supports a national-level information management system for integrating laboratory results and reporting to streamline information flow around the numerous tests used for dengue diagnosis. LAB is designed to improve quality control measures, simplify and automate some of the complexities of dengue diagnosis, and impact both the quality and the reliability of diagnostic results. Both quantitative and qualitative indicators were used in a mixed method evaluation of the pilot of these tools to assess their ability to support earlier and more accurate disease response and outbreak prevention. Following this design and testing phase, the goal is to extend the use of these tools to Mexico and to other Central American countries that are interested and able to adapt and implement "customized" versions of ALERT, SPECIALIST and LAB as appropriate in their country contexts.

726

DENGUE TORPEDO: A NOVEL APP TO MOTIVATE COMMUNITY-BASED DENGUE VECTOR CONTROL

Josefina Coloma[1], Rui Harayama[2], Marco Herrera-Valdez[3], Eva Harris[1], James Holston[3]

[1]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States, [2]LACS, Federal University of Minas Gerais, Minas Gerais, Brazil, [3]Department of Anthropology, University of California Berkeley, Berkeley, CA, United States

Dengue is a mosquito-borne viral disease that continues to expand geographically in part due to failed efforts in vector control. Residents of communities affected by dengue are potentially the best agents to control vector breeding sites, since the *Aedes* mosquitoes that transmit dengue virus breed in clean water in and around people's homes. We present the design and piloting of Dengue Torpedo (DT), an interactive cellphone and web platform that combines mobile technology and game concepts to motivate community residents to report and eliminate mosquito breeding containers. DT (a) crowdsources the identification and mapping of breeding sites (problem of information); (b) motivates residents to act (problem of execution); (c) develops a collaborative model of software production that involves local user-residents in researching, testing, and designing the application; (d) promotes civic engagement and active citizenship; (e) involves residents in public health education; and (f) engages youth in the creative development of communication technologies. Using a community-based collaborative model of software design and development, we created alpha and beta versions of DT in Rio de Janeiro, Brazil. The mobile and web interface is interactive and allows residents to create their own profiles and exchange information about dengue in their neighborhoods. DT also has an educational component that relates to other relevant issues in the community as well as specific information regarding dengue. Players earn badges and points that can be exchanged for community or personal prizes donated by local sponsors and small businesses. DT has 5 participatory features in dengue vector control that make it pioneering: it is interactive, connects mobile and web technologies, uses gameplay to motivate residents, institutes a community-based collaborative model of app development, and helps local institutions sponsor related educational activities. In parallel, we are developing DT for the Mexican health sector, incorporating contextually appropriate information and designing the interface with input from community members and researchers in Morelos, Mexico. DT can improve

public participation in combating dengue, generate new correlations and visualizations of scientific data of potentially great scale and low cost, reduce mosquito infestation, and eventually decrease rates of dengue infection and disease.

727

OPENHDS: USE OF TABLET COMPUTERS IN HEALTH AND DEMOGRAPHIC SURVEILLANCE

Aurelio Di Pasquale[1], Bruce MacLeod[2], Tobias Homan[3], Willem Takken[3], Richard Mukabana[4], Thomas Smith[1], Eveline Geubbels[5], Nicolas Maire[1]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]USM, Portland, ME, United States, [3]Wageningen University, Wageningen, Netherlands, [4]ICIPE, Mbita Point Township, Kenya, [5]IHI, Dar es Salaam, United Republic of Tanzania

Health and Demographic surveillance systems (HDSS) can provide essential information in areas where routine vital registration is absent or incomplete. also play an essential role in health intervention studies in such areas. Setting up and running an HDSS poses an operational challenge, and a reliable and efficient platform for data collection and management is a key prerequisite. OpenHDS is an HDSS data system that provides data entry, quality control, and reporting to support demo-graphic and health surveillance. It consists of two components: web and mobile. OpenHDS mobile is integrated with the Open Data Kit (ODK) a software platform for data collection using mobile devices running the Android operating system, and allows direct data entry using tablet computers. This offers a number of advantages over traditional paper-based systems: it reduces the workload of the data management team, no IDs need to be typed in (removing one of the biggest causes of errors on data collection in HDSS systems); it allows for near real-time quality control; and it can provide guidance for the project logistics. The web interface allow viewing of the data collected and correction of errors/perform eventual amendments. Here we present an overview of the openHDS/ODK software platform, and report on the experience of using this platform to set up a HDSS in support of a trial of odour baited traps as a malaria intervention study on Rusinga Island, Kenya, (Solarmal) Project. We also present the experience of migrating the data systems of established HDSS sites from an older system (HRS) to openHDS (Ifakara and Rufiji, Tanzania). We show how OpenHDS addresses specific operational problems and the use of the complete OpenHDS/ODK system for data collections offers a number of advantages over paper-based systems both with respect to data quality and cost savings.

728

AVAILABILITY OF SIGNAL FUNCTIONS AND QUALITY OF EMERGENCY OBSTETRICS CARE FOR POPULATION IN RURAL BANGLADESH

Wit Wichaidit[1], Mahbub Ul Alam[2], Amal K. Halder[2], Pavani K. Ram[1], Leanne Unicomb[2], Davidson H. Hamer[3]

[1]University at Buffalo, Buffalo, NY, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]Boston University, Boston, MA, United States

The maternal mortality ratio in Bangladesh is high at 220 deaths per 100,000 live births. Emergency obstetrical care (EmOC) interventions in health facilities are effective at reducing maternal mortality. Facility deliveries in Bangladesh have grown from 16% in 2000-2006 to 29% in 2007-2012. This study assessed the quality of obstetrical services in health facilities serving rural areas of Bangladesh in order to identify opportunities to improve EmOC. We randomly sampled 50 rural villages in 46 of 64 districts of Bangladesh and interviewed the administrator of 8-9 hospitals nearest to each sampled village. We categorized the quality of EmOC at each hospital according to its capacity to provide 6 basic EmOC signal functions (administrations of antibiotics, oxytocin, and anticonvulsant; assisted vaginal delivery; manual removal of placenta and removal of retained products after delivery), staffing, and availability of phone and ambulance for referring patients. EmOC quality categories included high (6 signal functions; ≥3 staff on call [24-hour coverage]; an ambulance and a phone), moderate (≥4 signal functions; ≥2 staff [or no 24-hour coverage]; a phone), low (≥2 signal functions; ≥1 staff; a phone), and sub-standard (no minimum criteria). Administrators of 432 hospitals were interviewed. Administration of antibiotics was available in 99% of the hospitals, whereas anticonvulsant administration was only available in 65%. The quality of EmOC was high in 31%, moderate in 55%, low in 4%, and sub-standard in 9% of the hospitals; 32% did not have 24-hour coverage of skilled birth attendance. Approximately one-third of health facilities providing obstetric care in rural Bangladesh lack anticonvulsants needed to manage eclampsia, an important cause of maternal mortality. Similarly, the lack of 24-hour availability of skilled birth attendants increases the risk of peripartum complications. Given the high rate of maternal mortality, there is a pressing need to improve provision of EmOC in health facilities in rural Bangladesh.

729

OCCURRENCE OF SEVERE DENGUE FEVER IN AN ENDEMIC CITY OF BRAZIL: AN ECOLOGICAL STUDY

Gerusa Belo Gibson dos Santos, Alexandre San Pedro, Reinaldo Souza-Santos, Nildimar Honório, Marilia Sá Carvalho

Oswaldo Cruz Foundation- FIOCRUZ, Rio de Janeiro, Brazil

Background: Brazil accounts for more than 70% of dengue cases notified on the American continent. In this context, Rio de Janeiro became one of the most endemic cities in the country during the last decades, presenting a long history of co-circulation of all four dengue serotypes with a recent trend for the clinical and epidemiological profile of the disease to change. Methods: This ecological study aimed to analyze the relationship between incidence of severe dengue during the 2008 epidemic in Rio de Janeiro City and socioeconomic, previous circulation of other dengue serotype and health service availability indicators. The data was incorporated into a negative binomial regression model. Results: Districts with more cases of dengue in the 2001 epidemic and where higher percentages of the residents who declare their skin color/race as black showed higher incidence rates of severe dengue in the 2008 epidemic. Meanwhile, districts with lower incidences of severe dengue in 2008 were those with more Family Health Strategy (FHS) clinics. Conclusion: The findings suggest persisting health inequities possibly due to greater socioeconomic vulnerability among black population. Additionally, the protective effect of FHS clinics may be due to facilitated access to other levels of health care or even by reducing vulnerability to transmission afforded by local practices in health promotion. These aspects reinforce the importance of better understanding of social determinants in order to identify key-points for developing and implementing interventions for dengue control.

730

CLIMATE-DRIVEN DENGUE EPIDEMIC EARLY WARNINGS FOR BRAZIL

Rachel Lowe[1], Christovam Barcellos[2], Luis R. Lage Rodrigues[1], Trevor Bailey[3], Marilia Sá Carvalho[2], Francisco Doblas-Reyes[1], Xavier Rodó[1]

[1]Institut Català de Ciències del Clima, Barcelona, Spain, [2]Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil, [3]University of Exeter, Exeter, United Kingdom

In this study, we address the potential to predict dengue fever epidemics across Brazil, during the peak dengue season (austral summer period). We produce probabilistic dengue forecasts for the 553 microregions of Brazil, from 2000 to 2014, using a novel spatio-temporal modelling framework. The dengue forecasts are driven by multi-model ensemble seasonal climate forecasts and the observed epidemiological situation in Brazil at the forecast issue date. The multi-model ensemble comprises several quasi-operational forecast systems, among them two systems

from the European EUROSIP initiative and six systems from the North American Multi-Model Ensemble (NMME) project. This precursory information allows dengue warnings to be released three months ahead. We evaluate the past performance of the dengue early warning system by verifying probabilistic predictions against out-of-sample observed data. Timely dengue early warnings provide the opportunity for the ministry of health and local authorities to implement appropriate, city-specific mitigation and control actions. This model framework could be applied to predict outbreaks of other climate-sensitive diseases in other parts of the world. This is especially pertinent as climate change is likely to make diseases, such as dengue and malaria, more widespread. The successful implementation of seasonal climate forecasts in disease early warning systems depends on close collaboration between public health specialists, climate scientists and mathematical modellers. The overall objective of this study is to bring awareness to scientists, policy makers and international health surveillance teams of the data and tools required in order to make timely predictions. We hope that this early warning framework will help to improve prevention strategies for vector-borne diseases and establish a landmark towards the use of climate data to benefit society.

731

COMMUNITY HEALTH WORKER TIME USE: METHOD EVALUATION AND TIME USE FINDINGS IN MALAWI

Emily Carter[1], Andrew Marsh[1], Spy Munthali[2], Gowokani Chirwa[2], Neff Walker[1], Ben Johns[3], Agbessi Amouzou[4]

[1]Johns Hopkins School of Public Health, Baltimore, MD, United States, [2]University of Malawi, Zomba, Malawi, [3]Abt Associates, Washington, DC, United States, [4]UNICEF, New York, NY, United States

Understanding community health worker (CHW) workload is critical for maintaining program quality in the face of competing tasks. Time motion studies are considered the gold standard in time use research, but are prohibitively time and resource intensive. Four alternative measurement methods (3 interview methods and time diary) were piloted to assess time use among CHWs as a subcomponent of a cost effectiveness evaluation of the Rapid Scale-up of MNCH in Malawi in 2013. Based on pilot results, 2 interview methods (week-long vs. year-long reference period) were deployed to measure time use among 248 CHWs (126 implementing community cases management (CCM)) in 6 districts. Reported total hours and hours spent on CCM activities in an average week were higher and more variable using the year-long vs. week-long interview method. Correlation between methods in estimated work hours/week (0.12) and CCM hours/week (0.27) was very low. Based on implausible variability in time use estimated by the year-long method, final results were reported using the week-long method only. On average a CHW reported working 42.29 (95% CI: 38.88, 45.70) hours/week and those who implemented CCM spent 18.08 (95% CI: 16.24, 19.92) hours/week on CCM. CCM (Adjusted coeff: 10.32; 95% CI: 5.09, 15.56) and each additional intervention implemented (Adjusted coeff: 1.86; 95% CI: 0.81, 2.90) increased total number of hours worked per week. CHWs who implemented CCM spent significantly less time on nutrition (Coeff: -2.24; 95% CI: -3.12, -1.37), HIV (Coeff: -3.49; 95% CI: -6.56, -0.42), and community based maternal and newborn health interventions (Coeff: -1.31; 95% CI: -2.33, -0.30), compared with CHWs implementing those services but not CCM. Findings highlight the importance of an accurate and efficient means of assessing CHW time use to determine feasibility and impact of introducing additional tasks. Time use interviews offer promise for collecting time use data when a large number of CHWs are targeted, although further refinement and validation against a gold standard time assessment is needed.

732

BREASTFEEDING PRACTICES AND CHILDHOOD DIARRHEA MANAGEMENT AMONG WOMEN IN RURAL COMMUNITIES IN MOZAMBIQUE

Victor W. Galson, Natasha Anandaraja, Sigrid Hahn

Icahn School of Medicine at Mount Sinai, New York, NY, United States

Early childhood nutritional practices, including exclusive breastfeeding for the first 6 months of life and adequate hydration of children with diarrhea, are critical for minimizing morbidity and mortality from infectious causes in the developing world. Gorongosa National Park in Mozambique is surrounded by rural communities with significant geographic and economic barriers to health resources. These communities are located in two groups: those located in the mountain region and those in the plains region, with the mountain communities being especially isolated. In 2011, a comprehensive household survey was conducted to study health behaviors in these regions as part of an ongoing Ecohealth Program. 1625 women of reproductive age were surveyed by trained interviewers in the local language: 1074 women from 4 mountain communities and 551 women from 2 plains communities. 192 women with children age 6 months or younger answered questions about breastfeeding, and 1035 women with children age 5 years or younger answered questions about previous nutrition and diarrhea education, and diarrhea management. Results showed that women in the plains communities were more likely to have received education about early childhood nutrition vs. women in the mountain communities (85% vs. 28%, p<.0001), but in both populations, there was no association between previous nutrition education and exclusive breastfeeding (plains: RR .85, CI .31-2.28; mountain: RR 1.01, CI .81-1.26). In the mountain communities, 46% of women withhold water completely during a child's episode of diarrhea; however, those who received previous education were significantly more likely to hydrate their child though the episode (RR 3.43, CI 2.19-5.38). No such association was found in the plains communities (RR 1.10, CI .92-1.32), where only 7% of women will withhold hydration. These results can be used to tailor a planned intervention involving a new nutrition educational program. The data suggest that previous programs did not effectively teach best breastfeeding practices, and that there is an opportunity to increase the number of women exclusively breastfeeding, especially in the plains communities where women are more likely to use breast milk alternatives. The data also suggest that prior education about rehydration in diarrhea has been successful in the mountain communities, and any future nutrition programs should model educational materials accordingly.

733

ACCEPTABILITY AND EFFECTIVE USE OF A RAPID HOME DIAGNOSTIC TEST FOR MULTIPLE INFECTIOUS DISEASES: FORMATIVE RESEARCH IN PERU AND CAMBODIA

Valerie A. Paz Soldan[1], Pamina M. Gorbach[2], Amy C. Morrison[3], Sopheab Heng[4], Jennie L. Mckenney[5], Chhea Chhorvann[4], Vonthanak Saphonn[6], Robert D. Hontz[7], Christopher A. Myers[8], Eric S. Halsey[7], Shawn P. Mulvaney[9], Patrick J. Blair[10], Erin McDonough[8], Shevin T. Jacob[11]

[1]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]University of California Los Angeles, CA, United States, [3]University of California Davis, CA, United States, [4]National Institute of Public Health, Phnom Penh, Cambodia, [5]University of California, Los Angeles, Fielding School of Public Health, Los Angeles, CA, United States, [6]University of Health Sciences, Phnom Penh, Cambodia, [7]U.S. Naval Medical Research Unit - 6, Lima, Peru, [8]Naval Health Research Center, San Diego, CA, United States, [9]Naval Research Laboratory, Washington, DC, United States, [10]Naval Medical Research Center-Asia, Singapore, Singapore, [11]University of Washington, Seattle, WA, United States

In 2012, the U.S. Defense Threat Reduction Agency Joint Science and Technology Office initiated a program to develop novel point-of-need

diagnostics for surveillance of emerging infectious diseases including dengue, malaria, plague, and melioidosis. Prior to distribution of devices to community members in Iquitos, Peru, and Phnom Penh, Cambodia, research was conducted to assess acceptability of use, determine conditions in which use would occur, examine incentives to use the device on someone with fever >5 years of age in their home, and assess device use competency. In February 2014, 9 focus group discussions (FGD) with community members and 5 FGD with health professionals were conducted in Iquitos, and 9 FGD with community members and 9 in-depth interviews with health professionals in Phnom Penh. In both places, participants agreed to use the device themselves (involving finger prick) or could identify someone to do so. The main incentive identified in Iquitos was the ability for tentative results to be used for care facilitation (as devices would not provide usable results for individuals), specifically reduced wait times to be seen or obtain a diagnosis. In Phnom Penh, the main incentives were monetary compensation (~US$2.50/test) or results from a simultaneous rapid test; also, free medicine for the sick was acceptable in lieu of usable results. To assess device use competency in Iquitos, participants were asked to read instructions and apply the device to research team members. Most steps were done correctly; the most difficult was proper recording of test results. In Phnom Penh, participants were asked to describe each step after reviewing the instructions; however, they struggled with comprehension. Health professionals' main concerns were their community's ability to accurately use the test, complicated instructions, and safety. Motivation and ability to use home diagnostic devices depended on local attitudes that varied between the two disease endemic sites, illustrating the value of formative research before deployment of novel technologies.

734

THE PERCEPTIONS OF PHYSIOTHERAPISTS AND PATIENTS ABOUT INTEGRATION OF HEALTH PROMOTION IN PHYSIOTHERAPY PRACTICE

Nomathemba P. Taukobong[1], **Helen Myezwa**[2], Jessica Freyman[3], Jessica Freyman[3], Jessica Freyman[3], Jean-Pierre J. Van Geertruyden[3]

[1]University of Limpopo-Medunsa Campus, Pretoria, South Africa, [2]University of Witwatersrand, Johannesburg, South Africa, [3]University of Antwerp, Wilrjik, Belgium

This study was undertaken to determine the status of integration of health promotion in physiotherapy practice from both physiotherapists and patients' perspectives. A qualitative research method was followed. A rich purposive sample of physiotherapists patients from different health care settings provided information for data collection during semi-structure interviews which lasted from 45 to 60 minutes. Data were collected over a period of six months. All interviews were audio taped followed by verbatim transcription. The Nvivo version 10 program was used. The following sequence was followed in data analysis: Familiarisation with data, code book construction, thematic analysis, synthesis and identification of similarities, identification of overarching themes and formulation of concept. A total of 35 physiotherapists, 69% females and 31% males, as well as 21 patients, 55% females and 45% males, participated in the interviews. All physiotherapists had a work experience of more than 5 years whilst patients' age ranged between 24 years to 60 years. Physiotherapists' workload comprised of in- and out-patients whilst patients attended physiotherapy sessions at hospital and Primary Health Care (PHC) settings. Concepts formulated for both groups were knowledge, attitude and practice. Patients do have an understanding of what health promotion means and expect health promotion service during physiotherapy treatment as well as aspire to be self-efficient in looking after their health. The physiotherapists are unable to differentiate between health education and health promotion though they have a positive attitude towards health promotion in practice. They rarely integrate health promotion in their daily practice. In conclusion, the need to integrate health promotion in practice exists. Hospital and PHC based physiotherapy differ. There are gaps between the patients' needs and physiotherapy practice requiring policy and guidelines to drive health promotion in physiotherapy. Focus should be on continuous professional development to improve knowledge. Education and training curriculum should be reviewed.

735

TREATMENT OF POSTPARTUM HEMORRHAGE WITH MISOPROSTOL BY GUATEMALAN TRADITIONAL MIDWIVES

Nora E. King[1], Peter Rohloff[2]

[1]Mayo Clinic, Rochester, MN, United States, [2]Brigham and Women's Hospital, Boston, MA, United States

Postpartum hemorrhage (PPH) due to uterine atony is a leading cause of maternal mortality in Guatemala, especially in rural areas where homebirth is common. In a pilot project, 36 Guatemalan traditional midwives received training on the recognition and treatment of PPH using 800 µg buccal misoprostol. Training was designed to be linguistically and culturally appropriate. Pre- and post-evaluation showed participants' knowledge of PPH signs, prevention and treatment using misoprostol significantly increased following training. Ongoing data collection, to be completed in June 2014, demonstrates that midwives are capable of recognizing PPH and using misoprostol for its treatment. We plan to expand our work to reach more midwives, further addressing the shortage of data on misoprostol for PPH treatment in homebirth, particularly in Latin America.

736

MAPPING THE ENVIRONMENTAL AND SOCIOECONOMIC COVERAGE OF THE 2012 INDEPTH INTERNATIONAL HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM NETWORK

Peng Jia[1], Osman Sankoh[2], Andrew J. Tatem[3]

[1]Louisiana State University, Baton Rouge, LA, United States, [2]University of the Witwatersrand, Johannesburg, South Africa, [3]University of Southampton, Southampton, United Kingdom

The International Network for the Demographic Evaluation of Populations and their Health (INDEPTH) was initiated in1998 and has produced reliable longitudinal data about the lives of people in low- and middle-income countries (LMICs) and the impact on those lives of development policies and programs through a global network of health and demographic surveillance system (HDSS) sites. Since stable and reliable health and demographic data are scarce across many LMICs, here we examine the environmental and socioeconomic similarities between existing HDSS field sites and the rest of the LMICs in Africa and Asia, so as to provide evidence in terms of levels of confidence in extrapolating the findings from HDSS field sites to other regions. A 'signature', consisting of 15 environmental and socioeconomic variables, was constructed for each HDSS field site. The field sites were then hierarchically grouped by the similarity of their signatures to quantify the variability in terms of environmental and socioeconomic conditions between sites, and these similarities were mapped. The current INDEPTH HDSS field site network covering Africa and Asia spans a wide range of climatic and environmental conditions. The similarity maps produced provide valuable information in determining the confidence with which relationships derived from present HDSS field sites can be extended to other areas, and to highlight areas where the location of new HDSS field sites would improve significantly the environmental and socioeconomic coverage of the network. The results also indicate suites of field sites that form cohesive groups and from which data can be logically summarized.

737

EVALUATION OF POINT-OF-CARE AND POINT-OF-NEED MULTIPLEX DIAGNOSTIC DEVICES FOR EASE OF USE AND FOR TRANSMITTING RESULTS THROUGH A SECURE ENCRYPTED REMOTE NETWORK

Robert D. Hontz[1], David A. Stenger[2], Shawn P. Mulvaney[2], Amy C. Morrison[3], Valerie A. Paz-Soldan[4], Pamina M. Gorbach[5], Christopher A. Myers[6], Erin A. McDonough[6], Kathlyn Santos[7], Andrea J. McCoy[8], Guillermo Pimentel[8], Eric S. Halsey[9], Patrick J. Blair[10], Shevin T. Jacob[11]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Naval Research Laboratory, Washington, DC, United States, [3]U.S. Naval Medical Research Unit - 6, Iquitos, Peru, [4]Tulane University, New Orleans, LA, United States, [5]University of California Los Angeles, Los Angeles, CA, United States, [6]Naval Health Research Center, San Diego, CA, United States, [7]Johns Hopkins University, Laurel, MD, United States, [8]Naval Medical Research Center, Frederick, MD, United States, [9]Centers for Disease Control and Prevention, Atlanta, GA, United States, [10]Naval Medical Research Center - Asia, Singapore, Singapore, [11]University of Washington, Seattle, WA, United States

The United States Department of Defense's (DoD) Defense Threat Reduction Agency recently initiated a program designed to drive development of novel point-of-need (PON) diagnostic devices within a biosurveillance network for capturing emerging infectious diseases in austere locations. DoD biomedical laboratories are evaluating two devices in South America (Peru), Australia, Southeast Asia (Thailand, Cambodia), and Africa (Sierra Leone) to mimic real-world settings similar to those encountered by deployed military personnel. These multiplex devices are designed to detect pathogens that cause dengue fever, malaria, plague, and melioidosis: a clinic/medic-based point-of-care (POC) molecular diagnostic device for use in health care clinics and hospitals with health-care providers as the end-user, and a patient-administered "buddy-test" PON device to be used in community households with the lay person as the end-user. For POC testing, 11 Investigational Use Only (IUO)-labeled systems and 1000-2000 test pouches will be distributed to each site. Nearly 1400 POC subjects will be enrolled in Peru with an expected dengue disease rate of 20-25%, and 2500 subjects will be enrolled in SE Asia with an expected melioidosis disease rate of 10%. For PON testing, 7000 to 9000 Research-Use Only (RUO)-labeled devices will be distributed across all locations to detect dengue (Peru), malaria, plague, and melioidosis (all other locations). De-identified and blinded results from these PON and POC tests will be compared to gold standard testing for study of device sensitivity and specificity (aiming for 85% for Role 1; 75% for Role 0), then wirelessly communicated through a secure encrypted remote network (SERN) using an FIO reader (success considered at 90% accuracy). Successful demonstration of delivery of de-identified and encoded test results through the SERN will enable forward operators direct access to surveillance and reach-back analysis remotely in locations devoid of established medical treatment facilities, and will provide information about infectious disease threats.

738

U.S. DEPARTMENT OF DEFENSE GLOBAL FEBRILE AND VECTOR-BORNE ILLNESS SURVEILLANCE PROGRAM

Jeffrey McCollum, Alaina C. Thomas, Clara G. Marshall, James F. Cummings

Armed Forces Health Surveillance Center, Silver Spring, MD, United States

Originally established by Presidential Directive in 1997, the Department of Defense (DoD) Global Emerging Infections Surveillance and Responsive System (GEIS) was expanded in 2006 and subsequently incorporated as a Division of the Armed Forces Health Surveillance Center (AFHSC) in 2008. AFHSC-GEIS divides its surveillance approach into five infectious disease categories: respiratory, gastrointestinal, febrile and vector-borne, antimicrobial resistant, and sexually transmitted. The goal of the GEIS febrile and vector-borne illness (FVBI) surveillance program is to integrate febrile illness, arthropod-vector, and pathogen discovery surveillance systems that contribute to Force Health Protection and global public health through: 1) characterizing the geographic distribution, transmission, and risk of FVBI pathogens and related illnesses; 2) outbreak detection and response; 3) generation of actionable surveillance data supporting informed patient care and FVBI disease risk assessments; 4) promoting FVBI-related research, training, and capacity-building initiatives. During fiscal year 2014, the FVBI program funded 36 competitive proposals supporting surveillance efforts in 32 countries. Recent program accomplishments include extensive global surveillance for drug-resistant malaria with initiation of a multi-site clinical trial evaluating *Plasmodium falciparum* artemisinin resistance in Thailand, Kenya, and Peru. Flea, chigger, and animal reservoir collections data (>90,000 data points) were also incorporated into the GEIS-funded VectorMap program, a publicly available repository for global arthropod vector collection data (www.vectormap.org). AFHSC-GEIS coordinates a worldwide surveillance network comprised of military and civilian laboratory partners who conduct FVBI surveillance in US military as well as foreign military and civilian populations. GEIS-funded initiatives yield enhanced recognition of the risks and threats from FVBI supporting the public health needs of military and associated populations in a growing number of partner nations.

739

PROGRAMMATIC ASSESSMENT TOOL FOR RISKS OF MEASLES OUTBREAKS IN THE PHILIPPINES

Eugene Lam[1], Maricel de Quiroz-Castro[2], Maria Joyce U. Ducusin[3], Dulce Cecilio-Elfa[4], W. William Schluter[5], Sigrun Roesel[2]

[1]Centers for Disease Control and Prevention, Center for Global Health, Global Immunization Division, Atlanta, GA, United States, [2]World Health Organization, Office of the World Health Organization Representative in the Philippines, Manila, Philippines, [3]National Center for Disease Prevention and Control, Department of Health, Manila, Philippines, [4]National Epidemiology Center, Department of Health, Manila, Philippines, [5]World Health Organization, Western Pacific Regional Office, Manila, Philippines

Measles is a highly contagious viral disease and remains an important cause of death and disability among children globally. In 2012, the World Health Organization (WHO) Regional Committee for the Western Pacific Region (WPR) reaffirmed its commitment to eliminate measles and urged member states to interrupt endemic measles virus transmission as rapidly as possible. Despite the Philippines' continued commitment to this goal and a nationwide supplemental immunization activity conducted with measles and rubella vaccine targeting children aged 9 months to <8 years in 2011, a total of 1499 cases of measles were reported in 2012. Identifying areas at risk of outbreaks can provide an effective strategy in targeting immunization efforts and preventing future outbreaks. Based on a polio risk assessment tool developed in WPRO and applied in the Philippines, an assessment tool was developed to assess risk of measles outbreaks. Overall risk of an outbreak was assessed as a function of indicator scores that fall into four main categories of risk components (with corresponding weights): population immunity (50%), surveillance quality (24%), program delivery performance (6%), and threat probability assessment (20%). Cut-off criteria for each indicator score were created to assign an overall risk category score (RCS). The RCS was categorized into very high, high, medium, or low risk using available data at the second subnational administrative level. Preliminary results of the risk assessment compared with reported measles cases in 2013 showed clusters of laboratory-confirmed measles cases in areas identified as high and very high risk. Risk assessments can be used for advocacy to communicate risk to policymakers, mobilize resources, and strategically guide immunization response accordingly to the level of risk. Ongoing evaluation of indicators is needed to effectively plan risk mitigation activities and to evaluate performance towards elimination of measles. Further studies are needed to pilot test the risk assessment tool in other countries and regions.

740

RISK FACTORS AND PREDICTORS OF PRETERM BIRTH IN DAR ES SALAAM, TANZANIA

Grace J. Chan[1], Ayesha Sania[1], Gernard I. Msamanga[2], Willy Urassa[2], Ellen Hertzmark[1], Donna Spiegelman[1], Wafaie W. Fawzi[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Muhimbili University College of Health Sciences, Dar es Salaam, United Republic of Tanzania

Preterm birth is associated with early life mortality and morbidity. In Tanzania, 1900000 babies are born each year of which 11.45% are preterm (less than 37 weeks gestation). Complications of preterm birth account for 40% of neonatal mortality and survivors are at high risk for long-term neurodevelopmental and behavioral sequelae. We aim to identify the incidence, risk factors, and predictors for preterm birth in Dar es Salaam, Tanzania. We prospectively followed 8428 mother-newborn pairs enrolled from 2001-2004. Maternal and socioeconomic characteristics, obstetric history, and medical illnesses were collected during pregnancy. Birth outcomes included small for gestational age (SGA, weight below the 10th percentile for gestational age) and preterm birth (less than 37 weeks gestation). We conducted bivariate and multivariate analyses using log-binomial regression models to examine the effect of predictor variables on outcomes. Of the 8428 pregnant women, 8003 (95.0%) gave birth to live newborns and were eligible for analysis. The incidence of preterm birth was 16.7%. Risk of preterm birth was associated with older maternal age greater than 34 years of age (RR=1.14 [unit=1 year]; 95%CI 1.07-1.20), wealth in the lowest quintile (RR=1.24; 95% CI 1.11-1.39), less than one year of education (RR=1.67; 95%CI 1.44-1.93), and lower mid-upper arm circumference (RR=1.06 [unit=1cm]; 95%CI 1.04-1.07). In Dar es Salaam, preterm birth is associated with maternal age, wealth, and education, which may be surrogates to unmeasurable factors affecting fetal development. Mid-upper arm circumference, a measure of maternal nutrition, is one modifiable factor associated with preterm birth.

741

PREDICTORS OF EARLY MODERATE TO SEVERE STUNTING IN BOLIVIAN INFANTS (0-6 MONTHS)

Paulina A. Rebolledo[1], Michael D. Garber[1], Rachel M. Burke[1], Rebecca Heritage[1], Rita Revollo[2], Juan S. Leon[1]

[1]Emory University, Atlanta, GA, United States, [2]Hospital Infantil Los Andes, La Paz, Plurinational State of Bolivia

Childhood malnutrition, particularly stunting (low length-for-age), can have long-term adverse effects like impaired cognitive function, increased risk of chronic disease, decreased economic potential, and increased risk of maternal mortality. Bolivia, a lower-middle-income country, has one of the highest prevalence of infant malnutrition in the Americas with an estimated 9.5% of six month old infants being moderately to severely stunted. The purpose of this study was to identify predictors of moderate-to-severe stunting (length-for-age Z score <-2) among urban and peri-urban Bolivian children in early infancy (0-6 months of age). Convenience sampling at outpatient well-child visits was used to recruit 185 mother-infant pairs in El Alto, Bolivia, from June to October 2011. Researchers collected anthropometric data from mothers (height, weight) and infants (length, weight, head circumference) at two visits (4 to 6 weeks apart) as well as baseline socio-demographic, clinical, and perinatal characteristics. Multivariable logistic regression was used to identify predictors of being stunted at both visits. The prevalence of being stunted at both visits was 15.7%. Multivariable logistic regression showed that breastfeeding (OR:0.29 95%CI[0.1-0.81]), preterm birth (OR: 10.25 95% CI[3.26-32.23]), small-for-gestational age (OR:6.67 95% CI[2.21-20.18]), and inter-birth spacing of less than 24 months (OR:7.21 95% CI[2.08-24.94]) were significantly associated with stunting in this study population. Although this study was limited in its small sample size (leading to large standard deviations), its results were consistent with prior literature identifying preterm birth and small-for-gestational age (SGA) as predictors of childhood stunting. These results emphasize the need for targeted interventions to foster optimal in-utero growth and prevent small-for-gestational age and preterm births, which would lead to lower rates of stunting in Bolivia.

742

THE PUBLIC HEALTH IMPACT AND COST-EFFECTIVENESS OF DIAGNOSTIC AND PROGNOSTIC TOOLS FOR CASE MANAGEMENT OF NON-MALARIAL FEBRILE ILLNESS IN CHILDREN UNDER FIVE

Lindsey Wu, Azra C. Ghani

MRC Centre for Outbreak Analysis and Modelling, Department of Infectious Disease Epidemiology, Imperial College London, London, United Kingdom

As malaria transmission declines globally, there is increasing recognition of the importance of correct identification and treatment of non-malarial febrile illness (NMFI). Studies have shown that in many regions, a decrease in antimalarial therapy has been accompanied by a simultaneous increase in antibiotic use. In the absence of appropriate diagnostic and prognostic tools, currently recommended guidelines for integrated management of childhood illness (IMCI) can potentially lead to overuse of antimalarials and antibiotics as well as under-referral of patients with early signs of severe illness requiring hospital treatment. We developed a Markov chain model to assess a range of diagnostic and prognostic tools for NMFI implemented at different levels of the healthcare system in terms of their impact on mortality and cost. The analysis was undertaken in a range of developing country healthcare infrastructure scenarios, including a comparison of factors such as high vs. low health facility access and public vs. private vs. community health systems. The model is paramaterised using data on healthcare access, care-seeking behaviour, and epidemiological and clinical characteristics of febrile illness across a range of countries. Sensitivity analysis is conducted using data on the sensitivity and specificity of diagnostic and prognostic tools for identifying NMFI etiology and symptoms of early severe illness. The methods outlined here can be used to optimise case management strategies across a portfolio of potential diagnostic and prognostic tools being considered for development and implementation.

743

STRENGHTENING MALARIA AND ANTENATAL CARE CONTROL PROGRAMS WITH A SYSTEM COMBINING AVAILABLE TECHNOLOGIES

Seif Shekalaghe[1], Humphrey Mazigo[2], Omary Juma[1], Salim Abdulla[3], Caroline Mavere[1], Kassim Kamaka[1], John Changalucha[2], Santiago Ferro[4]

[1]Ifakara Health Institute, Bagamoyo, United Republic of Tanzania, [2]NIMR, Mwanza, United Republic of Tanzania, [3]Ifakara Health Institute, Dar-es-Salaam, United Republic of Tanzania, [4]Fio Corporation, Toronto, ON, Canada

RDTs are considered a viable alternative to deliver accurate results at POC. However, there are still obstacles to the widespread implementation of this strategy, such as lack of proper quality assurance of RDT-based programmes at POC and reporting constraints, especially in remote areas of low-income countries where the strategy is mostly needed. In collaboration between IHI and NIMR, an implementation research study was conducted in the Geita District to test the feasibility and usability of a system designed to overcome the issues described above. In brief, the system called Fionet is designed to assist peripheral HCWs in the processing and interpretation of RDTs, and collect and transmit clinical data to a cloud information service using local cell phone networks. Information captured is useful for evaluation of quality of RDT by district authorities. HCWs were trained in the use of mobile devices which contained electronic survey forms easily completed through a touch-

screen interface and at the same time guide RDT processing and perform automated interpretation of the results. All information collected and a high-resolution image of the RDT was transmitted to a central database located in a cloud information service. Data were safely stored and organized according to predetermined reports and was accessed via a website . Two patient populations were included in the current study: 1- general population (children and adults, male and female) in whom a malaria test was required; and 2- pregnant women attending ANC clinics in whom screening for both malaria and syphilis was performed to prevent mother to child transmission and adverse outcome of pregnancies. HCWs at all government facilities participating in the pilot were able to operate the system and collected over 5,000 patients in the course of 8 weeks. Health program managers were able to login to the portal and review cases uploaded, aggregated and organized in predefined reports, enabling to make recommendations about program management including monitoring of RDT processing in the field. Scale up of a system such as Fionet to at least a full-district level is warranted after the encouraging results of the present study, to fully demonstrate health system strengthening opportunities in delivery of care, monitoring and evaluation and improvement in system efficiencies.

744

TRAINING AND CAPACITY BUILDING INITIATIVES IN SUPPORT OF GLOBAL HEALTH SECURITY, 2012-2014

Clara G. Marshall, Christopher L. Perdue, Matthew R. Kasper, James F. Cummings, Jose L. Sanchez

Armed Forces Health Surveillance Center, Silver Spring, MD, United States

The Global Health Security Agenda, launched on 13 February 2014, called upon US government agencies to work together towards a vision of "a world safe and secure from global health threats posed by infectious diseases" by engaging with partner nations to strengthen their capacity for preventing avoidable epidemics, detecting infectious disease threats early, and responding rapidly and effectively. The Global Emerging Infections Surveillance and Response System Division of the Armed Forces Health Surveillance Center (AFHSC-GEIS) supports programs that strengthen global networks for real-time biosurveillance capabilities, train an effective biosurveillance workforce, and strengthen laboratory systems. In fiscal years 2012-14, AFHSC-GEIS supported a global health capacity building portfolio that included 67 projects in 34 countries. Specifically, AFHSC-GEIS partners executed health system improvements in six main areas: 1) electronic disease and biosurveillance systems (29 projects in 15 countries); 2) workforce development in WHO-approved epidemiology and outbreak response methods (23 projects in 20 countries); 3) tropical medicine training for host-country civilian and military personnel (11 projects in 10 countries); 4) training in, and capacity for, entomological surveillance and control methods (10 projects in 7 countries); 5) accredited laboratory practices and quality assurance efforts (34 projects in 21 countries); and, 6) health care facility and laboratory diagnostic capacity development (18 projects in 16 countries). The results of qualitative analysis of quarterly and annual reports from AFHSC-GEIS partners indicate that, while challenging, there has certainly been a progressive evolution of health security capacities within the network.

745

DETERMINANTS OF DENGUE MORTALITY BEYOND BIOLOGICAL FACTORS: A SCOPING REVIEW

Mabel Carabali[1], Libia Milena Hernandez[2], Maria Jose Arauz[3], Luis Angel Villar[4], Valéry Ridde[5]

[1]Dengue Vaccines Initiative/International Vaccines Institute, Seoul, Republic of Korea, [2]Centro de Atencion y Diagnostico de Enfermedades Infecciosas (CDI-APRESIA), Bucaramanga, Colombia, [3]Department of Social and Preventive Medicine, University of Montréal, Montreal, QC, Canada, [4]Universidad Industrial de Santander, Bucaramanga, Colombia, [5]University of Montreal Hospital Research Centre (CRCHUM)/School of Public Health (ESPUM), University of Montreal, Montreal, QC, Canada

Dengue is a viral disease which clinical spectrum varies from unapparent to severe forms and fatal outcomes. Though dengue death is avoidable in 99%, every year around 20000 deaths are estimated to occur in more than 100 countries. Beyond biological factors, social determinants of health (SDH) are related to fatal outcomes. A scoping review and content analysis using QDA Miner was conducted to document the role of SDH in dengue mortality. Inclusion criteria were any document with information of dengue fatal cases in humans, written in English, Spanish, Portuguese or French, peer reviewed or grey literature. Using a set of key words related to dengue mortality, PubMed /LILACS /COCHRANE/Scielo/Science Direct/WHOLIS and Google Scholar, were the electronic databases used. From a total of 971 documents retrieved, 78 articles met the criteria and were reviewed. The documents were published in the Americas region (50%), Asia (38%), Europe (9%) and Africa (3%). The main article's source of information was hospital records (56%) followed by a mix of surveillance data and hospital records (33%). Ninety-three percent included any information about the SDH. Information about individual dimension was found in 88.5% of reviewed articles, where age, education and type of infection/immunological status were considered determinants for dengue deaths. Sixteen articles (20.5%) did mention about health systems and described determinants were access, opportunity, quality of attention and health staff knowledge. Three articles (3.8%) reported socio-economic and political context were poverty and social behavior were the determinants described. Gender and opportunity of attention were considered as dengue determinants dependent on social/personal health seeking behavior. Ethnicity was considered as biological determinant that also depends on cultural and socioeconomic context. These findings reveal the need for more studies about the role of SDH in dengue mortality, in order to design and implement interventions beyond biological factors in areas such education and the health systems (more data will be available at the presentation)

746

LABORATORY QUALITY MANAGEMENT SYSTEM: EXPERIENCE WITH IMPLEMENTATION IN LATIN AMERICA AND THE CARIBBEAN

Magaly Tejada, Drake H. Tilley, Diana Zorrilla, Jose Calderon, Nathanael Reynolds, **Silvia M. Montano**

U.S. Naval Medical Research Unit - 6, Callao, Peru

Laboratories are essential for public health activities to include disease surveillance and control and in the treatment of diseases. The Quality Management System (QMS) in clinical laboratories emphasizes efficient use of resources, processes and personnel to reduce errors and ensure reliable and safe results. The aim of the joint CDC DHAPP NAMRU-6 collaborative effort sponsored by DHP and PEPFAR is to strengthen QMS in clinical laboratories throughout Latin America and the Caribbean (LAC) to improve and measure compliance with quality laboratory services and prepare regional laboratories towards accreditation. Since 2012, 18 clinical laboratories in Belize, El Salvador, Guatemala, Honduras, Nicaragua, Dominican Republic, Colombia and Peru were enrolled in an improvement program. It began with a 5-week training workshop in Lima, Peru to include 2-weeks of training in Strengthening Laboratory

Management Towards Accreditation (SLMTA), followed by distance mentoring and technical assistant visits. We conducted baseline laboratory assessments using the Stepwise Laboratory Improvement Process towards Accreditation Checklist (SLIPTA WHO/AFRO), developed by WHO/AFRO for evaluating and monitoring the improvement progress of laboratories aiming to achieve the ISO 151889 standards. We report 12 laboratories which have now completed 12 months of the program with before and after evaluations. Among the 12 laboratories assessed, average baseline score was 118(78-144), representing 44% compliance with the SLIPTA WHO/AFRO checklist. The average score after 12 months was 14% higher at 151(120-180)representing 58% compliance. Among the 12 quality management systems evaluated, the three areas with greatest improvement included, customer management and customer service (28%), purchasing and inventory process control (16%), and Evaluation of internal and external quality control (15%). Information management (9%) and facilities and safety (4%) showed the least improvement. In conclusion, implementation of the QMS improved multiple areas of laboratory service and processes in diverse settings across LAC. Results of the initial post-testing evaluation will guide future training and resources for further improvement and help lead to the permanent adoption of effective laboratory practices.

747

CHARACTERIZATION OF *ANOPHELES GAMBIAE* HEME OXYGENASE

Christopher S. Spencer

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Heme is a double-edged sword, being vital for oxygen transport yet highly toxic at high concentrations. Heme oxygenase (HO) plays a key role in detoxification through heme catabolism. Present in many organisms it is particularly well characterised in humans. Surprisingly, however, HO is poorly characterised in hematophagous insect vectors such as mosquitoes, yet such organisms deal with enormous influxes of dietary heme in their bloodmeals. Thus one might expect the enzyme to play a key role in survival. Here we are investigating the role of HO in mosquitoes and tsetse flies, which transmit malaria and sleeping sickness respectively. HOs in both organisms were identified via genome database searches, amplified, cloned, and expressed in E. coli. Spectroscopic assays have been done to confirm the catalytic attributes of vector HO *in vitro*, as well as Western blotting, qRT-PCR and immunolocalisation studies to investigate tissue-specific and temporal variation in HO expression. HPLC analysis is being conducted to identify the products of the HO reaction pathway. Finally, inhibition of HO activity has been carried out *in vivo* to determine the physiological role of HO. Here, a competitive HO inhibitor (zinc protoporphyrin) was fed to *Anopheles gambiae* populations. This resulted in a significant reduction in oviposition, suggesting a key role for HO in egg production. It is hoped that the results of these studies will identify new targets for the development of novel vector control agents.

748

THE CIRCADIAN CLOCK AND LIGHT/DARK CYCLE INFLUENCE RNA EXPRESSION IN THE *AEDES AEGYPTI* MOSQUITO

Matthew T. Leming[1], Samuel S. Rund[2], Giles E. Duffield[1], Joseph E. O'Tousa[1]

[1]University of Notre Dame, Notre Dame, IN, United States, [2]University of Edinburgh, Edinburgh, United Kingdom

The biological rhythms of mosquitoes regulate and/or modify many behaviors and physiological processes underlying disease transmission, survivability, and fitness. The circadian clock regulation of RNA expression influences physiological and behavioral output on a daily basis. The *Aedes aegypti* mosquito is the major vector of Yellow fever and dengue virus, and exhibits 24 hr rhythms in biting, flight, and oviposition. To better understand the patterns of specific gene expression controlled by the daily light/dark (LD) cycle or the circadian clock in *Ae. aegypti*, we undertook a microarray analysis (>90% transcriptome) of adult female mosquito heads collected every 4 hr over 2 days maintained under LD or constant dark (DD) conditions. Data were subjected to cosine wave analysis (JTK_CYCLE) to determine for ~24 hr rhythmicity. We generated the *Aedes aegypti* Circadian Database, available at http://www.nd.edu/~bioclock/, in which these gene expression profiles and accompanying stastical analysis are searchable. This database provides the capability to examine the potential rhythmicity of the expression profile for each gene, as influenced by the LD cycle or the endogenous circadian clock. We identified 4 classes of genes present within the transcriptome: rhythmic only under LD cycle conditions, rhythmic only under DD conditions, rhythmic under both conditions, and non-rhythmic. The sets of genes active in vision, metabolism, and olfaction were investigated further, and those associated with vision were in good agreement with an earlier study on *An. gambiae* mosquitoes (Rund et al., 2011 PNAS 108:e421-30). Similarly, *Ae. aegypti* genes involved in metabolism and detoxification were under circadian control or non-rhythmic, as predicted by studies of *An. gambiae*. Our *Ae. aegypti* studies demonstrate similarities and differences in regulation of transcripts in different mosquito species, and suggest temporal modulation of processes such as vision and metabolism. These gene expression patterns likely identify both shared traits and the basis of species-specific behaviors.

749

DISSECTING THE FINE-SCALE MATING INTERACTIONS IN THE DENGUE VECTOR MOSQUITO *AEDES AEGYPTI*

Catalina Alfonso- Parra

Cornell University, Ithaca, NY, United States

Understanding mosquito mating biology is an important step towards identifying novel targets for mosquito control. We have begun dissecting the fine-scale mating biology in male *Aedes aegypti*. Seminal fluid proteins are produced in the reproductive tract tissues of male insects, primarily in the accessory glands, and are transferred _ along with sperm _ to females during mating. Seminal fluid proteins induce numerous physiological and behavioral changes in mated females, each of which might be targeted for novel control strategies. Our work on top candidates for genetic manipulation and the biology of male accessory gland function will be presented. Finally, and to further comprehend mosquito mating, we analyzed transcriptional changes within the lower female reproductive tract in response to male seminal fluid protein receipt. Our results suggest a wealth of new targets for control of these important disease vectors.

750

IMPACT OF INCREASED INSULIN SIGNALING IN THE FAT BODY OF *ANOPHELES STEPHENSI* AND *AEDES AEGYPTI* MOSQUITOES ON LIFESPAN AND REPRODUCTION

Lewis Hun, Anam Arik, Kendra Quicke, Patricia Scaraffia, Hemant Badgandi, Michael Piatt, Michael Riehle

University of Arizona, Tucson, AZ, United States

Lifespan is a key factor in determining the transmission efficiency of mosquito borne diseases. Finding a novel mechanism affecting mosquito lifespan could be a valuable tool to control mosquito-borne disease transmission. The insulin/insulin-growth factor signaling (IIS) pathway may provide a novel endogenous solution to vector control. In mosquitoes, the IIS plays an important role in the regulation of many physiological processes, including longevity and reproduction. Here we aimed to increase insulin signaling in the fat body of *An. stephensi* and *Ae. aegypti* mosquitoes by creating a transgenic line expressing an active form of Akt, a key component of the IIS, specifically in the fat body tissue. We observed the effects on longevity and reproduction in a heterozygous line. However, contrary to the expected results, we observed an increase in the life span of heterozygous females positive for the transgene. We also observed no significant difference in the reproductive potential of heterozygous positive versus heterozygous negative females, an effect that was opposite of the

anticipated result. Ongoing work on this transgenic mosquito may yield unique insights into how the insulin signaling cascade regulates lifespan in mosquitoes and other eukaryotes.

751

CROSSTALK OF IMD AND TOR SIGNALING PATHWAYS IN THE IMPORTANT DENGUE VECTOR *AEDES AEGYPTI*

Shin-Hong Shiao

National Taiwan University, Taipei, Taiwan

Mosquitoes transmit several devastating infectious disease such as malaria, dengue fever, yellow fever, Japanese encephalitis and filariasis. Due to the lack of effective vaccine and the increasing drug and insecticide resistance, alternative approaches for these mosquito-borne diseases are urgently required. It has been demonstrated that the Toll and the Immune Deficiency (IMD) signaling pathways play crucial roles in the production of antimicrobial peptides in the Drosophila. Recently, the JNK pathway was shown to be activated by transforming growth factor-β-activated kinase 1 (TAK1), the component of IMD pathway, and to play an important role in tissue modeling in Drosophila. On the other hand, Matrix metalloproteinase 1 (MMP1) was demonstrated to be essential for the embryonic development in the Drosophila. Our previous study showed that *Aedes aegypti* TAK1 (AaTAK1) is responsible for the production of Cecropin A. We also showed the novel role of *Aedes aegypti* Matrix metalloproteinase 1 (AaMMP1) in the regulation of vitellogenesis. Our results revealed that silence of AaTAK1 by RNA interference approach resulted in the inhibition of AaMMP1 in the translational level. In this study, we showed that the transcriptional pattern of AaTAK1 is highly expressed from 12 to 72 hours after a blood meal and particularly in the ovary and midgut. By RNAi-mediated silencing of AaTAK1, we showed that the egg production was reduced in the absence of AaTAK1. Interestingly, the expression of Vitellogenin (Vg) was inhibited in the absence of AaTAK1 or with the application of JNK inhibitor (SP600125) in the *in vitro* fat body culture system. In addition, by RNAi mediated silencing of AaJNK, the egg production was also reduced. Furthermore, we showed that silencing of AaTAK1 or AaJNK inhibit the phosphorylation of S6K, a key component of TOR pathway, and also inhibit the expression of Vitellogenin (Vg) and AaMMP1 in the fat body. Taken together, our data suggest a novel function of AaTAK1 and AaJNK in the regulation of vitellogenesis and AaMMP1 through TOR signaling pathway.

752

GENETIC BASIS OF PYRETHROID RESISTANCE IN A POPULATION OF *ANOPHELES ARABIENSIS*, THE PRIMARY MALARIA VECTOR IN LOWER MOSHI, NORTHEASTERN TANZANIA

Johnson J. Matowo[1], Chris Jones[2], Martin Donnelly[2], Bilali Kabula[3], Hilary Ranson[2], Keith Steen[2], Franklin Mosha[1], Mark Rowland[4], David Weetman[2]

[1]Kilimanjaro Christian Medical University College, Moshi, United Republic of Tanzania, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]National Institute for Medical Research, Tukuyu, Mbeya, United Republic of Tanzania, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom

Pyrethroid resistance has been slower to emerge in *Anopheles arabiensis* than in *An. gambiae* s.s and *An. funestus* and, consequently, studies are only just beginning to unravel the genes involved. Permethrin resistance in *An. arabiensis* in Lower Moshi, Tanzania has been linked to elevated levels of both P450 monoox ygenases and β-esterases. We have conducted a gene expression study to identify specific genes linked with metabolic resistance in the Lower Moshi *An. arabiensis* population. Microarray experiments employing an *An. gambiae* whole genome expression chip were performed on *An. arabiensis*, using interwoven loop designs. Permethrin-exposed survivors were compared to three separate unexposed mosquitoes from the same or a nearby population. A subsection n of detoxification genes were chosen for subsequent quantitative real-time PCR (qRT-PCR). Microarray analysis revealed significant over expression of 87 probes and under expression of 85 probes (in pairwise comparisons between permethrin survivors and unexposed sympatric and allopatric samples from Dar es Salaam (controls). For qRT-PCR we targeted over expressed ABC transporter genes (ABC '2060'), a glutathione-S-transferase, P450s and esterases. Design of efficient, specific primers was successful for ABC '2060'and two P450s (CYP6P3, CYP6M2). The primers for CYP4G16 used were previously used in a microarray study of *An. arabiensis* from Zanzibar islands. Over expression of CYP4G16 and ABC '2060'was detected though with contrasting patterns in pairwise comparisons between survivors and controls. CYP4G16 was only up regulated in survivors, whereas ABC '2060'was similar in survivors and controls but over expressed in Lower Moshi samples compared to the Dar es Salaam samples. Increased transcription of CYP4G16 and ABC '2060'are linked directly and indirectly respectively, with permethrin resistance in Lower Moshi *An. arabiensis*. Our study provides replication of CYP4G16 as a candidate gene for pyrethroids resistance in *An. arabiensis* though its role may not be in detoxification and this requires further investigation.

753

WNT SIGNALING IS ESSENTIAL FOR THE REGULATION OF TOR SIGNALING-MEDIATED EGG PRODUCTION IN THE MOSQUITO *AEDES AEGYPTI*

Shih-Che Weng

College of Medicine, National Taiwan University, Taipei, Taiwan

Mosquito-borne diseases are the most devastating agents for human being, due to its high diversity of transmissible pathogens like protozoan and viruses. Despite the efforts from government agencies that have contributed the eradication of the mosquito-borne diseases for several decades, the goal has not been achieved yet. Therefore, many research institutes turn their attentions toward the mosquito life cycle and immune system to halt the disease transmission. Previous studies have already been demonstrated that Target of Rapamycin (TOR) pathway plays an important role in mosquito vitellogenesis, whereas WNT pathway participates in the embryonic development and cell polarity in Drosophila. However, the interactions between these pathways are poorly understood. In this study, we propose a hypothesis that factors of TOR and WNT signaling pathway play synergistically in the mosquito vitelloginesis. We attempt to characterize Wnt signaling components in the mosquito, *Aedes aegypti*. Our results showed that silencing of *Aedes aegypti* Frizzled2 (AaFz2), a transmembrane receptor of Wnt signaling pathway, resulted in the decrease of fecundity in *Ae. aegypti*. We showed that AaFz2 is highly expressed in the mosquito fat body at 6 hours post blood meal in turns of transcriptional and translational level, suggesting the amino acid-stimulated feature of AaFz2. Interestingly, the phosphorylation of S6K and the expression of Vg were inhibited in the absence of AaFz2. These findings determine a direct link between Wnt and TOR signaling in the regulation of mosquito reproduction.

754

FUNCTIONAL ANALYSIS OF THIOESTER-CONTAINING PROTEIN COMPLEX IN DENGUE VIRUS INFECTION

Tsun Wang, Shin-Hong Shiao

National Taiwan University, Taipei, Taiwan

Dengue fever is one of the most devastating arthropod-borne diseases. The WHO reported some 2.5 billion people are now at risk from dengue and estimates that there may be 50 million cases of dengue infection worldwide every year. Up to now, no effective dengue vaccine or drug has been developed. Therefore, intensive study for potential host factors in the mosquito vector and construction of transgenic mosquito together with gene drive technique to replace the vector populations became an alternative strategy to combat dengue virus. Here, we made use of

the well-established reverse genetic approach by silencing potential host factors in the mosquitoes. Our results showed that two immune responsive genes, Thioester-containing protein 1 (TEP1) and Leucine-Rich Immune Gene 1 (LRIM1), were highly expressed in response to dengue virus infection in the mosquito midgut. Silenced of TEP1 or LRIM1 resulted in the over-expression of transcript of dengue virus 2 in the mosquito midgets. Immunofluorescent assay revealed that silenced of TEP1 or LRIM1 resulted in the over-expression of DENV E-protein in the mosquito midgets. We also demonstrated that the translation level of TEP1 is highly expressed in the mosquito midgut after an infectious blood meal. Taken together, our results suggest TEP1 or LRIM1 are important candidates for the establishment of transgenic mosquito against dengue virus. We are currently in the construction of gain-of-function TEP1 transgenic mosquito line with the blood meal inducible carboxypeptidase promoter. Our results will provide new insights into the understanding of dengue-vector interaction and new strategy for dengue eradication program. Data revealed by this proposal will be crucial for future studies on vector competence and vector control in the field.

755

MOSQUITOCIDAL PROPERTIES OF IGG TARGETING THE GLUTAMATE-GATED CHLORIDE CHANNEL IN THREE MOSQUITO DISEASE VECTORS (DIPTERA: CULICIDAE)

Jacob I. Meyers, Meg Gray, Brian D. Foy

Colorado State University, Fort Collins, CO, United States

Mosquito-borne diseases account for an estimated 1,434,000 deaths annually and 60,056,000 disability adjusted life years. Historically, the most successful strategies to control disease transmission have been by targeting mosquito vectors through the use of chemical insecticides. The glutamate-gated chloride channel (GluCl) is a conserved and highly sensitive target of the drug ivermectin. As an alternative to using chemical insecticides to kill mosquitoes, we tested the effects of purified immunoglobulin G (IgG) targeting the extracellular domain of GluCl from *Anopheles gambiae* (AgGluCl) on the survivorship of three mosquito disease vectors that vary in their sensitivity to ivermectin: *Anopheles gambiae* s.s. > *Culex tarsalis* > *Aedes aegypti*. AgGluCl IgG mixed in a single blood meal significantly reduced the survivorship of *An. gambiae* (LC_{50} =2.82mg/mL [2.68-2.96]), as did serially blood feeds containing 1/10th of the LC_{50} concentration. However, AgGluCl IgG did not affect the survivorship of *A. aegypti* or *C. tarsalis*. Transcriptional analyses showed AgGluCl mRNA was present in the *An. gambiae* adult female head and thorax, but not the abdomen and immunohistochemical staining showed AgGluCl expression only in the antenna, Johnston's Organ, supraesophageal ganglion and thoracic ganglia. Interestingly, injection of AgGluCl IgG into the hemocoel equally reduced the survivorship of *An. gambiae*, *A. aegypti* and *C. tarsalis*, suggesting that AgGluCl IgG sensitivity of blood fed *An. gambiae* is due to permissive antibody diffusion across the midgut. To analyze AgGluCl IgG's mode of action, we fed *An. gambiae* blood meals containing both AgGluCl IgG and ivermectin (a GluCl agonist). AgGluCl IgG attenuated the mosquitocidal effects of ivermectin, suggesting that AgGluCl IgG acts as a GluCl antagonist. These data characterize mosquito GluCl as an important insecticide and immunological target, further the science of developing mosquitocidal vaccines, and lend insight into the unique properties of the *Anopheles* midgut relative to other mosquitoes.

756

POST-INSEMINATION TRANSCRIPTIONAL RESPONSE IN THE LOWER REPRODUCTIVE TRACT OF *AEDES AEGYPTI* FEMALES

Ethan Degner, Catalina Alfonso, Laura Harrington

Cornell University, Ithaca, NY, United States

Insemination induces substantial changes in female *Aedes aegypti* mosquitoes. Physiologically, inseminated females accelerate blood digestion and oogenesis. Behaviorally, they increase oviposition, alter host-seeking behavior, and become refractory to subsequent insemination. While it is known that these changes are induced by male seminal fluid proteins, what happens in the female to produce these changes is largely unknown on the molecular level. In order to better understand these changes, their associated pathways, and the specific genes involved, we used RNA-seq to conduct a differential expression transcriptome analysis of the lower reproductive tract (LRT; i.e. the bursa copulatrix, spermathecae, common oviduct, and lateral oviducts) of female *Ae. aegypti*. Comparisons were made between virgin females and those at 0, 6, and 24 hours post insemination. Our results provide a framework for further investigation of the postcopulatory physiology and behavior of this important disease vector.

757

INVESTIGATION ON ENTOMOPATHOGENIC FUNGAL FAUNA OF MOSQUITOES IN BURKINA FASO: TRANSGENIC BIOCONTROL PERSPECTIVES

Etienne Bilgo

IRSS/Centre Muraz, Bobo Dioulasso, Burkina Faso

Unlike bacteria and viruses, fungi infect mosquitoes through direct contact with the cuticle and so lend themselves to strategies currently used for delivery of chemical insecticides, and in addition may provide environmentally preferred alternatives. Much attention has focused on the ascomycetes Metarhizium spp. and Beauveria spp. However it should be noted that not much is known about fungal pathogens of adult malaria mosquitoes in the field. In that respect this study aims to investigate entomopathogenic fungi which naturally infect mosquitoes in Africa and their potential use as genetically engineered biocontrol agents. The field collection of mosquitoes was carried out in Burkina Faso. Mosquitoes were collected alive and maintained in insectary conditions with access to sugar water till death. Every morning, dead mosquitoes were collected and isolated individually in a petri dish for fungal growth. Fungal isolation and culture was conducted in a sterile environment. Two different growth media were used. Potato Dextrose Agar, a common medium for isolating fungi, and Potato dextrose agar + CTAB + Chloramphenicol, selective medium. The analysis is ongoing to unravel an exhaustive list of entomopathogenic fungi. The natural virulence of these fungi toward malaria vectors will be subsequently tested. Final results will be available in coming months, before October 2014.

758

DETECTION OF POINT-MUTATIONS IN THE *KDR* GENE OF THE DENGUE VECTOR *AEDES ALBOPICTUS*

Jiabao Xu[1], Mariangela Bonizzoni[2], Daibin Zhong[2], Guiyun Yan[2], Xiaoguang Chen[1]

[1]Key Laboratory of Prevention and Control of Emerging Infectious Diseases of GD Higher Education Institute, Guangzhou, China, [2]Public Health, University of California Irvine, Irvine, CA, United States

The Asian tiger mosquito, *Aedes albopictus*, is the most invasive mosquito in the world and a main vector of dengue, chikungunya and other viruses. Though *Ae. albopictus* originated from east Asia, in recent 30 years, it has migrated to all of the continents except Antarctica. Treatment of public areas with high population density of *Ae. albopictus* with adulticides, mainly pyrethroids, is a recommended protective measure in response to Dengue epidemics and routine treatments with insecticides and is performed alongside source reduction through environmental modification. Intensive pyrethroid application poses selection pressure on mosquito populations for increased resistance. Physiological resistance to pyrethroids has been documented already in several *Ae. albopictus* populations, mainly from the native home range in Asia, abrading the sustainability of control programs based on these insecticides. Field collections of *Ae. albopictus* were conducted in six sites throughout the Guangdong province in South China and subjected to standard WHO tube assays with 0.045% deltamethrin based on discrimination dose determined by the *Ae. albopictus* reference susceptible strain. Between

375 and 658 females were tested per site. Mortality ranged between 28.3% and 84.9%, indicating wide-spread phenotypic resistance in south China. Apart from standard WHO tube assay, rapid and reliable detection of resistance is recognized as an important element of resistance management. Historically, the most used markers for pyrethroid resistance are mutations in the *para*-gated sodium channel gene (*kdr* gene) the pyrehtroid target site. Here we report the current polymorphism of the *kdr* gene in 10 world-wide populations across the world. A total of seven non-synonymous mutations were identified. Although the frequency of non-synonymous mutations was generally low, one population from southern China showed 41.9% frequency, suggesting that *kdr* point mutations are widely spread among *Ae. albopictus* population in various part in the world.

759

THE *ANOPHELES GAMBIAE* MOSQUITO MIDGUT TRANSCRIPTOME BY RNA-SEQ

Alvaro Molina-Cruz[1], Alejandro Padrón[1], Mariam Quinones[2], José M. Ribeiro[1], Urvashi Ramphul[1], Janneth Rodrigues[1], Kui Shen[2], Ashley Haile[1], José Luis Ramirez[1], Carolina Barillas-Mury[1]

[1]Laboratory of Malaria and Vector Research/National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States, [2]National Institutes of Health, Rockville, MD, United States

The mosquito midgut is the first organ to interact with malaria parasites. This tissue can mount effective antiplasmodial responses that limit parasite survival and disease transmission. RNA-Seq by Illumina sequencing of the midgut transcriptome was used to identify new genes and transcripts, contributing to refinement of *Anopheles gambiae* genome annotation. We sequenced ~223 million reads from *An. gambiae* midgut cDNA libraries generated from susceptible (G3) and refractory (L35) mosquito strains after *Plasmodium berghei* or *P. falciparum* infections. In total, 22,889 unique midgut transcript models were generated from both *An. gambiae* strain sequences combined, and 76% are potentially novel. Of these novel transcripts, 49.5% aligned with annotated genes and appear to be isoforms or pre-mRNAs of reference transcripts, while 50.5% mapped to regions between annotated genes and represent novel intergenic transcripts (NITs). Predicted transcripts were validated for midgut expression using qRT-PCR and microarray analysis, and novel isoforms were confirmed by sequencing. Coding potential analysis revealed that 43% of total midgut transcripts appear to be long non-coding RNA (lncRNA), and functional annotation of NITs showed that 68% had no homology to current databases from other species. Reads were also analyzed using *de novo* assembly and predicted transcripts compared with genome mapping-based models. Finally, variant analysis of G3 and L35 midgut transcripts detected 160,742 variants with respect to the *An. gambiae* PEST genome, and 74% were new variants. This in-depth sequencing and assembly of the *An. gambiae* midgut transcriptome doubled the number of known transcripts and tripled the number of variants known in this mosquito species. It also revealed existence of a large number of lncRNA and opens new possibilities for investigating the biological function of many newly discovered transcripts.

760

PYRETHROID SUSCEPTIBILITY OF MALARIA VECTORS IN FOUR DISTRICTS IN WESTERN KENYA

Eric O. Ochomo[1], Nabie M. Bayoh[2], Luna Kamau[3], Francis Atieli[2], John Vulule[2], Collins Ouma[1], Maurice Ombok[2], Kiambo Njagi[4], David Soti[4], Evan Mathenge[5], Lawrence Muthami[6], Teresa Kinyari[7], Krishanthi Subramaniam[8], Immo Kleinschmidt[9], Martin J. Donnelly[8], Charles Mbogo[10]

[1]Maseno University, Kisumu, Kenya, [2]KEMRI/Centers for Disease Control and Prevention Research and Public Health Collaboration, Kisumu, Kenya, [3]KEMRI–Centre for Biotechnology and Research Development, Nairobi, Kenya, [4]Division of Malaria Control, Ministry of Health, Nairobi, Kenya, [5]KEMRI-Eastern and Southern Africa Centre of International Parasite Control, Nairobi, Kenya, [6]KEMRI-Centre for Public Health Research, Nairobi, Kenya, [7]Department of Medical Physiology, University of Nairobi, Nairobi, Kenya, [8]Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [9]London School of Hygiene & Tropical Medicine, London, United Kingdom, [10]KEMRI-Centre for Geographic Medicine Research-Coast, Kilifi, Kenya

Implementation of insecticide resistance monitoring programs is necessary to ensure continued efficacy of insecticide-based interventions long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). This study was designed to investigate the extent and distribution of pyrethroid resistance in 4 districts of western Kenya (Nyando, Rachuonyo, Bondo and Teso). All four districts have received long lasting insecticide treated nets (LLINs) while Nyando and Rachuonyo districts have had indoor residual spray (IRS) campaigns for 3-5 years using a mix of pyrethroids. This study is part of a project (Implications of Insecticide Resistance) that aims to determine the impact of insecticide resistance on the efficacy of malaria control interventions. Three day old adult mosquitoes from either larval samples collected in the field, or F1s raised from blood-fed females collected from houses, were used for WHO tube bioassays with mortality recorded 24 hours post exposure. Resistance level was assigned based on the WHO 2013 guidelines. Once exposed, samples were identified to species using PCR. Results *Anopheles arabiensis* comprised at least 94% of all *An. gambiae* s.l. in Bondo, Rachuonyo and Nyando. Teso was a marked contrast case with 77% of all samples being *An. gambiae* s.s. Mortality to insecticides varied widely between clusters even in one district with mortality to deltamethrin ranging from 45-100%, while to permethrin the range was 30-100%. Mortality to deltamethrin in Teso district ranged from 44-95.4% in An arabiensis and 28-85.4% in *An. gambiae* s.s. To permethrin, mortality ranged between 5.9-95% and 34.6-100% in *An. arabiensis* and *An. gambiae* s.s. respectively although a Wilcoxon signed-rank test failed to show consistently higher or lower resistance in any one vector compared to the other ($Z = 0.1$, $P = 0.9203$). Cluster specific mortality of *An. arabiensis* between permethin and deltamethrin were not correlated ($Z = 2.9505$, $P = 0.2483$) . In conclusion, our results show high levels of pyrethroid resistance in western Kenya with intense spatial heterogeneity. The observation that insecticide resistance can vary within small geographical areas may allow evaluation of the impact of resistance on the efficacy of malaria control interventions within similar eco-epidemiological zones.

761

LIFE SHORTENING EFFECT OF OLYSET® DUO, A LONG-LASTING INSECTICIDAL NET INCORPORATING A MIXTURE OF PYRETHROID AND PYRIPROXYFEN, AGAINST PYRETHROID-RESISTANT MOSQUITO

John R. Lucas[1], John Invest[1], Kazunori Ohashi[2], Hayato Teshima[2], Yoshinori Shono[2]

[1]Vector Control Division, Sumitomo Chemical, UK, Plc., London, United Kingdom, [2]Health and Crop Sciences Research Laboratory, Sumitomo Chemical Co., Ltd., Hyogo, Japan

The increase in pyrethroid resistance in mosquitoes across Sub Saharan Africa has become a serious threat for malaria vector control, and threatens to reverse the recent gains achieved by the widespread use of bed nets. New bed net products are clearly needed to maintain these gains. It has previously been shown that contact of adult mosquitoes with pyriproxyfen treated materials can dramatically reduce egg laying and shorten the life expectancy of exposed females. Pyriproxyfen treated bed nets could therefore be used as a resistance management tool against to help prevent the selection of pyrethroid resistance. These findings led to the development of Olyset® Duo which is a long-lasting insecticidal mosquito net incorporating a mixture of 2% w/w permethrin and 1% w/w pyriproxyfen on all sides of the net. In this study we evaluate the life shortening effect of this net against susceptible and pyrethroid-resistant malaria vectors in the laboratory. Olyset Duo and PPF LN, a pyriproxyfen alone net, were washed 3 times according to WHO guidelines and exposed to a susceptible (Kisumu) and a kdr resistant strain (VK7) of *Anopheles gambiae* in the WHO tunnel test. Contact with the PPF LN significantly reduced the survival rate of blood-fed females of both strains, while contact with Olyset Duo killed the susceptible strain and reduced the longevity of surviving blood-fed females of the resistant strain, when compared with the effect of exposure to untreated netting. These results indicate that Olyset Duo could reduce vectorial capacity of malaria vectors through the life shortening effect of pyriproxyfen and thus disrupt the malaria transmission cycle (since a reduction in longevity of as little as 30% can reduce vectorial capacity by approximately 300 times). The cumulative impacts of mortality of mosquitoes from exposure to permethrin, and the suppression of progeny combined with the observed life shortening effects on females that survive permethrin exposure are anticipated to have significant impacts on malaria transmission under field conditions.

762

ANOPHELES GAMBIAE SENSU LATO POPULATION SUSCEPTIBILITY TO THE COMMONLY PUBLIC USED INSECTICIDES FOR MALARIA CONTROL IN MALI

Moussa Keita[1], Sitan Traore[2], Adama Sacko[1], Boubacar Coulibaly[1], Seydou Doumbia[1], Sekou Fantamady Traore[1], Nafomon Sogoba[1]

[1]International Center for Excellency in Research/Malaria Research and Training Center, Bamako, Mali, [2]National Malaria Control Program, Bamako, Mali

Current vector control strategies are mainly based on the use of chemical insecticides for insecticide-treated nets (ITNs) and indoor residual spraying (IRS) of houses. Mosquito resistance to at least one of the insecticides used for malaria control has been observed in many countries including Mali. Consequently, monitoring insecticide resistance is a key element of the implementation of insecticide-based vector control interventions. The aim of this study was to update data on vectors resistance to insecticides in National Malaria Control Program's sentinel sites in Mali. World Health Organization standard bioassay method was used to assess resistance in 3-5 days old F0 or F1 adult female *An. gambiae* s.l.. Insecticides were Lambda-cyhalothrin 0.05%, DDT 4%, Permethrin 0.75%, Deltamethrin 0.05%, Bendiocarb 0.1% and Fenitrothion 1.0%. Results: Suspected to confirmed phenotypic resistance of *Anopheles gambiae* s.l. population were observed for all tested pyrethroids and DDT in all sites except in Yanfolila where it was susceptible to lambda-cyhalothrin (98.0%) [IC 95%, 98_99.8] and to DDT (100%). *An. gambiae* s.l. was susceptible to the bendicarb in Gao, Bougouni, Djenné, Yanfolila and Tombouctou, while suspected resistance was observed in Kati, Niono, Bandiagara and Kita. Except in Niono, a rice cultivation area (92% [IC 95 % 88.3_94.8] mortality), *An. gambiae* s.l. population was fully susceptible to the Fénitrothion in all sentinel sites. These results showed resistance of *Anopheles gambiae* s.l. population to pyrethroids in the majority of the sentinel sites. Therefore, we suggest to the NMCP to alternate the pyrethroid with the Fenitrothion as management strategy to the current resistance.

763

EVALUATING THE EVIDENCE FOR EFFECTIVENESS OF VECTOR CONTROL OF DENGUE OUTBREAKS BY SYSTEMATIC REVIEW AND META-ANALYSIS

Leigh Bowman, Philip J. McCall

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Dengue is the most widespread mosquito-borne arboviral disease worldwide with an estimated 400 million cases occurring annually and almost half of the world's population at risk. At present, preventing and responding to dengue outbreaks both rely on vector control to suppress vector populations or reduce dengue virus transmission, most commonly by eliminating or larviciding mosquito breeding sites and space-spraying with insecticide, respectively. Other approaches are also available and used widely, but with none considered to be sufficiently proven or reliable enough for recommendation, perceptions or beliefs regarding the effectiveness of each approach vary widely among decision-makers in the public health community, often without foundation. Addressing this within the aims of the European Union supported IDAMS research consortium (International Research Consortium on Dengue Risk Assessment, Management and Surveillance; www.idams.eu), we undertook a systematic review and meta-analysis of the evidence available from published studies, to determine if specific vector control tools could impact on vector abundance and/or dengue incidence. A total of 945 studies were found during systematic searches of peer-reviewed databases and grey literature; 35 of these satisfied all inclusion and exclusion criteria and were subject to the Quality Assessment Tool for Quantitative Studies (QATQS). The PRISMA guidelines were followed to ensure rigorous application of review principles. Control approaches included insecticide fogging/space-spraying, insecticide-treated materials, vector trap devices, house screening, mosquito coils and other repellents, clean-up or environmental management and biological control agents. Initial analyses indicate that house screening and community-based programmes can potentially have a beneficial impact, while evidence suggests that repellents and traps do not. Complete results of all final analyses will be presented and the implications of the findings for dengue control in the face of today's realities will be fully considered.

764

INVESTIGATING THE MECHANISMS OF DDT AND DIELDRIN RESISTANCE IN FIELD POPULATION OF *ANOPHELES FUNESTUS* IN SENEGAL

Badara Samb[1], Charles S. Wondji[2], Ibrahima Dia[3], Lassana Konate[1], Ousmane Faye[1]

[1]Laboratoire d'Ecologie Vectorielle et Parasitaire, Département de Biologie Animale, Université Cheikh Anta Diop, Dakar, Senegal, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]Unité d'Entomologie Médicale, Institut Pasteur de Dakar, Dakar, Senegal

Insecticide resistance in *Anopheles funestus*, one of the main malaria vectors, is threatening malaria control in Africa. Elucidation of the mechanisms of resistance is crucial to the design of suitable resistance management strategies. Therefore, we have investigated the mechanisms responsible for DDT and dieldrin resistance in *An. funestus* population

from Senegal. Insecticide susceptibility assays were carried out using 2-5 day old F1 adults generated from indoor-collected, blood-fed female of *An. funestus* from Gankette, in Northern Senegal. WHO bioassays indicated that *An. funestus* is resistant to lambda-cyhalothrin 0.05% (74.64% mortality / n = 222), DDT 4% (83.36% mortality / n = 158) and deltamethrin 0.05% (88.53% mortality / n = 114). Suspected resistance was observed to permethrin 0.75% (91.19% mortality / n = 139), bendiocarb 0.1% (94.13% mortality / n = 157) and dieldrin 4% (96.41% mortality / n = 306). However this population is fully susceptible to malathion 5 % (100% mortality / n = 50) and fenitrothion 1% (100% mortality / n = 55). Genome-wide transcription analysis using microarray and quantitative RT-PCR revealed that the cytochrome P450 CYP6M7 was the detoxification gene most commonly over-expressed in DDT resistant mosquitoes and field unexposed to insecticide compared to a laboratory susceptible strain. In addition, several others genes with diverse functions including glutathione S-transferases were also overexpressed. Using the pyro-sequencing method, The A296S Rdl(R) target site mutation was detected in all dieldrin resistant mosquitoes but at a low frequency (14%) in the field sample. Our study has revealed a strong association between the dieldrin resistance phenotype and the presence of the Rdl mutation. TaqMan genotyping revealed that the L119F mutation in the GSTe2 gene conferring DDT resistance in Benin is completely absent in Senegal. This indicates a shift of DDT resistance mechanisms in West Africa *An. funestus*. These results could help to guide the implementation of suitable control interventions against this vector in Senegal.

765

EXTENSIVE ALTERNATIVE SPLICING IN THE VOLTAGE GATED SODIUM CHANNEL OF *ANOPHELES STEPHENSI*

Cherry L. Dykes[1], Martin J. Donnelly[1], Mark J. Paine[1], Om P. Singh[2]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]National Institute of Malaria Research (Indian Council of Medical Research), Delhi, India

Anopheles stephensi, a sub-tropical species distributed throughout the Middle-East and South Asia is one of the major malaria vectors in India mainly in urban areas. Resistance to DDT in this vector has been reported in India since 1965, and development of incipient resistance against pyrethroids has recently also been reported. One of the mechanisms of resistance in insects is reduced sensitivity of the insects to these two insecticides due to alteration in the target site_the voltage gated sodium channel (VGSC). A single amino acid substitution in the VGSC (*kdr* mutation) is known to significantly alter the susceptibility of vector to insecticides by altering channel kinetics. The role of alternative splicing which reorganizes a primary transcript to generate multiple transcripts, in insecticide resistance is not yet known in insects although such events are known to significantly alter the channel kinetics. To understand the extent of alternative splicing in *An. stephensi*, and the possible role in generating altered sensitivity to DDT and pyrethroids, the whole coding region of VGSC gene of DDT- and pyrethroid-resistant mosquitoes was amplified in two overlapping fragments, cloned and sequenced. Analysis of sequences revealed extensive alternative splicing events. These include seven different exon-skipping events, four alternative acceptor sites, two mutually exclusive exons and one intron-retention. In addition to these alternative splicing events, modification at the 5′ un-translated region has also been recorded where quadruplet repeats of 30 nucleotides are seen. Preliminary data in our study showed two splicing events to be pronounced in resistant clones. The role of such splicing events is being examined to determine correlation with DDT and pyrethroid resistance._

766

INSECTICIDE RESISTANCE IN *ANOPHELES FUNESTUS* IN SOUTHEASTERN AFRICA AND ITS IMPACT ON MALARIA CONTROL PROGRAM FAILURE

Maureen Coetzee

University of the Witwatersrand, Johannesburg, South Africa

Malaria parasites are transmitted to humans by anopheline mosquitoes. The parasite undergoes an obligatory sexual stage within the mosquito midgut that takes up to 14 days to complete. This presents a window of opportunity for us to control the mosquito populations before they have sufficient time to become infective. Unfortunately, both mosquitoes and parasites have been around a lot longer than humans and so far have managed to find ways of getting around all the insecticides and drugs that we throw at them. In Africa today there are approximately 140 recognised species of *Anopheles* mosquitoes. Only 4 of these are really good vectors of malaria parasites and of these 4, *Anopheles funestus* is the most important vector in the south-eastern African region. It is highly adapted to humans and human habitations and should, therefore, be easy to control using current technology. Unfortunately, it has developed high levels of resistance to the pyrethroid insecticides that are used for both treating bed nets and spraying on the inside walls of houses. Recent entomological surveys in Zimbabwe, Zambia, Malawi and Mozambique show an almost uniform profile of resistance in *An. funestus* with unpublished data from Zimbabwe indicating a definite impact on programme failure. There is an urgent need for resistance management strategies to be implemented in these countries.

767

UNDERPINNING SUSTAINABLE VECTOR CONTROL THROUGH INFORMED INSECTICIDE RESISTANCE MANAGEMENT

Edward Thomsen[1], Clare Strode[2], Kay Hemmings[1], Angela Hughes[1], Emmanuel Chanda[3], Mulenga Musapa[3], Mulakwa Kamuliwo[3], Faustina Phiri[3], Lucy Muzia[4], Javan Chanda[4], Alister Kandyata[3], Brian Chirwa[4], Kathleen Poer[4], Janet Hemingway[1], Hilary Ranson[1], Michael Coleman[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Edge Hill University, Ormskirk, United Kingdom, [3]National Malaria Control Centre, Lusaka, Zambia, [4]Abt Associates, Lusaka, Zambia

There has been a rapid scale-up of vector control for malaria in the last ten years. Both of the primary strategies, long-lasting insecticidal nets and indoor residual spraying, rely on the use of a limited number of insecticides. Insecticide resistance has rapidly increased in prevalence and has come to the forefront as an issue that needs to be addressed in order to maintain the sustainability of control efforts in the drive to elimination. Zambia's programme reported high levels of resistance to the insecticides it used in 2010. As a result, it increased its investment in resistance monitoring to support informed resistance management decisions. A national survey on insecticide resistance in Zambian malaria vectors, covering 26 districts in all 10 provinces, was performed using WHO bioassays to detect resistant phenotypes. Molecular techniques were used to detect target-site mutations and microarray to detect metabolic resistance mechanisms. *Anopheles gambiae* s.s. was resistant to pyrethroids, DDT and carbamates, with potential organophosphate resistance in one population. The resistant phenotypes were conferred by both target-site and metabolic mechanisms. *Anopheles funestus* s.s. was largely resistant to pyrethroids and carbamates, with potential resistance to DDT in two locations. The resistant phenotype was conferred by elevated levels of cytochrome p450s. Currently, the Zambia National Malaria Control Centre is using these results to inform their vector control strategy. The methods employed here can serve as a template to all malaria-endemic countries striving to create a sustainable insecticide resistance management plan.

768

INCREASED EXPRESSION OF METABOLIC RESISTANCE CANDIDATE MUTATIONS IN THE MALARIA VECTOR *ANOPHELES GAMBIAE* SENSU STRICTO IN DAR ES SALAAM, TANZANIA

Bilali I. Kabula[1], Johnson Matowo[2], Bernard Batengana[1], Craig S. Wilding[3], Emily J. Rippon[3], Keith Steen[3], William Kisinza[1], Stephen Magesa[1], Franklin Mosha[2], Martin J. Donnelly[3]

[1]National Institute for Medical Research, Tanzania, Muheza, Tanga, United Republic of Tanzania, [2]Kilimanjaro Christian Medical University College, Moshi, United Republic of Tanzania, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

One of the major challenges facing malaria control is the development of insecticide resistance in vector mosquitoes. *Anopheles gambiae*, which is a major vector of the malaria parasite *Plasmodium falciparum* in Africa, has over the years developed resistance to dieldrin, 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethane (DDT), and pyrethroids. The objective of this study was to determine the mechanisms contributing to DDT resistance in *Anopheles gambiae s.s.* in Dar es Salaam, Tanzania. Female mosquitoes of standard age, reared from larvae sampled across a variety of natural breeding sites, were used in the study. Members of the *An. gambiae* complex were PCR-identified and screened for target-site mutations (*Vgsc-1014S* and *Vgsc-1014F*). A DDT-resistant population of *An. gambiae s.s.* and controls (sympatric and allopatric controls) were screened for GSTe2 and P450 genes-expression profiles using real-time quantitative polymerase chain reaction (qPCR) tests. A significantly higher allelic frequency of the *Vgsc*-L1014S mutation was found in DDT-resistant *An. gambiae s.s.* than in the control mosquitoes ($p<0.001$). The cytochrome P450 genes *Cyp6m2*, *Cyp6p3*, *Cyp6z3* and *Cyp6z1* were significantly over-expressed in DDT-resistant *An. gambiae s.s.* compared with the control populations. We report increased expression of multiple DDT-associated resistance mechanisms in the primary African malaria vector, *An. gambiae s.s.* in Dar es Salaam. The presence of multiple resistance mechanisms in *An. gambiae* that are common to both DDT and pyrethroids may have confounding effect in resistance-management strategies. However, the geographical extent of the insecticide resistance mechanisms observed in this study needs to be investigated further.

769

EFFICACY OF LLIN MIXTURES OF CHLORFENAPYR AND ALPHACYPERMETHRIN AGAINST PYRETHROID RESISTANT *ANOPHELES GAMBIAE*: AN EXPERIMENTAL HUT TRIAL IN BENIN

Raphael N'Guessan[1], Corine Ngufor[1], Abibathou Odjo[2], Estelle Vigninou[2], Martin Akogbeto[2], Mark Rowland[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

Insecticide Treated Nets (ITNs) and Indoor Residual Spraying (IRS) have and will continue to save millions of lives in Africa. However, malaria vectors are developing incredible resistance to existing insecticides and current gains may be lost if new generation tools are not made available soon. The mixture of dual unrelated active ingredients on mosquito net maybe a solution as it presents an opportunity for improved control and management of pyrethroid resistance through the simultaneous exposure to both compounds. With industry and IVCC, we have come to develop a new generation of bednet incorporating alphacypermethrin and chlorfenapyr that may counter growing insecticide resistance in west Africa. Samples of these new nets treated with chlorfenapyr or chlorfenapyr-alphacypermethrin mixtures with binders were washed gradually, 10 and 15 times and evaluated each time in experimental huts against pyrethroid resistant *Anopheles gambiae* in Central Benin. Interceptor 1, an alphacypermethrin-impregnated polyester LLIN (WHO approved) washed to same extent was used as positive control. The nets were deliberately holed with six holes to examine the effect of wear and tear on protectiveness. Mortality rate of *A. gambiae* with Interceptor 1 was constantly less than 20% after 10 or 15 washes, and was presumably due to the high level of pyrethroid resistance in this species. Nets treated with the insecticide mixtures and washed 15 times induced 3 times higher mortality of *A. gambiae* (58-60%) than the Interceptor 1. The differences in mortality of *An. gambiae* between mixtures with low and high dosages chlorfenapyr were not significant, nor was the difference between low and high dosed binders. After washing 15 times, the LN treated with the mixtures inhibited *A gambiae* biting (32–44%) by a greater margin than the Interceptor 1 (20%). The study demonstrates the availability of a resistance-combating dual active ingredient mosquito nets that resist at least 15 washes of the WHO standard practices and restore the effectiveness of ITNs in areas compromised by the spread of pyrethroid resistance.

770

A NOVEL LONG-LASTING INSECTICIDAL NET BASED ON A MIXTURE OF CHLORFENAPYR AND ALPHACYPERMETHRIN CONTROLS MULTI-RESISTANT *ANOPHELES* MOSQUITOES UNDER FIELD CONDITIONS AFTER MULTIPLE WASHING: EXPERIMENTAL HUT RESULTS FROM EAST AFRICA AND IMPLICATIONS FOR WHOPES TESTING GUIDELINES

Richard M. Oxborough[1], Raphael N'Guessan[1], Rebecca Jones[1], Jovin Kitau[2], Corine Ngufor[1], Martin Akogbeto[3], Franklin W. Mosha[4], Mark W. Rowland[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Kilimanjaro Christian Medical Universtiy College, Moshi, United Republic of Tanzania, [3]Centre de Recherche Entomologique de Cotonou, Cotonou, Benin, [4]Kilimanjaro Christian Medical University College, Moshi, United Republic of Tanzania

The emergence and spread of pyrethroid-resistant *Anopheles* across Africa is a grave threat to malaria control. There is an urgent need for non-pyrethroid, wash-resistant LLINs to be developed. Chlorfenapyr is a pyrrole insecticide with a distinct mode of action which disrupts cellular respiration. Interceptor 2 is a 'coating' LLIN containing a mixture of chlorfenapyr (CFP) and alphacypermethrin-α. This study assessed wash-resistance in experimental hut field trials and explored the properties of CFP in laboratory tests based on current WHOPES guidelines and behavioural tests. Hut trials in Tanzania with the mixture LLIN killed 63% and 66% of *An. arabiensis* when unwashed and 10 times washed respectively, compared with 46% for the pyrethoid LLIN (Interceptor 1-α only) washed 10 times. HPLC showed that after 10 washes Interceptor 2 had 75% CFP and 95% α still remaining. This is the first report of a non-pyrethroid insecticide on a LLIN performing successfully after multiple washes. By contrast the standard WHO bioassays failed to predict the performance of chlorfenapyr and therefore need urgent revision otherwise suitable insecticides may be overlooked. The three minute bioassay of CFP nets produced <15% mortality while LLIN require >80% mortality to pass the LLIN test. In laboratory testing there was a strong positive correlation between temperature and mortality above and below standard test temperature. Bioassay tests conducted during the night produced consistently higher levels of mortality than the same tests in the day time. It appears that conversion of CFP to the active metabolite and its disruption of respiratory pathways is enhanced at night when the host seeking mosquito is more metabolically active during the active phase of its circadian rhythm. Testing according to WHOPES guidelines are not suitable for certain types of non-neurotoxic insecticide which, though highly effective in field trials, would be overlooked at the screening stage of evaluation through conventional bioassay.

771

A NEW PROMISING LONG LASTING INSECTICIDE NET IN THE CONTROL OF INSECTICIDE RESISTANT VECTORS: OLYSET DUO®: A PYRIPROXYFEN AND PERMETHRIN MIXTURE NET

Natacha Protopopoff[1], Richard Oxborough[1], Seth Irish[2], David Malone[3], Franklin W. Mosha[4], Mark Rowland[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Innovative Vector Control Consortium, Liverpool, United Kingdom, [4]Kilimanjaro Christian Medical University College, Moshi, United Republic of Tanzania

Insecticide resistance is threatening the reduction in malaria burden achieved by the massive scale up of vector control measures. One of the most effective vector control tool is the long lasting insecticidal nets (LNs). LNs rely on pyrethroid insecticides to provide a repellent barrier between humans and mosquitoes and kill mosquitoes before they can transmit malaria. Pyrethroid resistance is now occurring in most countries and can undermine the effectiveness of the LN. The development of new tools combining chemical with different mode of action is a necessity to control resistant vector and sustain the gain achieved in controlling malaria. Olyset Duo is a new LN combining a permethrin and pyriproxyfen an insect growth regulator. Pyriproxyfen can sterilize adult mosquitoes and incorporated on a net reduce the vector density. The efficacy and wash resistance of Olyset Duo is currently evaluated in Lower Moshi, Tanzania. Standard WHO cone, cylinder and tunnel test has been performed in the laboratory to evaluate mortality, blood feeding inhibition, reduction in fecundity and fertility of adult female anopheles exposed to Olyset Duo. Experimental hut trial has been also carried out to evaluate the impact of the mixture net on the wild free flying An.arabiensis compare to standard LNs. *Anopheles* exposed to unwashed Olyset Duo in cone, cylinder and tunnel show higher mortality and lower blood feeding rates than to the standard Olyset Net (permethrin alone). The surviving *Anopheles* exposed to Olyset Duo were all sterilized. This should have impact on the population size of the next generation. Initial results of this novel LN's mixture show great potential for the control of resistant malaria vectors in Sub Sahara Africa. The full outcomes of the study will be presented.

772

FIELD TESTING OF A PYRETHROID QUANTIFICATION KIT (PQK) IN TANZANIA - AN EASY-TO-USE TOOL FOR MONITORING THE QUALITY OF INDOOR RESIDUAL SPRAY CAMPAIGNS

Alexandra Wright, Harparkash Kaur, Natacha Protopopoff, Mark Rowland

London School of Hygiene & Tropical Medicine, London, United Kingdom

Insecticide treated nets (ITN) and indoor residual spraying (IRS) are two of the primary methods of malaria prevention in Africa. In order for these methods to be effective it is essential that adequate concentrations of insecticide are present on nets and wall surfaces to kill mosquitoes. There is no easy assay to quantify insecticide levels without expensive laboratory equipment and procedures. To address this, LSHTM has developed a simple field-applicable kit for monitoring pyrethroid residues on insecticide-treated nets- the Pyrethroid Quanitification Kit (PQK)- which can be adapted to other types of treated surfaces. During the initial trial the PQK kit was calibrated against a variety of sprayed surfaces and with different concentrations of lambdacyhalothrin before being taken into the field. Mosquito cone bioassay was conducted to show whether the surface concentrations of insecticide detected by the PQK were sufficient to kill a susceptible strain of mosquitoes. Houses in six villages were visited 3 months after IRS had been conducted in Muleba, Tanzania. The samples were analysed in the field using a handheld spectrophotometer. In each house, five areas of the wall were examined to give an indication of insecticide distribution and within-wall variation. Results showed that the actual spraying results differed from expectation. Preliminary results showed that only 28% of houses had all rooms sprayed, leaving 72% of houses partially sprayed, and insecticide concentration varied dramatically across sprayed walls. The PQK is an easy to use quality assurance tool for monitoring of pyrethroid application rates and improving the quality of IRS campaigns.

773

INSECTICIDE RESISTANCE AND COPY NUMBER VARIATION IN THE *ANOPHELES GAMBIAE* ACETYLCHOLINESTERASE (*ACE-1*) GENE

David Weetman[1], Luc Djogbenou[2], John Essandoh[3], Constant V. Edi[4], Martin J. Donnelly[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]IRSP, Ouidah, Benin, [3]University of Cape Coast, Cape Coast, Ghana, [4]CSRS, Abidjan, Côte D'Ivoire

The *Anopheles Ace-1* gene encodes the neurotransmitter acetylcholinesterase, the target of organophosphate and carbamate insecticides, which are of growing importance for vector control. A single base mutation (G119S), which causes a major conformational change in the protein, gives resistance to both insecticide classes in mosquitoes. However, the nature, evolution and phenotypic consequences of the more recently identified copy number variation (CNV) of *Ace-1* are far less well understood in *An. gambiae*. We show that CNV is present throughout West Africa and appears to be ubiquitously associated with resistant (119S), but is much less commonly associated with wild-type susceptible (119G) alleles. In *Culex pipiens Ace-1* CNV is well known and involves duplication which pairs a 119G and a 119S allele on the same chromosome to create a permanent heterozygote. However, in *An. gambiae* many *Ace-1* allele copies can be found in 119S homozygotes and sequence analysis suggests that resistant alleles may have originated, or at least spread, following gene duplication. Duplicated alleles are expressed though not necessarily in direct proportion to copy number, and *Ace-1* overexpression is linked to high-level carbamate resistance. Diagnostic assays and neutral markers show that *Ace-1* 119S duplicated resistant alleles are strongly selected and increasing in frequency. Given strong predictive links with carbamate and organophosphate resistance, molecular monitoring should be a key component of programmes using these insecticide classes for control of *An. gambiae*.

774

A RETROSPECTIVE EVALUATION OF THE DURABILITY OF LONG-LASTING INSECTICIDAL NETS FROM TWO NATIONAL CAMPAIGNS IN TANZANIA

Lena M. Lorenz[1], Sarah J. Moore[2], Dennis Massue[3], Zawadi Mageni[4], Renata Mandike[5], John Bradley[1], Jason D. Moore[4], William Kisinza[3], Hans J. Overgaard[6]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Swiss Tropical and Public Health Institute, Basel, Switzerland, [3]National Institute for Medical Research, Muheza, United Republic of Tanzania, [4]Ifakara Health Institute, Bagamoyo, United Republic of Tanzania, [5]National Malaria Control Programme, Dar-es-Salaam, United Republic of Tanzania, [6]Norwegian University of Life Sciences, As, Norway

Long-Lasting Insecticidal Nets (LLINs) are the mainstay of malaria control, particularly in sub-Saharan Africa. However, despite many National Malaria Control Programs (NMCPs) adopting partial or universal LLIN coverage, there is still only limited knowledge of the effective life of nets after user conditions - commonly known as LLIN durability. Our ABCDR study retrospectively investigates four aspects determining LLIN durability - attrition, bioefficacy, chemical content and physical degradation - of national campaign LLINs in eight districts in Tanzania covering a range of epidemiological and ecological settings. Nets were collected from 3,420 households, and a questionnaire was conducted to determine net ownership and net use. The number, size and location of holes and bioefficacy against anopheline mosquitoes were measured in a sub-sample

of identified campaign nets. A total of 6,832 nets were collected, though not all of them were LLINs. Preliminary results suggest that net ownership, coverage and use are highly variable between districts (household with at least 1 net per sleeping space ranged from 29 - 66%) and by equity (those in lower socio-economic quintiles used nets less frequently). 27% of nets were reported not to have been used the night before the survey, mainly because there were no mosquitoes, the primary user did not sleep at home that night and net age. The results of the bioefficacy and physical degradation analysis are still outstanding, but will be presented and correlated with net use characteristics. Increasing the data on LLIN durability after user conditions to understand the median lifespan of nets for programmatic and cost-effectiveness decisions is becoming a priority for the World Health Organization and NMCPs. In addition to informing the Tanzanian NMCP about the nets distributed during national campaigns between 2009 and 2011, we are also extending the ABCDR study prospectively to compare the durability of three LLIN products over at least three years.

775

DIRECT EVIDENCE OF CHEMICAL CONTAMINATION OF *ANOPHELES GAMBIAE* S.L. BREEDING SITES UNDERLYING THE SELECTION OF PYRETHROID RESISTANCE IN COTTON GROWING AREAS REVEALED BY GPC: POTENTIAL IMPACT ON THE EFFICACY OF VECTOR CONTROL TOOLS IN BURKINA FASO

Dabire K. Roch[1], Hien Aristide[1], Namountougou Moussa[1], Soma D. Dieudonne[1], Combary Patrice[2], Diabate Abdoulaye[1]

[1]Centre Muraz/IRSS, Bobo-Dioulasso, Burkina Faso, [2]National Malaria Control Programme, Ministry of Health, Ouagadougou, Burkina Faso

Since the detection of the first case of *Anopheles gambiae* resistance to pyrethroid recorded in Ivory Coast in 1993, several studies had reported the role of agriculture in the selection and the spread of pyrethroid resistance in natural populations of *An. gambiae* s.l. Unfortunately no direct evidence was reported enhancing the presence of such chemicals in anopheline breeding sites. It was what we addressed in the current study performed in Dano, a cotton growing area located in the South West of Burkina Faso by monitoring the insecticide content both in water and sediments sampled from randomly selected breeding sites using GC analysis from August to October 2013. The resistance status of local populations of *An. gambiae* s.l. was estimated using standard WHO tube assays. Early in August some herbicides as Diouron were detected from the soil residue in concentrations ranged from 22.63 to 105.5 mg/Kg of soil without any insecticide in the water. In October two pyethroids namely lambacyalothrin and deltamethrin were found in the breeding water at concentrations ranging from 0.0147µg/l to 1.49 µg/l together with other chemicals occurring in very low concentration from the soil residue (benzoypropenyl, dioxacarb, chloroneb). A reduced mortality rate was observed both with deltamethrin 0.05% and bendiocarb 0.1% reaching 52.04% and 66.67% respectively. High *kdr* allele frequencies reaching 0.95 and 0.4 respectively for 1014F and 1014S alleles and 0.12 for the ace-1 allele accompanied this strength resistance phenotype. Data on the efficacy of long lasting insecticide treated bednets (LLINs) in use in the region obtained by WHO cone test, showed mortality rates ranged from 10% to 83% depending to the type of LLIN. The significance and the impact of such resistance on the efficacy of malaria vector control strategy in short and long terms were discussed.

776

ANALYSIS OF THE DNA SEQUENCE OF ACE.1 GENE IN *ANOPHELES GAMBIAE* S.S: DUPLICATION AND RECOMBINATION HISTORY

Luc S. Djogbénou[1], John Essondoh[2], Constant Edi[2], Keith Steen[2], Benoit Assogba[3], David Weetman[2], Martin Donnelly[2]

[1]Institut Régional de Santé Publique/UAC-Liverpool School of Tropical Medicine, Cotonou, Benin, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]Institut Régional de Santé Publique/UAC, Cotonou, Benin

Gene duplication is a source of molecular innovation throughout evolution. However, even with massive amounts of genome sequence data, correlating gene duplication with other events in natural history can be difficult. This is especially true in its most interesting cases, where rapid and multiple duplications are likely to reflect adaptation to rapidly changing environments and life styles. This may be so for the *Anopheles* ace-1 gene which encodes for the neurotransmitter acetylcholinesterase, the target of organophosphate and carbamate insecticides. *Anopheles gambiae*-resistant is known to possess only one mutation on the acetylcholinesterase (AChE) gene (ace) that is involved in insecticide (carbamates and organophosphate) target site insensitivity. To better understand a preferred model for the natural history of ace.1 duplication in the main malaria vector, we proceeded to the cloning and sequence analysis of DNA fragment including the G119S mutation from four West Africa countries. Here we show that phylogenetic trees produced from the nucleotide sequences of 34 individual ace.1 gene consisted of 4 main clusters, with ace.1 copies of different specimens grouping together in three out of the four clusters as expected if there was multiple duplication events. Furthermore, Phylogenetic analysis displayed an individual mosquito bearing more than two different resistant haplotypes with unexpectedly high copy number (at least six) of susceptible haplotype in the same individual. Our data indicate that this high copy number in both susceptible and resistant haplotype have evolved through gene duplication following by recombination event.

777

EVALUATING THE EFFICACY OF ORGANOPHOSPHATE-COMBINED PAINT AGAINST PRYRETHROID-RESISTANT *ANOPHELES GAMBIAE* S.L. IN VALLÉE DU KOU, BURKINA FASO

Dieudonné D. Soma

IRSS/Centre Muraz, Bobo-Dioulasso, Burkina Faso

Malaria control is currently treated by the rapid spread of insecticide and drug resistance among mosquito and parasite respectively, and operational difficulties on the field. It is necessary to find alternative and innovative tools for more effective malaria control. In the current study we evaluated the efficacy of organophosphate-combined paint containing insect growth regulator in indoor application on the walls in Vallée du Kou where *Anopheles gambiae* is resistant to pyrethroids. The main entomological parameters such as mortality rates and paint insecticidal residual efficacy were compared in a village scale between treated and untreated huts. The residual insecticidal efficacy of the paint tested both with the susceptible *An. gambiae* "Kisumu" and wild local populations of *An. gambiae* s.l. showed high mortality rates ranged 98 to 100% even six months after the treatment. The indoors catches of wild *An. gambiae* performed during four consecutive days per month from July to December 2013 revealed that all mosquitoes entered in the paint-treated houses were died reaching 100% mortality rates throughout the six months collection. The frequency of kdr-L1014F (98%) mutation which was higher in this area did not differ between treatments. That of the Ace-1R remained low less than 5% and did not differ whatever the treatment. These results are very promising in terms of new perspectives to control resistant malaria vectors. Contrary to the classic indoor sprays, this tool is very simple and does not require

any special equipment or qualified personnel. But a large-scale assay is required to address its impacts on malaria transmission and the perception and acceptability of targeted human communities.

778

INSECTICIDE RESISTANCE PROFILES IN *AEDES AEGYPTI* STRAINS FROM THE CARIBBEAN REGION OF COSTA RICA

Olger Calderón-Arguedas, **Adriana Troyo**

Universidad de Costa Rica, San Jose, Costa Rica

Dengue is the most important vector-borne disease in Costa Rica. Although control of the vector, *Aedes aegypti*, includes repeated use of pyrethrins and temephos, insecticide resistance is not monitored continuously. In this study, resistance profiles to deltamethrin, cypermethrin, and temephos were evaluated in strains of *Ae. aegypti* from the cities of Guacimo and Limon in the Caribbean Region. Bioassays were carried out by exposing groups of 20 larvae for 24 hours to each of the insecticides at a series of concentrations that would generate from 2% to 100% mortality. Tests were performed by quintuplicate using larvae from the second to fourth generations, and a 50% lethal concentration (LC50) was estimated. A 50% resistance ratio (RR50) was calculated using the LC_{50} of the Rockefeller strain as a susceptible control. When resistance occurred, the enzymatic mechanism was evaluated by exposing the larvae to the synergists piperonyl butoxide (PB) and S, S, S, tributylphosphorotrithioate (DEF) and repeating the assays. No resistance to temephos or deltamethrin was detected in *Ae. aegypti* strains from Guacimo and Limon. Emerging resistance to cypermethrin was detected in both Guacimo (LC50 = 0.00845 mg/L, range = 0.00664 to 0.01038 mg/L; RR50 = 6.07) and Limon (LC50 = 0.01016 mg/L, range = 0.00876 to 0.01177 mg/L; RR50 = 7.30). The analysis of cypermethrin with PB resulted in synergism ratios of 19.2 and 8.7 for Guacimo and Limon strains, respectively. Synergism ratios for DEF were 0.9 for the Guacimo strain and 1.8 for the Limon strain. Results show that *Ae. aegypti* in both areas studied are in the process of developing resistance to cypermethrin, which is associated at least in part with the activity of cytochrome P450 monooxygenase. Therefore, local authorities should begin replacement of cypermethrin to ensure effectiveness of vector control and limit the development of resistance.

779

USE OF NEXT GENERATION SEQUENCING (NGS) TO IDENTIFY NOVEL SNPS ASSOCIATED WITH PYRETHROID RESISTANCE IN *AEDES AEGYPTI*

Karla L. Saavedra-Rodriguez, Patricia Penilla-Navarro, Corey Rosenberg, Saul Lozano-Fuentes, Farah Vera-Maloof, William C. Black, IV

Colorado State University, Fort Collins, CO, United States

The identification of single nucleotide polymorphisms (SNPs) associated with insecticide resistance has being performed mainly through genomic DNA and cDNA sequencing. Several SNPs conferring insecticide resistance have being identified in target site genes and in less extent at some detoxification genes. Recent Next Generation Sequencing (NGS) technology allows screening the whole genome to identify SNPs associated with insecticide resistance. In this study we identified SNPs associated with pyrethroid survival in an *Aedes aegypti* field population collected in Viva Caucel, Yucatan, Mexico, which already has high frequency for pyrethroid associated mutations in *para*. Four genomic DNA libraries were built following the recommended Illumina HiSeq protocols. Two replicate libraries contained DNA from mosquitoes that had survived one hour exposure to 25 µg permethrin (active ingredient per bottle). The remaining two libraries contained DNA from mosquitoes that died from the same exposure. Sequences were obtained from an Illumina HiSeq2000/2500 sequencer. We constructed a reference library including the total transcript sequences released by Vector Base. We aligned the paired read data to our locally build reference library using the GSNAP software. SNPs with coverage <15 or >1000 were excluded. SNPs association with resistance was assessed using LOD scores. SNPs in 38 genes were associated with permethrin resistance; none of these belonged to the expected target site or putative detoxification genes. Instead, we identified genes involved with GTP and ATP binding, calcium ion binding, zinc ion binding, glutamate ion channel transport and enzymes with specific activity. These candidate genes could become markers that will allow tracking pyrethroid resistance in *Ae. aegypti*.

780

INSECTICIDE SUSCEPTIBILITY, CHARACTERIZATION OF BREEDING SITES AND COMMUNITY PERCEPTIONS ON MALARIA VECTOR CONTROL INTERVENTIONS ON KNUST CAMPUS

Ruth C. Brenyah

Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Control of malaria vectors with insecticides remains an essential component to fight and eliminate malaria. There have been reports of insecticide resistance to all four classes of insecticides. Spread of resistance is said to be due to the excessive use of insecticides in Public health and in Agriculture. Insecticide-treated nets (ITN) and Indoor residual spraying (IRS) have proven to be the two most powerful and most broadly applied vector control interventions over the years. Despite the reports of resistance to pyrethroids, a lot of ITNs are still being mass distributed for free in most communities in Ghana.This study seeks to find out the level of resistance in *Anopheles* mosquitoes, to insecticides on the University campus which has an urban ambience, to determine other chemical vector control methods used by inhabitants and their perception on ITN use. Due to the extensive agricultural practices on the campus, the breeding sites of the *Anopheles* mosquitoes were characterized using physico-chemical parameters to find out the impact of spraying on the larvae. For the susceptibility tests, 2-5 days old non blood fed female *Anopheles* mosquitoes were tested against 0.05% deltamethrin, 4% DDT, 0.1% fenitrothion and 1% bendiocarb using standard WHO tube assay. A well-structured questionnaire was administered to residents to determine their knowledge, attitudes and practices to malaria. A total of 3,766 mosquitoes were identified as *An. gambiae* s.l (98.9%) and *An. funestus* (1.2%). Resistance was recorded to all classes of insecticides with mortality rates of 15-54% deltamethrin, 10-50% bendiocarb, 7.5-38.75% DDT and 5-42.5% fenitrothion. Overall knockdown rates was 60-21% for deltamethrin, 36.25-11.25% for fenitrothion, 26.25-12.5% DDT and 55-10% for bendiocarb across all breeding sites. Susceptibility status of mosquitoes indicate most are resistance. There was no association between susceptibility status and physical parameters of breeding sites. Inhabitants use ITN's, aerosol sprays, mosquito coils and repellents, impregnated curtains and screens on windows amongst other traditional methods; knowledge on use of ITNs was adequate. Knock down resistance (kdr) genes must be assessed. Knowledge on ITN usage was not converted into practice. This study shows the need for continuous monitoring of resistance to mosquitoes in this area to enable better control methods to be formulated and practiced.

781

MULTI-COUNTRY PROFILE OF INSECTICIDE RESISTANCE ON MALARIA VECTORS IN THE PRESIDENT'S MALARIA INITIATIVE (PMI/USAID) SUPPORTED COUNTRIES UNDER THE AFRICA INDOOR RESIDUAL SPRAYING PROJECT

Aklilu Seyoum[1], Dereje Dengela[2], Bradford Lucas[2], Allison Belemvire[3], Kristen George[3], Christen Fornadel[3]

[1]Abt Associates Inc., Accra, Ghana, [2]Abt Associates Inc., Bethesda, MD, United States, [3]United States Agency for International Development, Washington, DC, United States

The Africa Indoor Residual Spraying project implemented indoor residual spraying (IRS) in twelve African countries, namely Angola, Benin, Burkina Faso, Ethiopia,Ghana, Liberia, Madagascar, Mali, Mozambique, Nigeria,

Rwanda and Zimbabwe withsupport from the President's Malaria Initiative in 2012/2013. The project regularly collects data on susceptibility of the malaria vectors to the four classes of insecticides approved by WHO for IRS with the aim to guide selection of appropriate insecticides for IRS programs. WHO tube tests were used for susceptibility tests, and most of the tests were carried out against *Anopheles gambiae* s.l. (144 and 188 tests in 2012, and 2013 respectively); and a total of 14 tests were also carried out against the *An. funestus* in Mozambique and Zimbabwe. Data were entered into the database and collated to inform the decision making processes. *An. gambiae* s.l. was found to be fully susceptible (98-100% mortality after 1 hour exposure and 24 hrs. holding period) to pirimiphos-methyl in all the sites where the tests were conducted. However, potential resistance to fenithrothion was observed in sites in Benin, Ethiopia, Ghana, Liberia, Madagascar and Mozambique. Resistance to malathion has been observed in Ethiopia. Variable levels of susceptibility were reported for the carbamate bendiocarb against *An. gambiae* s.l. Resistance to pyrethroid insecticides is widespread for *An. gambiae* s.l. in most countries.However, in Angola, Mozambique, Madagascar and Zimbabwe, *An. gambiae* s.l. is susceptible to deltamethrin for most sites. A high level of resistance to DDT (0-26% mortality after 1 hour exposure and 24 hrs. holding period) has been reported in most countries, except Zimbabwe, Mozambique, and in some areas of Madagascar. *An. funestus* was resistant to pyrethroids in Zimbabwe and in some sites of Mozambique. It was,however, susceptible to organochlorine and organophosphate insecticides in the southern parts of Africa. The results are discussed in the context of malaria vector control interventions, geographical locations and epidemiology of malaria in the countries.

782

A SEASONAL COMPARISON OF HIGH AND LOW EPIDEMIOLOGICAL CLUSTERS OF DENGUE TRANSMISSION IN SURINAME

Diana Hamer[1], Helene Hiwat[2], Maureen Lichtveld[1]

[1]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Bureau of Public Health Suriname, Paramaribo, Suriname

The primary vector of the dengue virus, *Aedes aegypti*, is well established in the increasingly urbanized capital of Paramaribo, Suriname. Due to the complex interaction of the environment and the mosquito, dengue transmission risk differ within the geographic area of concern. This study characterizes dengue-related entomological and environmental factors affecting disease transmission. Previously, high and low epidemiological rate clusters of dengue were identified in Paramaribo. Within the clusters, houses were randomly selected for inspection before and after the short rainy season. An environmental survey was conducted and entomological indices were calculated for each cluster. Presence of larva and pupae were recorded and samples of pupae were collected during the surveys. All inspected locations were geospatially coded. In total, 536 houses were surveyed in Paramaribo: 242 during the pre-rainy season and 294 post-rainy season. The findings show significant difference between pupae-positive houses and pupae-positive containers (house & container index) pre- (37.60% & 16.53%) and post- (24.83% & 7.73%) rainy season, p=0.001 and p=0.033. The Breteau indices pre- and post- rainy season were 0.68 v. 0.27. These pupae indices were higher in the low cluster pre-rainy season but higher in the high cluster post-rainy season. The dispersion index remained unchanged between the seasons (3.99 and 3.70 for pre- and post-rainy season, respectively) but decreased from 5.10 to 3.81 in the high cluster and increased from 2.95 to 3.16 in the low epidemiological cluster between the rainy seasons. The variation in the dispersion index indicates that factors within clusters and seasonality affect the proportions in which pupae are present among different categories of containers. This is the first attempt to demonstrate how entomological and environmental data influence the rate of dengue transmission in Suriname.

783

DENGUE VECTOR COMPETENCE STUDIES IN *AEDES* MOSQUITOES

James Whitehorn[1], Duong Thi Hue Kien[2], Nguyet Minh Nguyen[2], Hoa L. Nguyen[1], Tran Nguyen Bich Chau[2], Long Vo Thi[2], Le Thi Dui[2], Nguyen Tan Truong[3], Luong Thi Hue Tai[3], Bridget Wills[2], Chau Van Vinh Nguyen[3], Marcel Wolbers[2], Cameron P. Simmons[2]

[1]London School of Hygiene & Tropical Medicine, Ho Chi Minh City, Vietnam, [2]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, [3]Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam

Dengue is globally the most important arboviral infection of humans. The main vector worldwide is *Aedes aegypti*. The Asian tiger mosquito, *Ae. albopictus*, is also a competent vector of dengue viruses and its presence in continental USA and southern Europe raises the concern that dengue will spread further into new regions. This study aimed to directly compare the susceptibility of *Ae aegypti* and *Ae albopictus* to dengue by conducting blood feeding experiments on viremic dengue patients. This study directly compared the susceptibility of these two mosquito types by conducting blood feeding experiments on 118 viremic dengue patients on 232 independent exposure events. The likelihood of saliva infection (and thus potential infectiousness) in *Ae. albopictus* was approximately half that in *Ae. aegypti* (OR=0.49; CI: 0.34-0.70). When stratifying by serotype, the odds of saliva infection were significantly lower for *Ae. albopictus* with DENV-2, DENV-3 and DENV-4 , but not with DENV-1. We have demonstrated that disseminated DENV infections and thus transmission are less likely to occur in *Ae. albopictus* than *Ae. aegypti*. These results have implications for understanding the spread, distribution and control of dengue.

784

DOES DENGUE VIRUS ENHANCE ITS OWN TRANSMISSION FROM HUMANS TO MOSQUITOES?

Lauren B. Carrington[1], Nguyet M. Nguyen[1], Hue Tai T. Luong[2], James Whitehorn[1], Cameron P. Simmons[1]

[1]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, [2]Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam

Among other cues, host-seeking mosquitoes are known to be responsive to carbon dioxide and body temperature. Due to a higher body temperature, dengue virus-infected patients, like other febrile patients, may be more attractive to *Aedes aegypti* mosquitoes compared to healthy individuals. That is, the clinical effects of dengue may aid its transmission to further susceptible mosquitoes. An altered distribution of mosquito bites between infectious and non-infectious hosts may impact predictions of transmission dynamics during epidemic periods with a high force of infection. We test the hypothesis, H_0 = both febrile and afebrile hosts are similarly attractive to naïve *Ae. aegypti* mosquitoes, by exposing febrile dengue patients and healthy matched volunteers to uninfected *Ae. aegypti* mosquitoes. We released five *Ae. aegypti* into the center of a large cage (~4m x $2m^2$), with the participants at each end. The time it takes for each participant to be first chosen, and landed upon by a mosquito, is measured, with mosquito landing used as a surrogate for biting. Anticipated completion of enrollment will occur in December 2014. Results from this study will inform us whether dengue virus has the capacity to enhance its own transmission (whether it be directly or indirectly) by increasing the attractiveness of infected human hosts to naïve mosquitoes.

785

DO SEASONAL TEMPERATURES MATTER IN THE LARVAL HABITAT? EFFECTS ON ADULT SIZE, LONGEVITY, AND R' IN THE MOSQUITO *AEDES TRISERIATUS*, VECTOR OF LA CROSSE VIRUS

Katie Westby, Steven A. Juliano

Illinois State University, Normal, IL, United States

Mathematical models of mosquito transmitted diseases suggest that female longevity is an important factor for determining disease risk. As ectothermic organisms, temperature regulates mosquito growth and development. It is well understood that bellow a critical maximum, hotter temperatures decrease development time and adult size. Adult size is correlated with fecundity, but it is less well understood how size is related to longevity and how temperatures experienced in the larval habitat influence adult longevity. Additionally, most experimental studies that have tested effects of temperature use constant temperatures and ignore daily or seasonal temperature fluctuations that mosquitoes encounter under natural conditions. I simulated fluctuating daily temperature and photoperiods consistent with those experienced in St. Louis, MO in June and August to test the hypotheses that 1) *Aedes triseriatus* mosquito larvae developing under different seasonal conditions will differ in size and adult longevity 2) that fluctuating temperatures yield adults that differ in size and adult longevity from those produced in stable conditions. There was no effect of size on female longevity. Despite early differences in survival probability and different ages at senescence, there was no statistically significant difference between August and June treatments. The August treatment was significantly different from the constant temperature control, which experienced reduced longevity. Simulated August, June, and constant treatments also differed in larval survivorship, median day to eclosion, size, and r' (a cohort performance index). The June and constant treatments only differed in female size. These results suggest seasonal temperature fluctuations in the larval habitat do not affect adult longevity in *Ae. triseriatus*, but do affect other measures of performance typically reported in studies that investigate down- stream effects of the larval habitat.

786

TRANSGENIC *ANOPHELES STEPHENSI* FITNESS AND SUSCEPTIBILITY TO VARIOUS INFECTIONS

Andrew Pike, George Dimopoulos

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Various mosquitoes from the genus *Anopheles* spread the causative agents of human malaria. Traditional malaria control efforts have been unable to eliminate the disease, mainly because of insecticide and drug resistance arising among both the mosquitoes and the *Plasmodium* spp. parasites that cause the disease. Therefore, new tools to combat the disease are needed, and one potential new technology to limit malaria transmission is the use of transgenic mosquitoes refractory to malaria infection. Multiple laboratories have created multiple mosquito lines that do not transmit the malaria parasite, but have not yet reached the stage of releasing such mosquitoes, partly due to concerns about their fitness relative to wild-type conspecifics and their ability to resist a broad range of malaria parasites and other human pathogens. We studied various aspects of mosquito fitness in five separate transgenic lines representing different transgenes, insertion points and promoters in order to determine the fitness costs that may arise due to transgenesis. Of the five lines tested, four have shown no fitness cost and the fifth has some fitness reduction due to position effects. We also challenged our mosquitoes with different *P. falciparum* strains. Our results indicate that the mosquitoes are highly resistant to infection by multiple *P. falciparum* strains and do not suffer a large fitness cost as a result of transgenesis, thereby illustrating their potential utility as part of a larger malaria control program and warranting further testing in large cage or field trials. Ongoing studies are focused on testing transgenic mosquito resistance to the *P. vivax* parasite, O'nyong'nyong virus and the filarial worm *Brugia malayi*.

787

AEDES AEGYPTI POPULATION STRUCTURE IS DRIVEN BY BOAT TRAFFIC IN THE PERUVIAN AMAZON

Sarah Anne Guagliardo[1], Yoosook Lee[2], Jacklyn Wong[3], Yui Yin Chu[4], Helvio Astete[5], Gonzalo Vazquez-Prokopec[1], Thomas W. Scott[6], Uriel Kitron[1], Steven T. Stoddard[6]

[1]Emory University, Atlanta, GA, United States, [2]University of California Davis School of Veterinary Medicine, Davis, CA, United States, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Stanford School of Medicine, Palo Alto, CA, United States, [5]U.S. Naval Medical Research Unit - 6, Iquitos, Peru, [6]University of California Davis, Davis, CA, United States

In the Americas, as in much of the rest of the world, the dengue vector *Aedes aegypti* is predominantly found in urban areas. Its presence in rural areas is more limited, and the factors favoring its potential geographic expansion to rural and smaller urbanized settings are poorly understood. In the Peruvian Amazon, this vector has been expanding its range into rural communities over the last 5-10 years. Understanding *Ae. aegypti* dispersal patterns is important for anticipating the future range expansion of dengue and other viruses transmitted by this mosquito. To examine if human transportation networks play a significant role in the population expansion of this mosquito, we analyzed *Ae. aegypti* population structure using a panel of 10 microsatellite markers. Adult and immature *Ae. aegypti* (>20 individuals per site) were collected from the city of Iquitos (pop: 380,000) and several neighboring communities that are connected by river transport, including Nauta (pop: 14,000), Indiana-Mazan (pop: 7,000), Barrio Florida (pop: 750), Tamshiaco (pop: 4,500), and Aucayo (pop: 800). We detected significant departures from Hardy-Weinberg Equilibrium in all study sites due to lower than expected number of heterozygotes. FST statistics show significant differentiation for the majority of study site pairs. Population structure among *Ae. aegypti* in different towns is not correlated with the geographic distance between towns, suggesting that human transportation networks may be responsible. Furthermore, *Ae. aegypti* gene flow among sub-populations is greatest between locations with heavy boat traffic such as Iquitos-Nauta (which also has heavy road traffic) and Iquitos-Indiana-Mazan, and lowest between locations with little or no boat (or road) traffic such as Barrio Florida-Iquitos interior. Bayesian clustering analysis using Structure program suggested definite admixture, with 5-6 genetic clusters. Our results provide strong evidence for the hypothesis, especially via boats, is responsible for *Ae. aegypti* spread in the Peruvian Amazon.

788

ADVANCEMENTS AND CHALLENGES IN USING NEAR INFRARED SPECTROSCOPY (NIRS) TO DETERMINE THE AGE OF FEMALE *AEDES AEGYPTI* MOSQUITOES WITH VARYING LARVAL AND ADULT DIETS

Kelly Liebman[1], Isabel Swamidoss[1], Lucrecia Vizcaino[1], Audrey Lenhart[1], Floyd Dowell[2], Robert Wirtz[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]United States Department of Agricultulture, Manhattan, KS, United States

Interventions targeting adult mosquitoes are often used to combat the transmission of vector-borne diseases, including dengue. In the absence of a vaccine, we rely on control measures targeting the primary dengue vector, *Aedes aegypti*, to prevent transmission. Due to the 7 day extrinsic incubation period of the virus in the mosquito, older mosquitoes (>7 days)are of most concern in the dengue transmission cycle. Identifying the age of female mosquitoes is therefore a crucial step in determining if vector control interventions are altering the age structure of the mosquito population, thereby reducing transmission potential. We have developed

a model using near infrared spectroscopy (NIRS) to determine the age of adult female *Ae. aegypti.* This technique quantitatively measures organic compounds, and has previously been used successfully to age-grade other mosquito species. To determine if the larval and/or adult diet of female *Ae. aegypti* affects the ability of NIRS models to predict mosquito age, 2 groups of mosquito larvae were raised in identical settings and fed either fish food or infant cereal. Emerged adult females were separated and fed either blood or sugar, resulting in four experimental groups. These adult females were then killed 1, 4, 7, 10, 13 or 16 days post-emergence. The head and thorax of each mosquito were scanned using a LabSpec 5000. The spectral scans from each group were analyzed independently and collectively, to determine if the diet of the mosquito affected the spectrum. Multiple models were developed using GRAMS PLSPlus/IQ, with different spectral ranges. The best model included all experimental groups, and was able to positively predict the age group (< or ≥7 days) of 88.8% of mosquitoes. Models using a single experimental group to predict the others were less robust. These results indicate both larval and adult diet affect the predictive ability of models developed using the spectral scans of female *Ae. aegypti.* This potentially complicates the application of NIRS age grading to field populations, as larval and adult nutrition can vary greatly in the field.

789

SPECIES DIVERSITY, SEASONAL, AND SPATIAL DISTRIBUTION OF MOSQUITOES (DIPTERA: CULICIDAE) CAPTURED IN *AOTUS* MONKEY-BAITED TRAPS IN A FORESTED SITE NEAR IQUITOS, PERU

Michael J. Turell[1], Elizabeth S. Andrews[1], Alfonso Gonzalo[2], Faustino Carbajal[3], Victor Lopez-Sifuentes[4], George B. Schoeller[5]

[1]United States Army Medical Research Institute for Infectious Diseases, Fort Detrick, MD, United States, [2]Comparative Medicine Branch, Bethesda, MD, United States, [3]Unidad de Vigilancia y Control de Artrópodos y Reservorios, Minsa, Peru, [4]Department of Entomology, Callao, Peru, [5]U.S. Navy Bureau of Medicine and Surgery, Falls Church, VA, United States

This study was conducted to determine the relative abundance, diversity, seasonal, and vertical distributions of potential mosquito vectors in the Amazon Basin, Peru. A total of 66,097 mosquitoes (50 mosquito species from 12 genera) were collected from May 2001 through March 2002 at a forested site near Iquitos, Peru. Mosquitoes were collected using *Aotus nancymae* Hershkovitz monkey-baited CDC light traps set for 12-h day and night periods at varying heights (*e.g.* ground and canopy) in the forest. Of the 12 genera, three accounted for 75% of all mosquitoes collected: *Culex* (33%), *Aedes* (23%) and *Psorophora* (18%). The most prevalent species collected were *Aedes serratus* (Theobald), *Culex pedroi* Sirivanakarn & Belkin, *Psorophora albigenu* (Peryassu), and a combination of *Mansonia indubitans* Dyar & Shannon and *Mansonia titillans* (Walker), which accounted for 56% of all mosquitoes captured. In general, mosquitoes were collected more often at night and on the ground. Exceptions include *Coquillettidia venezuelensis* (Theobald), which were collected in relatively even numbers at both day and night and most *Mansonia* and some species of *Anopheles*, which were collected more often in the canopy. Total mosquito populations had two peaks, June-July (*Ma. indubitans/titillans, Cq. venezuelensis*) and December-January (*Ps. albigenu*, *Cx. pedroi, Ae. serratus*). Observations of the eight most collected mosquitoes indicated that behavioral shifts were not observed between collection months. These data provide a better understanding of the species diversity, population density and seasonal distribution of potential mosquito vectors within the Amazon Basin region and allow for the development of appropriate vector and disease prevention strategies.

790

HUMAN IGG ANTIBODY RESPONSE AGAINST RECOMBINANT *AEDES AEGYPTI* SALIVARY PROTEINS MODIFIED DURING DENV2 INFECTION

Berlin L. Londono-Renteria[1], Michael K. McCracken[1], Daniel M. Chisenhall[1], Jenny C. Cardenas[2], Miguel A. Sanabria[2], Lucio D. Cardenas[3], Omar G. Perez[3], Christopher N. Mores[1]

[1]Louisiana State University, Baton Rouge, LA, United States, [2]Hospital Local Los Patios, Los Patios-Norte de Santander, Colombia, [3]Universidad de Pamplona, Pamplona-Norte de Santander, Colombia

Arthropod saliva has been shown to modulate the success of infection success for the microorganisms they transmit. Our group has found that DENV infection of *Ae. aegypti* salivary gland induces changes in the expectorated salivary content. We have produced by recombinant technology three of the proteins we found to be reduced in saliva. The immunogenicity against these proteins was evaluated in subjects from a DENV endemic area in Colombia by ELISA. We found a significant increase in the IgG antibody levels after exposure to mosquito bites. We also found a differential antibody response among healthy, febrile DENV (-) and DENV (+) subjects. We hypothesize that the decrease of these proteins in saliva may be advantageous to the virus. Differential IgG response between DENV (+) and negative subjects suggests 1) dengue positive subjects are significantly at higher exposure/risk to mosquito bites 2) the immune response against these proteins may play an important role in disease progression.

791

ALTERNATIVE MOSQUITO SAMPLING TO MONITOR LYMPHATIC FILARIASIS TRANSMISSION IN PAPUA NEW GUINEA

Daniel J. Tisch[1], Naomi Vincent[2], John Keven[2], Lisa Reimer[3], Edward D. Walker[4], Peter Siba[5], James W. Kazura[1], Peter A. Zimmerman[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Papua New Guinea Institute for Medical Research, Madang, Papua New Guinea, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [4]Michigan State University, East Lansing, MI, United States, [5]Papua New Guinea Institute for Medical Research, Goroka, Papua New Guinea

Entomological markers of lymphatic filariasis (LF) transmission provide key information regarding progress toward LF elimination and subsequent monitoring. However, "gold standard" approaches of human landing catches (HLC) can be inefficient and expensive as transmission approaches zero. In addition, HLC can be complicated by and/or violate cultural and ethical standards. Recently barrier screens have been used with success in the Pacific region to estimate human blood index and quantify malaria transmission in anopheline mosquitoes. Since Wuchereria bancrofti (Wb) is exclusively transmitted by anopheline vectors in Papua New Guinea, this study compares HLC, light traps, and barrier screens with regard to mosquito species captured, anopheline infectivity (as detected by microscopy for stage 3 Wb larvae), and presence of Wb DNA. 2mx40m barrier screens were sampled every 15 minutes by trained collectors in communities with simultaneous HLC collections (plus light traps in one sampling week). Collections occurred between 6pm and 6am in four communities for one week in November 2013, March 2014, and July 2014. Mosquitoes were separated according to hour captured, capture method, and the side of the screen collected (village side vs. bush side). Anopheline capture density and composition will be compared for each mosquito sampling method. Xenomonitoring for LF elimination will be quantified by both microscopy of anopheline mosquitos and PCR for Wb DNA in all bloodfed mosquitoes. Human blood index and presence of Wb DNA will be compared across the study communities. Negative binomial models will be fit to compare agreement between mosquito capture

methods for mosquito species densities and presence of L3. The results are relevant to the selection of the most efficient mosquito collection techniques for xenomonitoring LF during elimination programs in PNG.

792

IGG ANTIBODY SUBCLASSES AGAINST VECTOR SALIVARY PROTEINS AS A MEASURE TO RISK OF *AEDES AEGYPTI* BITE EXPOSURE AFTER IMPLEMENTATION OF ATTRACTIVE LETHAL OVITRAPS

Berlin L. Londono[1], Daniel M. Chisenhall[1], Samuel B. Jameson[2], Amy C. Morrison[3], Robert D. Hontz[4], Christopher N. Mores[1], Dawn M. Wesson[2]

[1]Louisiana State University, Baton Rouge, LA, United States, [2]Department of Tropical Medicine, Tulane University, New Orleans, LA, United States, [3]Department of Entomology, University of California Davis, CA, United States, [4]U.S. Naval Medical Research Unit - 6, Lima, Peru

Aedes salivary proteins induce specific immune responses in the vertebrate host that can be used a tool to measure vector host-contact. Specifically, an increase in IgG antibody subclasses (IgG1 predominantly associated with the anti-microbial response and IgG4 representing chronic exposure to an antigen) has been associated with chronic exposure to mosquito bites and the risk for arbovirus infection. We evaluated the levels of antibodies against *Aedes aegypti* salivary gland extract in subjects from dengue endemic areas in Iquitos-Peru, before and after the implementation of Attractive Lethal OviTraps (ALOTs). Serum samples were collected from the household occupants before (Time 0) and after one year of the treatment (Time 12) in the ALOT treated and the untreated control areas. PRNT70 tests for DENV were performed on all sera for determination of viral exposure. Our results showed that IgG4 antibody concentrations were significantly higher than IgG1 concentrations for both groups at both times. When comparing the antibody response between times, we found that the concentration of both IgG1 and IgG4 decreased in subjects from the treated area over 12 months. No significant differences were observed in the control area during that time. The relationship between the exposure to DENV and IgG4/IgG1 will be discussed. Our results suggest that IgG4 levels are indicative of cumulative exposure to mosquito bites and it represents a useful tool for monitoring vector control interventions.

793

INFECTION EVALUATION ON FIELD-CAPTURED MOSQUITOES FROM THREE COLOMBIAN CITIES WITH DIFFERENT ENDEMICITY PATTERNS OF DENGUE

Víctor H. Peña, Sair O. Arboleda, Ana M. Mejía-Jaramillo, Francisco J. Díaz, Omar Triana-Chávez

Universidad de Antioquia, Medellín, Colombia, Colombia

Dengue fever is endemic in Colombia, where the four virus serotypes circulate in highly variable epidemiological scenarios. This heterogeneity is illustrated in the following three cities: Riohacha, with the highest values of Breteau index (BI) and lowest number of dengue cases; Villavicencio, which has the highest number of dengue cases and the second highest BI values; and Bello, which has the lowest BI values, but exceeds Riohacha in number of dengue cases. To explain these differences, adult mosquitoes were captured in four neighborhoods in each city over 3 to 5 sampling periods across 2012 and 2013. Virus presence and serotype were detected through examination of pooled mosquitos from each sampled house. We found that mosquitoes from Bello have the highest infection rate, followed by Villavicencio and finally Riohacha. In samples from Riohacha and Villavicencio, we detected the serotypes DENV-1, 3 and 4, while in Bello we detected serotypes DENV-2, 3 and 4, and the highest proportion of pooled mosquitos with more than one serotype. 13.3% of pools presented infection with more than one serotype, including two pools of just one single mosquito, which is the first reported case of a mosquito co-infected with two dengue serotypes in Colombia. DENV-4 serotype is the most prevalent in pools from the three cities (75.5%), followed by DENV-3 (28.8%), DENV-1 (6.6%) and DENV-2 (2.2%). Our results differ from those of dengue serotype presence in humans from the whole country reported by the Colombian National Institute of Health, where DENV-1 is the most prevalent and DENV-4 the least. This suggests that human populations are more refractory to DENV-4 infection, the most abundant serotype, while being more susceptible to DENV-1 and DENV-2 infection. These patterns highlight the importance of including evaluation of mosquito infection when developing dengue control strategies.

794

VALIDATION OF A PREDICTIVE MODEL: ENVIRONMENTAL INFLUENCES ON THE PREVALENCE OF WEST NILE VIRUS IN CULEX MOSQUITOES, LONG ISLAND, NEW YORK

Eliza A. Little[1], Scott Campbell[2], Jeffrey Shaman[1]

[1]Columbia University, New York, NY, United States, [2]Suffolk County Department of Health Services, Riverhead, NY, United States

To better understand the dynamics of West Nile Virus (WNV) transmission, we revisit a model describing the spatial-temporal distribution of positive Culex mosquito pools collected in Suffolk County, Long Island, New York. The original statistical model (m09) used meteorological and hydrological conditions to simulate WNV infection in Culex mosquitoes during 2001-2009. Here, we use Culex mosquito WNV infection data collected during 2010-2012 to validate m09 predictions for these years and to explore whether inclusion of additional environmental variables improves model performance. The m09 predictions for 2010-2012 are well correlated with observed WNV activity during the same time period (0.65). In evaluating the full 2001-2012 record (m12), we explored smoothing the meteorological and hydrological data temporally over 3-month seasons, increasing the spatial resolution of the analysis to the locations of the mosquito traps rather than the aggregated scale of the meteorological and hydrological data, and incorporating other socioeconomic and environmental variables (median household income, population density, a metric of the built environment, and household occupancy status); however, the results remained consistent, with wetter winter conditions, wetter and warmer spring conditions, and drier summer conditions favoring increased prevalence of WNV among Culex mosquitoes. In general the socioeconomic variables did not further constrain the models, suggesting that the distribution of WNV activity in Culex is most intimately linked to climate. Temporal cross validation of the m12 model confirmed consistency of the associations between environmental variables and WNV activity. The different spatial and temporal considerations of these analyses indicate robust associations between meteorological and hydrological conditions and WNV activity. Validation of this model indicates that as meteorological observations and hydrology model estimates of land surface wetness are collected, areas at heightened risk for WNV activity can be predicted.

795

OCHLEROTATUS GENICULATUS, A NATIVE EUROPEAN MOSQUITO WITH A HIGH POTENTIAL FOR TRANSMISSION OF CHIKUNGUNYA VIRUS

Aurélien Mercier[1], Ada Shukullari[2], Jean-Charles Gantier[1], Laure Diancourt[1], Henri Bonnabau[3], Francis Schaffner[4], Enkelejda Velo[2], Paul Reiter[1]

[1]Institut Pasteur, Paris, France, [2]Institute of Public Health, Tirana, Albania, [3]Institut de Santé Publique, d'Épidémiologie et de Développement, Bordeaux, France, [4]National Centre for Vector Entomology, Zurich, Switzerland

Ochlerotatus (Finlaya) geniculatus (Olivier), a tree-hole mosquito that bites humans aggressively, is present throughout Europe, North Africa and Asia Minor. In Albania it shares a common habitat with *Aedes (Stegomyia) albopictus* and our ovitrap collections have confirmed its abundance to at least 1350m. In the laboratory, both species were highly susceptible

to chikungunya virus (quantitative titration of saliva, head and body) but reached a higher titre in the head and thorax of *Oc. geniculatus*. Titer of infectious virus in the saliva was also higher and more sustained. Interestingly, in Ae. albopictus, virus was present in the saliva within three days, whereas in *Oc. geniculatus* it was not detectable until day seven, after which the profile of increase in titre was akin to that of dengue virus in *Ae. aegypti*. Given the widespread presence of Oc. geniculatus in peri-urban areas, its propensity to feed on humans and the rapid rise in the frequency of imported cases of chikungunya, we suggest that this species should be considered as a potential vector in the Palearctic region.

796

OPTIMIZATION OF FEEDING ASSAYS TO EVALUATE MALARIA TRANSMISSION-BLOCKING VACCINES IN MALI

Ibrahima Baber[1], Mahamadoun H. Assadou[1], Liam Donnelly[2], Junhui Duan[2], Adama Sacko[1], Lakamy Sylla[1], Omely Marte[2], Kun-lin Lee[2], Merepen A. Guindo[1], Issaka Sagara[1], Mamadou B. Coulibaly[1], Ogobara K. Doumbo[1], Sekou F. Traore[1], Sara Healy[2], Yimin Wu[2], Patrick Duffy[2]

[1]Malaria Research & Training Center, Bamako, Mali, [2]Laboratory of Malaria Immunology and Vaccinology/National Institutes of Health, Rockville, MD, United States

Transmission-blocking vaccines (TBV) are critical tools for malaria elimination. However, the assays used to measure TBV functional activity, including direct membrane feeding assays (DMFA) and direct skin feeds (DSF) involve multiple biological systems with inherent variability. The objective of this research study is to establish optimal conditions for our assays used in clinical trials in Mali. Since January 2014, field experiments have been carried out to address different aspects of the feeding assays (DSF/DMFA). Experiments have been conducted to confirm optimal feeding parameters for DMFA including membrane selection (parafilm versus baudruche), mosquito age, and mosquito starvation duration. The impact of anatomical location (arm, calf, ankle) on subsequent mosquito infectivity by DSF is being assessed. Finally, we want to assess the effect the time of day the DSF is performed has on mosquito infectivity by DSF and DMFA. The evaluated endpoints for the exploration of these variables includes: mosquito feeding rates, mosquito survival rates, and mosquito infectivity rates. Optimal observed parameters will be applied to improve the feeding assays conducted for transmission-blocking activity (TBA) measures. Data from pilot experimental hut (EH) assays, procedure by which wild, blood-fed mosquitoes are collected from the room where the participant slept alone overnight, will be presented. First, molecular tools will be deployed to pair fed mosquitoes with the individual volunteer participating in the EH assay. If fed mosquitoes captured are consistently matched to the EH volunteer, the EH assay will be expanded to evaluate TBV impact on naturally occurring transmission.

797

DESIGN AND ASSESSMENT OF A MOBILE DATABASE MANAGEMENT SYSTEM FOR ARTHROPOD-BORNE DISEASE SURVEILLANCE IN BELIZE

Michael Clark[1], John Grieco[2], Nitesh Chawla[1], Nicole Achee[1]

[1]University of Notre Dame, Notre Dame, IN, United States, [2]Uniformed Services University of the Health Sciences, Bethesda, MD, United States

Successful implementation of information and communication technologies for development (ICT4D) hinge on dynamic collaboration among all key stakeholders - particularly intended users. In dengue endemic areas, where vector control practices have shown diminishing returns, the gap between surveillance practices and ministerial response represents a unique opportunity for mobile health technologies to fill a need. The incorporation of real-time data collection and archiving, open-source QGIS mapping capabilities, and regression tools into a single platform aims to support ministry officials in streamlined action for both preventive and reactionary measures. This study presents a three-tiered, workshop-based approach to the adoption of a novel mobile application and database management system by the Northern Region of the Ministry of Health (MOH) in Belize. In general, the first phase consists of training MOH field technicians on theory and technical use of the mobile application; the second phase simulates field conditions in an outdoor setting and extends to risk map generation; while Phase III monitors real surveillance conditions in village settings. Shortcomings of the mobile system identified by MOH officials and field technicians at each phase guides reprogramming, ensuring a user-oriented product. Data will be available by mid-July 2014.

798

MOSQUITO VECTOR MICROBIOME DIVERSITY ACROSS HABITATS IN CENTRAL THAILAND ENDEMIC FOR DENGUE AND OTHER ARTHROPOD-BORNE DISEASES

Panpim Thongsripong[1], Amy Green[2], James Chandler[3], Pattamaporn Kittayapong[4], Durrell Kapan[5], Bruce Wilcox[6], Shannon Bennett[3]

[1]Department of Tropical Medicine, Tulane University, New Orleans, CA, United States, [2]Department of Microbiology, University of Hawai'i at Manoa, Honolulu, HI, United States, [3]Department of Microbiology, California Academy of Sciences, San Francisco, CA, United States, [4]Center of Excellence for Vectors and Vector-Borne Diseases, Nakhon Pathom, Thailand, [5]Center for Comparative Genomic, California Academy of Sciences, San Francisco, CA, United States, [6]Integrative Research and Education Program, Mahidol University, Bangkok, Thailand

Anthropogenic environmental change is among the most frequently identified factor linked to emergence of infectious diseases. The proposed mechanism by which anthropogenic environmental changes affect infectious disease spread is through the perturbation of the ecological communities involved in transmission, resulting in changing distribution and relative abundance of the key organisms. The objective of this study is to characterize the diversity of mosquitoes, relative abundance of vectors, and their associated microbial communities along a forest-agro-urban habitat gradient in Thailand to determine how habitat changes affect multi-level communities. Various adult mosquito traps were set-up along a habitat transect to capture components of mosquito communities in each characterized habitat. Over 62,000 female mosquitoes were identified morphologically and selected vector species, *Aedes aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus*, were pooled by species and habitat type. Segments of 16s and 18s rRNA genes were sequenced from the pools using 454 pyrosequencing technology to assess genetic and taxon-based diversity of the non-eukaryote and eukaryote microbiota. Female mosquito abundance was highest in rice fields and lowest in forests. Based on extrapolated species richness estimators (Chao1 and ACE), forest and fragmented forest habitats had the most diverse mosquito communities, followed by the rural, rice field, suburban and urban habitats. Interestingly, the relative abundance of two vector species, *Ae. aegypti* and *Cx. quinquefasciatus*, was negatively correlated with mosquito diversity. Furthermore, the microbiota community assembly and diversity of selected vector species were associated with habitat types suggesting that habitat factors differentially affect mosquito microbiota. In this study, mosquito community and vector microbiota diversity varied across a continuum of habitat types in a pattern reflecting habitat change. Moreover, changes in the abundance of important mosquito vectors tended to follow a predictable pattern. Collectively, these findings help illuminate how mosquitoes and their associated microbial communities vary across habitat types and how these dynamics could contribute to emergence of arboviral diseases in Thailand.

799

STUDYING THE EXTRINSIC INCUBATION PERIOD IN DENGUE VIRUS INFECTED MOSQUITOES

Elizabeth A. McGraw

Monash University, Clayton, Victoria, Australia

In the past 20 years, dengue has become the most prevalent arthropod-borne virus affecting humans today. This exponential increase in disease incidence has brought with it significant health, social and economic problems. Vectorial capacity, which is a measurement of the efficiency of vector-borne disease transmission, is influenced by a few key factors. Extrinsic incubation period (EIP), which is the interval of time from the ingestation of an infectious bloodmeal to the time of transmission, is a key determinant of vectorial capacity. Here we have used a repeat sampling technique for saliva to study individual variation in EIP in dengue-infected *Aedes aegypti*. We show significant heritability (~40%) for EIP and a strong positive correlation between length of EIP and mosquito lifespan. We also examine the effect of the biocontrol agent, Wolbachia pipientis on EIP under different temperature regimes.

800

RIFT VALLEY FEVER OUTBREAK IN SAUDI ARABIA ANTICIPATED FROM AFRICA OUTBREAKS AND TIME-SPECIFIC SATELLITE DATA

Abdallah M. Samy[1], Mohamed A. Kenawy[2], A. Townsend Peterson[1]

[1]Kansas University, Lawrence, KS, United States, [2]Ain Shams University, Cairo, Egypt

Rift Valley fever (RVF) is an acute zoonotic viral disease that affects domestic animals and humans in sub-Saharan Africa. More recently, a RVF outbreak was first identified outside Africa in the Arabian Peninsula. We used ecological niche modeling to assess the ability of models to identify areas of disease risk in Saudi Arabia using 8-day composite Land Surface Temperature (LST) and monthly Normalized Difference Vegetation Index (NDVI) data from the Moderate Resolution Imaging Spectroradiometer (MODIS) satellite imagery (1 km spatial resolution) for January to December 2000 (i.e., the time of disease outbreak in Saudi Arabia). The model was calibrated based on records from African outbreaks, and transferred to Saudi Arabia to anticipate suitable sites of RVF across Arabia. Results suggested suitable areas in western Saudi Arabia in Gizan region. We evaluated models using occurrence points from the 2000 outbreak in Saudi Arabia. Models calibrated in Saudi Arabia revealed similar spatial patterns. This study revealed the potential of niche modeling approaches to anticipate suitable areas for disease emergence in areas with no previous disease history, opening the possibility of genuine prediction of risk, particularly in future studies that add data on availability of vectors.

801

UNDERSTANDING DENGUE TRANSMISSION AND RISK FACTORS IN BANGLADESH

Parnali Dhar-Chowdhury[1], C. Emdad Haque[1], Robbin Lindsay[2], Shakhawat Hossain[3], Abdullah Brooks[4], Antonia Dibernando[2], Kai Makowski[2], Michael A. Drebot[2]

[1]University of Manitoba, Winnipeg, MB, Canada, [2]National Microbiology Laboratory, Winnipeg, MB, Canada, [3]University of Winnipeg, Winnipeg, MB, Canada, [4]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Southeast Asian countries including Bangladesh have remained hyperendemic for dengue due to unplanned urbanization, overcrowding, poverty and health inequalities. In recognition of the need for an multidisciplinary, scientific research on this problem, we applied an "Ecohealth Approach" to understand dengue virus (DENV) transmission and social-ecological risk factors in Dhaka, Bangladesh. Multiple disciplinary aspects were encapsulated by examination of: i) rates of human exposure to DENV by identifying individuals (via a serosurvey in 1200 households and 47 clinical samples) with IgM and IgG antibodies to DENV; ii) abundance of dengue vector during monsoon and dry seasons in the same households; iii) self-risk perception by the community members; and iv) human organizations responsible for interventions. Data included in the analysis are: a) two vector surveys [i.e., pupal surveys conducted in 847 households (monsoon season 2011) and 459 households (dry season 2012)]; b) two serosurveys [i.e., serosurveys conducted in 1128 households (pre monsoon season 2012) and 1130 households (630 paired sera and 500 replacement sera during post monsoon season 2012)]; c) socio-demographic survey of 300 households; and d) 12 focus group discussions and 12 key informant interviews. Competent dengue vectors were detected in >40% and 12% of households during the monsoon and dry seasons respectively. The monsoon and dry seasonal pupal index were 0.40 and 0.33 respectively for the selected 12 wards. Only 8 types of key containers and two types of ecological clusters are responsible for 72% of pupal distribution. More than 80% IgG and nearly 3% IgM were positive during pre- and post-monsoon serosurvey. Among the IgM positives, in-house PRNTs, using a serial dilution of sera mixed with a DENV serotype, are being carried out. There are significant variations in dengue risk perception between lower and higher socioeconomic groups. Also, experts ranked dengue risk at a much lower level than lay persons and experts emphasized the need for stronger institutional measures to control dengue outbreaks. The overall findings of the study will contribute to the advancement of DENV transmission knowledge, forecast the disease burden as well as socioeconomic burden in the City of Dhaka, Bangladesh.

802

INVESTIGATING THE EFFICACY OF MONOVALENT AND TETRAVALENT LIVE-ATTENUATED DENGUE VACCINE FORMULATIONS AGAINST DENV-4 CHALLENGE IN AG129 MICE

Jeremy Fuchs[1], Haiyan Chu[1], Subash Das[1], Nigel Bourne[2], Gregg Milligan[2], Dan Stinchcomb[1], Alan Barrett[2], **Charalambos Partidos[1]**, Jorge Osorio[1]

[1]Takeda Vaccines, Inc., Madison, WI, United States, [2]University of Texas Medical Branch, Galveston, TX, United States

Dengue virus (DENV) causes a rapidly spreading mosquito-borne human viral disease that has major impact on global health and economics. Currently, there is no licensed vaccine against DENV. We have developed a live attenuated tetravalent dengue vaccine candidate based on an attenuated dengue 2 virus (DENV-2) and three chimeric viruses containing the pre-membrane and envelope genes of DENV-1, -3 and -4 expressed in the context of the attenuated DENV-2 genome. In a mouse model, we investigated the immune responses to monovalent DENV-4, DENV-2 or tetravalent DENV vaccine formulations, and their efficacy against challenge with a newly mouse adapted DENV-4 strain 703. Single doses of the tetravalent or monovalent vaccines elicited neutralizing antibodies and cellular responses to DENV-2 backbone. Monovalent DENV-2 vaccine also elicited cross-neutralizing antibody responses to DENV-4. When tested against a lethal challenge of DENV-4 sixty days post-primary immunization all mock-vaccinated animals (n=15) died, but all vaccinated animals survived except for 1 of 15 DENV-2 vaccinated mice. Investigation of DENV-4 viremias post-challenge showed that only the placebo-treated animals had high viremias on day 3 post-challenge. Overall, these data highlight the immunogenicity and efficacy profiles of our candidate dengue vaccine in the mouse model.

803

EFFECT OF MIGRATION STATUS ON RISK OF DENGUE VIRUS INFECTION IN PUERTO MALDONADO, PERU

Gabriela Salmon-Mulanovich[1], Zonia Rios[1], Angelica Espinoza[1], Carolina Guevara[1], David L. Blazes[2], Joel M. Montgomery[3], Daniel G. Bausch[1], William K. Pan[4]

[1]U.S. Naval Medical Research Unit - 6, Callao, Peru, [2]Navy Medicine Professional Development Center, Bethesda, MD, United States, [3]Division of Global Disease Detection, Centers for Disease Control and Prevention, Nairobi, Kenya, [4]Duke Global Health Institute and Nicholas School of Environment, Duke University, Durham, NC, United States

Dengue virus (DENV) affects more than 100 countries worldwide. DENV has been reported in Puerto Maldonado (population ~80,000) in the Peruvian southern Amazon Basin since 2000. This region also has the highest human migration rate in the country, mainly from non-endemic areas for DENV. The risk of DENV infection may vary between recent migrants (RM) and long-term residents (LTR) due to both biological and sociologic factors. We explored the prevalence of past DENV infection and knowledge, attitudes and practices (KAP) related to dengue disease control and prevention of RM versus LTR, defined respectively as residency in Puerto Maldonado for less than or greater than 5 years. In 2012 we conducted a cross-sectional serosurvey and administered a KAP questionnaire to members of randomly selected households. Sera were screened for antibody to DENV by ELISA and confirmed by plaque reduction neutralization test (PRNT). We created *ad hoc* indices for KAP (KAPi), household infrastructure and services (CFSi) and assets (Ai). PRNT results were analyzed against migration status and the various indices with an ordered logistic regression. Five hundred and five participants from 309 households provided a blood sample and completed a questionnaire. RM comprised 12% of the study population and were more likely to be DENV antibody negative than LTR on bivariate analysis (42% vs. 56%, p=0.043). However, after controlling for the other variables in the multivariate analysis, KAPi (p=0.032), commercial activities (p=0.023) and CFSi (p=0.017) were associated with antibody positivity, while migration status was not (p=0.226). The higher KAPi is likely consequence of experiencing the disease. Commercial activities and CFSi may be related to the location or infrastructure of areas where participants spend long periods of time. We conclude that risk of DENV infection in Puerto Maldonado relates more to socio-economic status, especially infrastructure and services available in the household, than to migration status *per se*. These findings should help tailor specific prevention and control strategies for dengue diseases in the area.

804

HOST CELL RESPONSES ATTENUATE DENV-2 PDK53 BUT NOT DENV-3 PGMK30FRHL3

Kenneth C. Goh[1], Diana C. Norton[2], Kuan Rong Chan[1], October M. Sessions[1], Eng Eong Ooi[1]

[1]Duke-NUS Graduate Medical School, Singapore, Singapore, [2]Duke University School of Medicine, Durham, NC, United States

The global prevention of dengue is challenging without a vaccine, the development of which has been limited by an incomplete understanding of pathogenesis. In particular, efforts to develop a live attenuated tetravalent vaccine encountered problems in balancing immunogenicity with reactogenicity. Such vaccines have been derived through serial passage of dengue viruses (DENVs) in various primary cell cultures followed by selection of strains that meet specific but empirically-derived phenotypic criteria such as small plaque size. Unfortunately, these empirically-derived criteria do not invariably inform on attenuation. The Mahidol University-developed DENV-2 PDK53 and DENV-3 PGMK30FRhL3 strains both fully met these empirically-derived phenotypic criteria. However, while PDK53 was shown to be safe and immunogenic, PGMK30FRhL3 was reactogenic and vaccine recipients developed symptoms consistent with dengue fever. In this study, we asked if a molecular approach might be able to distinguish a more accurate basis of attenuation. We observed that Huh-7 cells infected with PDK53 upregulated the expression of many genes in the innate immune system compared to wild-type DENV-2 16681 from which it was derived. In contrast, the pattern of expression of these same genes in both PGMK30FRhL3 and the parental DENV-3 16562 were similar and mostly low. Functional studies suggest that the innate immune responses restrict the plaque size of PDK53 while that of PGMK30FRhL3 plaque size is limited by its slower replication. We suggest that a molecular definition could be developed for a more accurate identification of viral attenuation.

805

DENGUE VIRUS TYPE 3 EVOLUTION AND EPIDEMIC ACTIVITY IN INDONESIA, A HISTORICAL STUDY OF OUTBREAKS FROM 1976-1979

Sean V. Edgerton[1], Duane J. Gubler[2], Shannon N. Bennett[1]

[1]California Academy of Sciences, San Francisco, CA, United States, [2]Duke-NUS Graduate Medical School, Singapore, Singapore

Dengue viruses are one of today's most significant vector-borne disease agents threatening human health throughout the tropics and subtropics, infecting hundreds of millions of people annually. Dengue is primarily transmitted by *Aedes aegypti* mosquitoes. There are four known serotypes circulating in humans (DENV-1 to -4) all of which can cause a febrile illness known as dengue fever that can progress to more severe and potentially fatal disease involving hemorrhage or shock (DHF/DSS). We report here follow up sequence data on DENV-3 strains isolated during epidemics that occurred in Indonesia between 1976 and 1979. The epidemics began with the detection of fatal DHF/DSS associated with DENV-3 in Jakarta in Jan-Mar, 1976. The virus spread to Bantul, Central Java in Oct. 1976, and to Surabaya, East Java and Pontianak, West Kalimantan in 1977. All of these were explosive epidemics with associated severe disease. A smaller outbreak with more sporadic transmission, milder illness and much lower viremia levels occurred in Sleman, Central Java in 1978. Viruses were isolated by one of us (DJG) from all of these epidemics and stored in infected mosquitoes at -70 C for nearly 40 years. The viruses had not been passaged in mice nor mammalian cell cultures. Full genomic sequence analysis suggests that a single strain of DENV-3 with greater epidemic potential and possibly virulence emerged in Jakarta and spread rapidly along the main transportation routes to Central and East Java, and to Kalimantan. Interestingly, the Sleman DENV-3 viruses were genetically distinct, belonging to a separate clade from the other strains. There were two unique Bantul isolates that also belonged to the Sleman clade, suggesting that the Sleman virus descended from these Bantul viruses. Our findings emphasize the importance of dengue evolution and genetic variation as a contributor to epidemic intensity and severe dengue disease.

806

ANALYSIS OF HUMAN DENGUE-IMMUNE SERA USING WHOLE VIRUS PROTEOME PEPTIDE ARRAYS

Felix Loeffler[1], Angela M. Green[1], Alexander Nesterov-Mueller[2], Angel Balmaseda[3], Thomas Jaenisch[4], Frank Breitling[2], Eva Harris[1]

[1]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States, [2]Department of Peptide Arrays, Institute of Microstructure Technology, Karlsruhe Institute of Technology, Karlsruhe, Germany, [3]Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua, [4]Department of Infectious Diseases, Section Clinical Tropical Medicine, Heidelberg University Hospital, Heidelberg, Germany

Dengue, a mosquito-transmitted viral disease, has become a major worldwide public health burden. Four distinct serotypes of dengue virus (DENV1-4) contribute to the challenge of developing a safe and effective tetravalent vaccine. We have exploited high-density peptide arrays for the readout of serum antibodies from dengue patients from ongoing pediatric hospital-based and community-based cohort studies

in Managua, Nicaragua. First, we selected a set of 3,172 overlapping 15-mer peptides, covering the proteomes of 20 different DENV genotypes (3 DENV1, 7 DENV2, 5 DENV3, 5 DENV4). Then, we generated five peptide arrays applying our novel particle-based peptide synthesis method using a custom-built laser printer, which allows for complete content flexibility on every array. Each array enables simultaneous analysis of a patient's IgG and IgM antibody reactivity to all four DENV serotypes. We first analyzed acute, convalescent, and 12-month samples from primary and secondary DENV2 cases. The results from the arrays identified the acute infecting serotype and revealed time-dependent waning of antibody responses in patients' sera. We also observed an increase in reactivity to a few peptides after 12 months, specifically, to peptides in NS1 and NS3. To confirm these results, we are currently analyzing 46 additional serum samples of primary and secondary DENV infection with a set of 2,000 overlapping 20-mer peptides, derived from 361 different DENV strains representing all known Nicaraguan genotypes in DENV1-3 and 9 Colombian DENV4 strains. In addition, to obtain a profile of the immunogenic DENV proteome map, we incubated one peptide array with a pool of 100 patient sera from adult Red Cross donors from Nicaragua who had experienced one or more DENV infections. From this broad polyclonal response, we identified immunogenic pan-serotype protein regions, as well as serotype-specific regions. In contrast to protein arrays, our peptide-based approach allows us to obtain a fine resolution map of the proteome immunogenicity. These results can help to elucidate structural and functional differences among the DENV serotypes and point to possible diagnostic biomarkers and vaccine targets.

807

ECONOMIC IMPACT OF WORK ABSENTEEISM DUE TO NON-SEVERE DENGUE VIRUS INFECTION IN A CITY IN THE PERUVIAN AMAZON

Stalin Vilcarromero[1], Sandra Olkowski[2], Amy C. Morrison[3], Percy A. Rojas-Ferreyra[4], Cristiam Carey[5], Alex Vasquez-Valderrama[1], Wieslawa Alava[1], Regina Fernandez[1], Brett M. Forshey[6], Eric S. Halsey[6], Thomas W. Scott[2], Tadeusz J. Kochel[7], V. Alberto Laguna-Torres[6]

[1]U.S. Naval Medical Research Unit - 6, Iquitos, Peru, [2]Department of Entomology, University of California Davis, Davis, CA, United States, [3]Department of Entomology, University of California and U.S. Naval Medical Research Unit - 6, Lima and Iquitos, Peru, Davis, CA, United States, [4]Hospital Regional de Loreto, Iquitos, Peru, [5]Direccion Regional de Salud Loreto, Iquitos, Peru, [6]U.S. Naval Medical Research Unit - 6, Lima and Iquitos, Peru, [7]U.S. Naval Medical Research Unit - 6/National Biodefense Analysis and Countermeasures Center, Lima and Iquitos, Peru

Dengue fever is the most prevalent mosquito-borne viral disease globally. The public health impact of this disease is compounded by the fact that it occurs primarily in developing countries, adding a significant burden for local economies and individuals that are already under-resourced, undermining both regional and national development. To assess the economic impact at the community-level in Iquitos, the largest city in the Peruvian Amazon basin, we collected information on work absenteeism due to dengue virus (DENV) infection. Clinical and epidemiological data were obtained from outpatients > 18 years-old who sought care for acute febrile disease at one of twelve health facilities in Iquitos during July 2009 to June 2010, when DENV-4 was the predominant circulating serotype. Dengue fever was confirmed by PCR, IFA or by a four-fold rise in IgM titer between acute and convalescent blood samples. Housewives were not included in the study, since they are not formally financially compensated. Daily wage estimates were assigned based on the reported occupation (the average of reported salary ranges in the city), then multiplied by the number of work days reported lost due to illness by each case. The effect on the local economy was estimated using data from the Regional Government. Of the 504 enrolled patients who engaged in wage-earning employment and provided absenteeism information, 199 (39%) had laboratory-confirmed dengue fever. Dengue fever patients missed an average of 3.9 work days translating to a mean income loss of US$45.Twenty-six percent of those sampled earned US$151-199 monthly, significantly less than the Peruvian minimum wage of US$283. The average income loss in this group was US $48, representing 24-32% of their monthly income. Despite the fact that these dengue fever cases were not severe because they were all outpatients, in the context of a local economy where many people are independently employed and have subsistence-level income, DENV infection posed a considerable financial burden.

808

IDENTIFYING HOST INNATE IMMUNITY FACTORS CRUCIAL FOR PROTECTION AGAINST DENGUE VIRUS

Diana C. Norton[1], Kenneth C. Goh[2], Hwee Cheng Tan[2], Eng Eong Ooi[2]

[1]Duke University School of Medicine, Durham, NC, United States, [2]Duke-NUS Graduate Medical School, Singapore, Singapore

Dengue virus (DENV) is a mosquito-borne human pathogen with no existing vaccine. Previous vaccine development efforts have relied on serial passage in cell culture to derive attenuated strains. This approach by Mahidol University led to a tetravalent vaccine with all strains meeting *in vitro* phenotypic criteria of attenuation, such as small plaque size. In clinical trial, DENV-3 PGMK30FRhL3 caused symptoms consistent with dengue fever, whereas DENV-2 PDK53 did not. As such, the traditional phenotypic criteria are not able to accurately predict human response to vaccines. Defining the molecular basis of attenuation could thus provide for more objective means of assessing candidate vaccine strains. We hypothesized that the PGMK30FRhL3 strain maintains its wild-type ability to evade host antiviral defenses, while the PDK53 strain elicits protective antiviral sensors and interferon signaling. To study host innate immunity defenses, we performed RNAi knockdown of key genes of antiviral sensors and interferon signaling cascade--MAVS, IRF3, TRIF, STAT1, and NF-kB--in Huh-7 and BHK-21 cells. Following infection with PDK53 and PGMK30FRhL3, we compared plaque and focus size between cells with normal and impaired antiviral defenses. We then quantified viral spread via plaque size, immunofluorescence foci, and flow cytometry. As expected, plaque size of attenuated PDK53 was smaller than wild type DENV-2 16681. Upon knockdown of IRF3 or p50, the plaque size of PDK53 but not 16681 increased significantly. Similar observations could be made in the human Huh-7 cell line. These findings indicate that the plaque size of PDK53 is because of its inability to overcome the innate immune response, whereas the replication of wild type 16681 does not benefit from the knockdown of these innate immune genes. Our results suggest that the inability of PDK53 to escape innate immune recognition is the molecular basis of attenuation.

809

THE SPATIAL PATTERNS AND INFLUENCES OF CLIMATE VARIATIONS ON DENGUE OUTBREAKS IN SOUTHERN TAIWAN THROUGHOUT 1998 TO 2012

Ting-Wu Chuang

Taipei Medical University, Taipei, Taiwan

Southern Taiwan has been a hotspot of Dengue Fever (DF) transmission since 1998. The incidence of dengue fever in Taiwan shows strong seasonality. Mosquito ecology and the transmission of dengue fever are influenced by multiple environmental factors, especially for climate variations. Thus, interannual variability in climatic conditions could be important drivers for annual outbreaks. This study explored the spatial patterns of dengue outbreaks in Tainan and Kaohsiung city in Southern Taiwan. Multiple climatic indices generated from weather stations at these two study regions were used to develop a model to evaluate the risk of dengue transmission. In view of disease early warning system, the climate variations in the early season is emphasized in the analysis rather than the conditions during the summer time. Non-linear generalized additive models (GAMs) were used to evaluate the influences of monthly weather

variables, including average temperature (TAVG), maximum temperature (TMAX), minimum temperature (TMIN), precipitation (PREC), and relative humidity (RH), on interannual variations of DF incidence from 1998 to 2012. The significant temporal and spatial heterogeneity of large scale DF outbreaks were shown in the two cities. Kaohsiung experienced significant outbeaks in 2002, 2010, and 2011; however, 2007 and 2012 are two outbreaks years in Tainan. The spatial patterns vaired in different year for both regions. The best-fitting model highlighted the importance of temperature, especially for TMIN, on the transmission of DF. Warmer TMIN during the preceding winter indicated elevated DF risk for the next summer and fall in both regions (p=0.03 for Kaohsiung, p=0.001 for Tainan). The outbreak of DF in Tainan is also associated with TMIN in February (p=0.007); however, DF outbreaks in Kaohsiung could be determined by the TMIN in April (p=0.009). The different responses to temperature variations in the two connected cities might be affected by other environmental factors, such as urban stuctures or land use types. Our modeling approach can provide useful information for establishing dengue early warning system in Taiwan.

810

CLIMATE VARIABILITY AND DENGUE EPIDEMICS IN PUERTO RICO

Roberto Barrera

Centers for Disease Control and Prevention, San Juan, PR, United States

Inter-annual climate variability is driven by climate systems such as El Niño-Southern Oscillation (ENSO) and the North Atlantic Oscillation (NAO). Warm ENSO events have been found to be associated with dengue epidemics in the Caribbean, whereas the impact of NAO variability on dengue dynamics has not been investigated. These climate systems affect the local climatology (air temperature, rainfall) of Puerto Rico, which in turn may affect dengue virus transmission. This study investigated the influence of inter-annual climate systems at various temporal scales (decadal, yearly, monthly) on local meteorological variables and dengue dynamics in Puerto Rico from 1987-2013. It was found that the impact of El Niño on dengue incidence may be modulated by the phase of the NAO, and that both climate systems can interact to exacerbate or reduce the magnitude of epidemics. For example, the largest dengue epidemic ever registered in Puerto Rico in 2010 occurred after a strong El Niño that peaked in December 2009 / January 2010, bringing anomalous warmer conditions and a strong negative NAO that brought exceptionally wetter conditions. Annual dengue incidence was significantly associated with temperature and dengue incidence during the winter. Warmer winters allow higher than normal dengue virus transmission that may cause epidemics during the rainy season when mosquito populations increase.

811

USE OF HOUSEHOLD CLUSTER SURVEYS TO QUANTIFY UNDER RECOGNITION OF DENGUE DURING THE 2013 EPIDEMIC IN LUANDA, ANGOLA

Tyler M. Sharp[1], Rosa Moreira[2], Maria J. Soares[3], Luis M. da Costa[3], Elizabeth Hunsperger[1], Jorge L. Munoz-Jordan[1], Ryan Hemme[1], Jennifer Mann[4], Victor Luteganya[5], Gilberto Santiago[1], Mark DeLorey[6], Harold S. Margolis[1], Adelaide de Caravalho[3], Kay M. Tomashek[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]Centers for Disease Control and Prevention, Luanda, Angola, [3]Angola Ministry of Health, Luanda, Angola, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]World Health Organization, Luanda, Angola, [6]Centers for Disease Control and Prevention, Fort Collins, CO, United States

Dengue is endemic throughout the tropics and is under recognized in Africa. In March 2013, a dengue epidemic was identified in Luanda, the capital of Angola. In total, 1,214 dengue cases were reported, of which 811 (67%), including 11 fatal cases, tested positive with a dengue rapid diagnostic test (RDT). Only dengue virus (DENV)-1 was identified. To estimate the percent of individuals infected during the epidemic and identify risk factors for infection, we conducted household cluster surveys within a 25-meter radius of RDT(+) dengue case-patients' and randomly selected households. All participants reported demographic data, medical history, and individual and household mosquito avoidance practices, and provided a serum specimen for dengue diagnostic testing by RT-PCR and anti-DENV IgM ELISA. Of 453 specimens collected, anti-DENV IgM was detected in 41 (9%); none were positive by RT-PCR. Of 173 individuals from 67 households in 21 RDT+ clusters, 16 (9%) were anti-DENV IgM(+). Of 247 individuals from 90 households in 26 random clusters, 25 (10%) were anti-DENV IgM(+). There were no statistically significant differences in frequency of detection of anti-DENV IgM among individuals, households, or clusters. Of the 41 anti-DENV IgM(+) individuals, 13 (32%) reported fever in the past 30 days, of which 5 (38%) and 1 (8%) reported symptoms consistent with dengue with warning signs and severe dengue, respectively. Seven (54%) of the recently infected febrile individuals sought medical care; 1 (14%) was hospitalized, and none were diagnosed with dengue. Factors associated with protection against recent DENV infection identified in this sample of individuals included bed net use in the past 30 days (p = 0.04) and delivery of household water by truck (p = 0.01). Identification of recent DENV infection among 10% of survey participants and lack of accurate diagnosis of participants that sought medical care suggest under recognition and underreporting of dengue. Clinical awareness of dengue should be strengthened to better define the epidemiology of dengue in Angola.

812

COMMUNITY-BASED PARTICIPATORY RESEARCH FOR PREVENTION OF DENGUE FEVER FROM THE APPROACH TO HEALTH COMMUNICATION: THE EXPERIENCE IN THE CARIBBEAN SLOPE OF COSTA RICA

Lissette Marroquin[1], **Adrian E. Avendaño-López**[2], Carlos Murcia[1], Yanet Martinez[1], Elsy Vargas[3], Gabriela Murillo[4], Viviana Ramirez[5], Mugur Geana[6]

[1]Centro de Investigación en Comunicación, Universidad de Costa Rica, San José, Costa Rica, [2]Centro de Investigación en Enfermedades Tropicales, Universidad de Costa Rica, San José, Costa Rica, [3]Escuela de Comunicación Colectiva, Universidad de Costa Rica, San José, Costa Rica, [4]Escuela de Salud Pública, Universidad de Costa Rica, San José, Costa Rica, [5]Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica, [6]William Allen White School of Journalism and Mass Communication, University of Kansas, Lawrence, KS, United States

Dengue fever is the most important tropical vector-borne disease in Costa Rica. In 2013, the country had the largest outbreak in its history when 49 993 new cases were reported. A Community-based participatory research (CBPR) was developed between years 2012-2014 at La Roxana of Pococí, an endemic locality at the caribbean slope. Social, cultural and health public components of two communities (Luis XV and San Antonio) were studied in 2012 with the purpose of promoting community organization for Dengue prevention during 2013. Focal groups and etnomethodology were used as qualitative tools meanwhile knowledge, attitudes and practices survey has been employed as quantitative approach. Qualitative data has suggested that Dengue Fever has not been an important health public trouble since community perception. Communities were more interested in problems as water disposal and availability, and waste management. This has caused difficulties for the implementation and acceptance of vector control and other community actions. Although, quantitative data has suggested that communities have a lot of knowledge about dengue, its vector and specific actions for the disease control. Knowledge has been obtained from mass media, national education system and the neighborhood. Data analyzed were used to promote women organization in Luis XV and young people organization in San Antonio for implementation of a public health strategy for control of dengue based in health promotion and waste management. Indicators have suggested success in implemented actions. CBPR has demonstrated

the importance of community awareness to increase the success of communication strategies for the prevention of dengue. Also, when CBPR is based on the health needs and interests, it can promote the community self-organization. In the case of La Roxana women's empowerment and leadership of community groups were considered essential for success.

813

A NOVEL ALLOSTERIC SMALL MOLECULE INHIBITOR OF INDUCIBLE HSP70 REDUCES DENGUE VIRUS INFECTION

Matthew Howe, Brandt Levitt, Brittany Speer, Khaldon Bodoor, Philip Hughes, Mariano Garcia-Blanco, Timothy Haystead

Duke University, Durham, NC, United States

The inducible protein chaperone Heat shock protein 70 (Hsp70i) is important in maintaining protein folding and cellular homeostasis. Hsp70i is utilized throughout the viral lifecycle for replication and propagation of the virus. Dengue virus, HIV, and rotavirus are a few of the viruses that exploit Hsp70i for infection and replication. However, the complete role of Hsp70i in dengue virus pathogenesis remains unclear. Previous studies have shown that Hsp70 may act as a receptor complex for virus internalization. Additionally, Hsp70 siRNA knockdown reduced dengue virus load, and Hsp70 aids in propagating the virus following internalization. Dengue virus is now endemic in over 100 countries and there are currently no approved vaccines or treatments for dengue virus infection. To date, few Hsp70 inhibitors have been identified and characterized, and their efficacy in clinical settings is unknown. We have identified a novel allosteric small molecule inhibitor of Hsp70i, HS-72, using FLECS (fluorescence linked enzyme chemoproteomic strategy). Inhibition of Hsp70i in a monocyte cell lines reduces dengue virus infection, while maintaining cell viability. Additionally, HS-72 leads to a reduction in the binding and entry of dengue virus in monocytes. Hsp70i is expressed at low levels preceding infection, but intracellular Hsp70i expression is rapidly induced upon dengue virus infection, while surface Hsp70i is unaffected. Furthermore, increasing intracellular Hsp70i expression prior to infection through Hsp90 inhibition, leads to an increase in dengue virus infection. This work highlights an essential role for Hsp70 in dengue virus pathogenesis and identifies a potential therapeutic anti-viral agent for dengue virus infection.

814

NEUTRALIZATION OF DENGUE VIRUSES IN VIREMIC HUMAN BLOOD; MAPPING THE MOST POTENT VIRUS NEUTRALIZING HUMAN MABS

Cameron Simmons[1], Tuan Trung Vu[2], Tran Bich Chau Nguyen[2]

[1]University of Melbourne, Melbourne, Australia, [2]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam

A better understanding of the characteristics of antibodies that neutralise dengue viruses *in vivo*, rather than *in vitro*, is a research priority. We established an experimental human-to-mosquito dengue virus (DENV) transmission system for measuring the potency of human mAbs to neutralise dengue virions in DENV viremic human blood collected from Vietnamese dengue cases. In this assay we have characterized the potency of a panel of 15 human mAbs that target a range of epitope regions on all four DENV serotypes. The results demonstrated that selected serotype-specific mAbs, recognizing a quaternary epitope, were the only mAbs able to neutralize DENV in the "*in vivo*" environment of viremic blood. These results identify a class of antibodies that dengue vaccines should aim to elicit.

815

ASSOCIATION OF NUTRITIONAL STATUS AND DENGUE COMPLICATIONS IN CHILDREN: RESULTS FROM A PROSPECTIVE COHORT STUDY CONDUCTED IN COLOMBIA

Idali Amado[1], Eduardo Villamor[2], Oscar Herran[1], Claudia Romero[3], Lyda Osorio[4], Beatriz Parra[4], Víctor Herrera[1], Luis Villar[1]

[1]Universidad Industrial de Santander, Bucaramanga, Colombia, [2]University of Michigan, Ann Arbor, MI, United States, [3]Universidad del Norte, Barranquilla, Colombia, [4]Universidad del Valle, Cali, Colombia

Undernutrition is an important predictor of complications in communicable diseases; however, the evidence of its role in pediatric patients with dengue remains controversial. We conducted a prospective cohort study (in three recruitment waves between 2003 and 2011) among out-patients aged 5-19 years old from Colombia, who had fever and serologic or virologic evidence of dengue infection. Socio-demographics, warning signs, and nutritional status were ascertained at baseline. Nutritional status was determined by calculating height for age (HFA) and body mass index for age (BFA) z-scores using the software WHO-ANTHRO. Patients were followed for complications defined as new-onset of shock (age-specific tachycardia and pulse pressure <20 mmHg or systolic or mean blood pressure below PAHO's age-specific cut-points for hypotension) or severe hemorrhage (hematemesis, melena, hematochezia or hematuria). We evaluated 330 children free of complications at baseline (mean age: 12.3 years; 57% male; mean disease's duration: 3.5 days). During a median follow-up of 3 days, 75 patients (22.7%) developed complications: 63 (19.1%) shock, 9 (2.7%) severe hemorrhage, and 3 (0.9%) shock and severe hemorrhage. Baseline HFA and BFA were lower among incident than non-incident cases: -0.1 vs. 0.3 (p=0.021) and -0.3 vs. 0.1 (p=0.007), respectively. After controlling for age, gender, and disease's duration, an increase in 1 z-score unit of HFA and BFA were independently associated with 23% (OR=0.77; 95%CI: 0.61, 0.98) and 25% (OR=0.75; 95%CI: 0.61, 0.92) lower probability of complications, respectively. There was no evidence of HFA-by-BFA interaction (p=0.377). Further adjustment for baseline warning signs did not attenuate associations. Our results suggest that, from a public health perspective, tackling undernutrition in dengue endemic countries might reduce the burden of the disease in the pediatric population.

816

ESTIMATING CROSS-ENHANCEMENT AND CROSS-PROTECTION OF DENGUE VIRUSES USING TIME SERIES DATA FROM THAILAND

Hannah E. Clapham[1], Nick G. Reich[2], In-Kyu Yoon[3], Richard G. Jarman[3], Krzysztof Sakrejda[2], Stefan Fernandez[3], Ananda Nisalak[3], Siripen Kalayanarooj[3], Derek A. Cummings[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]University of Massachusetts, Amherst, MA, United States, [3]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Using serotype specific case data from Queen Sirikit National Institute for Child Health in Bangkok, previous work estimated the length of cross protection to be 1-3 years. We have extended this work in two directions. First, we have developed models to consider whether there are serotype-specific differences in the cross-protection period and secondly to estimate the population-level impact of any enhancement of susceptibility or symptom severity of secondary infections, concurrently with cross-protection. We estimate whether the length of cross protection depends on the serotype of the primary or secondary infection, or the combination of both. Early results show that it may be difficult to detect any differences in the length of cross-protection by serotype. However our work continues to define what we can estimate, and what the impact of differential infection-to-case ratio or transmissibility by serotype will have on this estimate. We derive estimates of both cross protection and enhancement by combining data from multiple sources, including serotype specific and non-serotype specific case data from multiple locations in

Thailand. Early results show that the estimates of the duration of cross-protection from previous work are robust to the inclusion of susceptibility enhancement in the model. Further work is currently underway to further test these preliminary results. The model framework also provides estimates of the seasonality in transmission. We correlate these estimates of seasonality in multiple places with climatic variables throughout the year. From this we can determine drivers of seasonality in transmission, how this relationship varies depending on serotype, and how seasonality in transmission interacts with immunological processes. Methodologically, the extension of this framework to use multiple data sources will give us more power to estimate parameters that govern transmission, by making use of the similarities and differences observed in multiple locations. Further consideration of these processes of dengue transmission will enhance our understanding of what drives the observed dynamics of dengue cases. This, in turn, could lead to improvements in future dengue control efforts.

817

AN EARLY-LIFE DENGUE VACCINE CANDIDATE INDUCES EFFECTIVE IMMUNITY IN A MOUSE MODEL

Syed Muaz Khalil[1], Daniel R. Tonkin[2], Melissa D. Mattocks[2], Andrew T. Snead[2], Robert E. Johnston[2], Laura J. White[2]

[1]The University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]Global Vaccines Inc., Research Triangle Park, NC, United States

Dengue viruses (DENV1-4) cause 50-100 million clinical infections every year, several hundred thousand of which progress to severe hemorrhagic and shock syndromes. Preexisting immunity resulting from a previous DENV infection is the major risk factor for severe dengue during secondary heterologous infections. During primary infections in infants, maternal antibodies pose an analogous risk. At the same time, maternal antibodies are likely to prevent induction of endogenous anti-DENV antibodies in response to current live, attenuated vaccine (LAV) candidates. Any effective early life dengue vaccine has to overcome maternal antibody interference (leading to ineffective vaccination) and poor induction of antibody responses (increasing the risk of severe dengue disease upon primary infection). Here we demonstrate the feasibility of a non-propagating VEE virus replicon vector (VRP) expressing DENV E protein as an early life vaccine platform for dengue. We previously showed that a DENV-VRP vaccine is immunogenic even in the presence of maternal antibodies that otherwise interfere with a live virus vaccination in weanling BALB/c mice. In this report, we observed that a single immunization in 7-day-old neonatal BALB/c mice with a VRP vaccine expressing E ectodomain of DENV induced neutralizing antibody (NAb) titers by 6 weeks, which remained stable until at least 15 weeks post-immunization. DENV-specific cell-mediated immunity was also induced in these immunized mice. Furthermore, the NAb levels induced to each serotype by a tetravalent VRP formulation were equivalent to those of each monovalent vaccine components, suggesting that this vaccine modality can overcome serotype interference. VRP immunization in neonatal mice was durable and could be boosted later in life to further increase NAb and T-cell responses. Although the neonatal immune response was lower in magnitude than responses in adult BALB/c mice, we demonstrate that, both monovalent and tetravalent VRP vaccines generated protective immunity from a lethal intracranial challenge after a single neonatal immunization. In summary, VRP vaccines expressing DENV antigens were immunogenic and protective in neonates, and hence are promising candidates for safe and effective vaccination in early life.

818

DEFINING THE TARGETS OF DENGUE VIRUS INFECTION IN HUMAN SKIN

Parichat Duangkhae[1], Amanda P. Smith[1], Ernesto T.A. Marques, Jr.[1], Kate D. Ryman[2], Simon C. Watkins[3], Simon M. Barratt-Boyes[4]

[1]Department of Infectious Diseases and Microbiology; Center for Vaccine Research, University of Pittsburgh, Pittsburgh, PA, United States, [2]Department of Microbiology and Molecular Genetics, Center for Vaccine Research, University of Pittsburgh, Pittsburgh, PA, United States, [3]Center for Biologic Imaging, University of Pittsburgh, Pittsburgh, PA, United States, [4]Departments of Infectious Diseases and Microbiology; and Immunology, Center for Vaccine Research, University of Pittsburgh, Pittsburgh, PA, United States

Dengue is the most important arthropod-borne viral infection of humans causing an estimated 50-100 million cases worldwide every year. Dengue virus (DENV) is introduced into human skin, its replication site, by the bite of infected mosquitoes. While Langerhans cells have been implicated as the main targets of DENV infection the dynamics of infection of human skin remain ill-defined. Here, we exposed skin explants from healthy human donors to DENV to determine the targets of infection. Virus was inoculated into skin explants using a stabbing method with a bifurcated needle, and mock-infected and infected skin was analyzed by immunofluorescence after intervals of incubation using antibodies to cell-specific markers and viral protein NS3. Time course experiments showed that the first targets of DENV infection were basal keratinocytes, with infection first detected within 8 hours. From 12-48 h of infection abundant virus replication was detected in epidermal Langerhans cells and dermal macrophages as well as tubular structures consistent with lymphatic endothelium. Quantitative analysis indicated that exposure to DENV resulted in significant infiltration of macrophages into dermis. These preliminary data suggest that DENV initially infects basal keratinocytes which may release factors promoting the mobilization of infected Langerhans cells out of the epidermis, and the influx of macrophages into the dermis, which subsequently replicate DENV to high levels. Ongoing experiments are designed to determine the mechanism for macrophage recruitment. These studies are revealing that DENV infection of human skin is a dynamic process involving sequential infection and recruitment of distinct cellular targets.

819

USE OF INTEGRATED DISEASE SURVEILLANCE AND RESPONSE SYSTEM (IDSR) TO DETERMINE THE EXTENT OF DENGUE FEVER OUTBREAK ALONG THE COASTAL REGION OF KENYA

Daniel Ondari Mogeni[1], Melvin Ochieng[1], Lilian W. Waiboci[2], Leonard Cosmas[2], John Neatherlin[3], Rosemary C. Sang[4], Barry Fields[3], Joel M. Montgomery[3]

[1]Kenya Medical Research Institute-Centre for Global Health Research, Nairobi, Kenya, [2]Centers for Disease for Control and Prevention-Kenya, Nairobi, Kenya, [3]Global Disease Detection Branch, Division of Global Health Protection, Center for Global Health, Centers for Disease Control and Prevention, Nairobi, Kenya, [4]Kenya Medical Research Institute-Centre for Virus Research, Nairobi, Kenya

Integrated Disease Surveillance And Response System (IDSR) is a comprehensive strategy used for capturing information on communicable diseases by the Ministry of Health (MOH) in Kenya. From January to May 2013, MoH reported laboratory confirmed cases of dengue (DEN) along the Kenyan coast in Mombasa County. The Kenya Medical Research Institute (KEMRI) and the US Centers for Disease Control and Prevention (CDC) confirmed the presence and co-circulation of three DEN serotypes (DEN1, DEN2 and DEN3). On May 29th, 2013, KEMRI and CDC together with MoH initiated enhanced passive surveillance of DEN in six public sites in Coastal Kenya; Likoni, Port Reitz, Lamu, Kilifi, Msambweni and Taveta district hospitals and in 3 private hospitals; Bomu, Pandya and

Mombasa Hospital. The surveillance was from 21st June to 30th September, 2013. All persons presenting at the healthcare facilities with symptoms consistent with the case definition of suspect DEN; temperature ≥38.0°C for up to five days AND did not meet criteria for acute respiratory illness, was to be reported using IDSR outbreak report forms. A blood sample was taken where possible from two suspect DEN cases per day per site for five days a week in the public hospitals and in the private hospitals, as cases were identified. Real-time RT-PCR was performed at the KEMRI/CDC laboratories. Blood samples were collected from all 67 suspected cases identified. Two-thirds (44/67) were from the public hospitals. Of the 67 cases, 14 (21%) tested positive for DEN, 78.6% from private hospitals and 21.4% from public hospitals. Use of IDSR report forms varied across facilities and partially conformed to the MoH guidelines. We found varying rates of positivity and compliance between the public and private facilities. IDSR is the strategy adopted by WHO-AFRO for disease surveillance and outbreak response in the region; however it needs to be strengthened to ensure that the planned objectives are met while ensuring optimal resource use. In Kenya, IDSR implementation following devolvement of government may need to be decentralized to the county level.

820

SPATIAL ANALYSIS AND MODELING OF DENGUE SEROPREVALENCE IN VENEZUELA

Maria F. Vincenti[1], Zoraida I. Velasco-Salas[1], Erley Lizarazo[2], María E. Grillet[3], Manuel Amarista[4], Gloria M. Sierra[2], Guillermo Comach[2], Adriana Tami[1]

[1]Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands, [2]Instituto de Investigaciones Biomédicas de la Universidad de Carabobo (BIOMED-UC), Maracay, Bolivarian Republic of Venezuela, [3]Instituto de Zoología y Ecología Tropical, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Bolivarian Republic of Venezuela, [4]Centro de Estudios de Enfermedades Endémicas, Instituto de Altos Estudios "Dr. Arnoldo Gabaldón", Ministerio del Poder Popular para la Salud, Maracay, Bolivarian Republic of Venezuela

Dengue has become one of the most important public health problems of urban areas in Venezuela. Control of dengue and of its mosquito vector has proven challenging in settings of uncontrolled urban growth and unreliable water supply. The ability to identify high-risk areas of dengue transmission can be used to target surveillance and control measures to those locations in a cost-effective manner, particularly in countries where resources are scarce. We used mapping technology and spatial statistics to identify clusters ("hot spots") of transmission within a community-based cohort of 2012 individuals living in 840 households in the hyperendemic city of Maracay, Venezuela. Two spatial analyses of epidemiological and dengue seroprevalence data were conducted: 1) at house level, and 2) at block level. Risk-maps drawn at a fine scale determined that dengue seroprevalence is highly heterogeneous at the studied spatial scale. We detected significant hot spots (Gi*[d] >2.79, P≤0.05), at each spatial scale in all neighbourhoods. Our results also suggest that dengue transmission is very focal (20-80 meters). To better explain what determines dengue spatial clusters, we did comparative analyses of risk factors within and outside of hot spots areas using logistic regression modelling. Finally, we applied spatial regression modelling to identify which variables (demographic, socioeconomic and environmental) were more relevant to explain local dengue dynamics in Venezuela. Results will be presented.

821

LONG-TERM EPIDEMIOLOGICAL ANALYSIS OF PEDIATRIC DENGUE IN VENEZUELA (2000-2011)

Jaime R. Torres[1], Luis R. Echezuria[2], Mariano M. Fernandez[3], Alejandro Risquez[2]

[1]Tropical Medicine Institute, Caracas, Bolivarian Republic of Venezuela, [2]Luis Razetti School of Medicine, Caracas, Bolivarian Republic of Venezuela, [3]Public Health School, Caracas, Bolivarian Republic of Venezuela

Dengue is a major public health problem in Venezuela. It exhibits a variable epidemiological pattern in time, and associates with a significant annual number of cases, ±50% of which, are seen in children. A retrospective morbidity-mortality and disease burden study of dengue in Venezuelan pediatric population, based on official data from the Ministry of Health and National Institute of Statistics from 2000 to 2011, was carried out. Statistical analysis was performed by a SPSS20 program. Significance tests were performed as required. Burden of disease was estimated as DALYs associated with cases and deaths. Of the 632,066 dengue cases registered in the general population, 316,404 (50.05 %) were seen in children <15 Y/O. Sixty nine per cent of the cases occurred in six epidemic years (2001/2005-2007/2009 and 2010), the latter with a historical record of over 110,000 cases. Whereas the average General Morbidity Rate for the period was 194.87, it was 1.7 higher in children (332.19). The most affected age group was that of 5-9 Y/O (364.98). The <15 Y/O experienced 28,065 cases of severe dengue (56.60% of all severe dengue). Mortality Rate in children was 1.42 times higher than in adults (0.27 vs. 0.19, respectively) ($p < 0.05$), and in the group under 1 year it was 4.10 times as much (0.78) ($p <0.01$). Lethality rate was 20% lower in children than in adults (0.08 vs. 0.10, respectively) ($p <0.01$). However, in <1 Y/O it was 2,4 times higher than in adults and 3,4 times more than in all other pediatric age groups ($p <0.01$). Of note, during the last half of the analyzed period both mortality and lethality rates significantly increased for all pediatric age groups, especially in 5-9 Y/O. Disease burden was estimated in 3,793.65 DALYs per clinical cases and 9,796.78 per deaths. In Venezuela, dengue exhibits both endemic and epidemic cycles. Epidemics were frequent in the studied period and associated with majority (±70%) of reported cases, reflecting failures in the control and prevention programs. Lethality in children was lower than in adults and that reported internationally. The marked increase in the mortality and lethality during the last lustrum is unexpected, and requires to be explained. Although the most affected groups were the 5-14 Y/O, mortality and lethality rates were much higher in those less than 1 year. These results may be useful to understand better the epidemiology of the disease in the country and improve the effectiveness of disease control.

822

THE HUMAN CD8+ T CELL RESPONSES INDUCED BY A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE ARE DIRECTED AGAINST HIGHLY CONSERVED EPITOPES AND ARE SIMILAR IN MAGNITUDE AND BREADTH TO THOSE FOLLOWING NATURAL INFECTION

Daniela Weiskopf[1], Michael A. Angelo[1], Derek J. Bangs[1], John Sidney[1], Sinu Paul[1], Bjoern Peters[1], Aruna D. de Silva[1], Janet C. Lindow[2], Stephen Whitehead[3], Anna Durbin[4], Beth Kirkpatrick[2], Alessandro Sette[1]

[1]La Jolla Institute for Allergy and Immunology, La Jolla, CA, United States, [2]University of Vermont College of Medicine and Vaccine Testing Center, Burlington, VT, United States, [3]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [4]Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States

The incidence of infection with any of the four dengue virus serotypes (DENV 1-4) has increased dramatically in the last few decades, and the lack of a treatment or vaccine has contributed to significant morbidity and mortality worldwide. The recent failure of a candidate vaccine to protect

against disease despite induction of antibody responses against all DENV serotypes in most subjects suggests that better correlates of protection are needed. A recent comprehensive analysis of the human T cell response against wild-type DENV suggested an HLA-linked protective role for CD8+ T cells. Here, we characterize for the first time CD8+ T cell responses after live attenuated dengue vaccination and compare them to responses observed in natural infection with dengue virus. We have collected one-unit blood donations from study participants receiving the monovalent or tetravalent live attenuated DENV vaccine (DLAV), developed by the U.S. National Institutes of Health. PBMCs from these donors were screened in IFNγ ELISPOT assays with pools of predicted, HLA matched, class I binding peptides covering the entire DENV proteome. CD8+ T cell responses in vaccinees were readily detectable with a magnitude and breadth similar to natural dengue infection. Interestingly, while broad responses to structural and non-structural (NS) proteins were observed after monovalent vaccination, T cell responses following tetravalent vaccination were, dramatically, focused towards the highly conserved non-structural proteins NS3 and NS5. Epitopes from these proteins are highly conserved in a vast variety of field isolates and are able to elicit multifunctional T cell responses. Live attenuated vaccines against dengue virus are able to induce a CD8+ T cell response comparable to responses seen in natural infection. Detailed knowledge of the T cell response will contribute to the identification of robust correlates of protection in natural immunity and following vaccination against DENV.

823

HISTOLOGIC CHARACTERIZATION OF THE LOCAL IMMUNE RESPONSE TO DENGUE VIRUS INFECTION IN INTRADERMALLY INOCULATED MICE

Brad A. Goupil, Michael K. McCracken, Daniel M. Chisenhall, Christopher N. Mores

Louisiana State University, Baton Rouge, LA, United States

Dengue virus (DENV) is an emerging arboviral pathogen transmitted primarily by the mosquito *Aedes aegypti*. Approximately 40% of the world's population lives in regions that are at risk for DENV transmission. This virus is associated with a high morbidity of febrile disease and in a small percentage of cases, disease can progress to more severe manifestations. Several models of DENV infection have been developed to study the pathogenesis of this disease in various organ systems. While some experiments have examined the role of dendritic cells following intradermal inoculation of DENV, none have attempted to qualify the local inflammatory response and potential differences between viral serotypes *in vivo* to elucidate the pathogenesis that leads to disease-causing viremia. In this study, mice were intradermally inoculated with either DENV 2 [strain 1232] or DENV 4 [strain 1228] in the hindlimb footpad after allowing mosquitoes to feed at this site. For negative control mice, mosquitoes were allowed to feed, but no viral inoculum was injected. Mice were sacrificed at 3 hours and 18 hours post-inoculation (hpi). The distal hindlimbs, as well as the associated popliteal lymph nodes, were harvested, formalin-fixed and processed for histologic evaluation. At 3 hpi, the dermis was mildly expanded and the inflammatory response consisted predominantly of small numbers neutrophils, fewer mast cells and occasional macrophages. The popliteal lymph nodes contained small numbers of mast cells and fewer neutrophils. There were no observable differences associated with serotype and the inflammatory infiltrates in virus inoculated mice were similar to negative control mice. Histologic evaluations of the 18 hpi mice are pending, as are immunofluorescence assays to correlate location of viral antigen with the inflammatory responses. Characterization of the local immune response following intradermal DENV infection will help elucidate the early pathogenesis of disease and determine any potential differences associated with serotype.

824

ASSESSMENT OF INCIDENT DENGUE VIRUS INFECTIONS AMONG FEBRILE PATIENTS IN WESTERN KENYA

Alastair I. Matheson[1], Joshua R. Lacsina[1], Melvin Ochieng[2], Allan Audi[3], Godfrey Bigogo[3], John Neatherlin[2], Harold S. Margolis[4], Barry Fields[2], Judd L. Walson[1], Joel M. Montgomery[2]

[1]University of Washington, Seattle, WA, United States, [2]Centers for Disease Control and Prevention, Nairobi, Kenya, [3]Centers for Disease Control and Prevention, and the Kenya Medical Research Institute/Centers for Disease Control and Prevention Public Health and Research Collaboration, Kisumu, Kenya, [4]Centers for Disease Control and Prevention, San Juan, PR, United States

Although dengue occurs in Africa, it is poorly identified and there is uncertainty about its geographic distribution. In many settings, a lack of diagnostic capacity has resulted in use of syndromic guidelines for the management of febrile illnesses. In East Africa, dengue outbreaks have been reported recently and seroprevalence studies suggest dengue virus (DENV) transmission. A retrospective study was conducted to estimate the incidence of DENV infection among febrile patients in western Kenya. Serum was collected from febrile (≥38°C) patients in an ongoing population-based disease surveillance study in Asembo District, Kenya, from Sept.-Nov. 2011 and Mar.-July 2013; rainy seasons when DENV transmission was expected to be high. We excluded patients with alternative clinical or lab diagnoses (e.g., bacteremia, positive urine culture, virus-positive nasal swab), or >5 days of fever. Testing for DENV RNA was performed by real-time RT-PCR and RNA integrity was verified by a human RNAse P control. DENV positive and negative controls performed as predicted. A total of 688 febrile patients met the inclusion criteria and 615 (89.4%) of them had samples available for testing. The median age was 4.5 years and 53% were female. No samples were DENV RNA positive. In this study, no DENV infections were detected using molecular diagnostic testing, despite samples being collected in what would be the viremic period of dengue. Possible reasons include inaccurate reporting of fever onset, a predominantly rural study site resulting in lower vector density than in coastal Kenya where incident dengue has been detected, or a genuine absence of DENV. If subsequent IgM analyses are also negative, the combined results will support the idea of uneven distribution of dengue in East Africa. Such variation, combined with the possible spread of disease in the area, reinforces the need for including dengue diagnostics in febrile illness surveillance to better inform management of fever.

825

RISK FACTORS OF BLEEDING IN HOSPITALIZED ADULT DENGUE PATIENTS

Joshua G. Wong, Tun-Linn Thein, Linda K. Lee, Yee-Sin Leo, David C. Lye

Tan Tock Seng Hospital, Singapore, Singapore

Hemorrhagic manifestations are undesirable clinical outcomes and important criteria of dengue prognosis. Clinical bleeding is required for the classification of dengue hemorrhagic fever. Severe bleeding is a form of severe dengue. However, risk factors for bleeding in dengue patients are not well studied. We conducted a retrospective study of adult dengue patients hospitalized at Communicable Disease Center, Singapore from 2005 to 2008, confirmed by positive PCR or dengue serology with World Health Organization 1997/2009 probable dengue criteria (n=6070). Clinical bleeding was defined as bleeding excluding petechiae and severe bleeding include gastrointestinal bleeding or menorrhagia or requiring blood transfusion. Those with no prior bleeding were included into the analysis (n=4383).Through statistical modelling, we aim to identify factors associated with clinical and severe bleeding. There were 869 (19.8%) and 148 (3.4%) patients who developed clinical and severe bleeding respectively. Variables with a p-value of <0.2 in the univariate analysis were entered into the multiple logistic regression model. The final model was derived using manual backward elimination and adjusted for age, gender,

disease severity, Charlson's score, hematocrit and aspartate transaminase. Fever on admission (aOR:1.4 [1.2-1.7]), absence of rash (aOR:0.8 [0.7-0.99]), anorexia (aOR:1.2 [1.01-1.4]), neutrophilia (aOR:1.01 [1.008-1.02]), leukopenia (aOR:0.86 [0.82-0.9]) and thrombocytopenia (OR:0.996 [0.993-0.998]) were significantly associated with clinical bleeding. Fever on admission (aOR:2 [1.3-2.9]), neutrophilia (aOR:1.02 [1.005-1.03]) and abdominal pain (aOR:1.44 [1.003-2.05]) were significantly associated with severe bleeding. Our findings warrant further validation in different cohorts, including other countries, children and different serotype outbreaks.

826

STUDY OF CROSS-REACTIVE ANTIBODIES AGAINST DENGUE VIRUS ENVELOPE PROTEIN FOLLOWING HETEROTYPIC IMMUNIZATION AND SECONDARY INFECTION

Wen-Yang Tsai[1], Jih-Jin Tsai[2], Anna Durbin[3], Stephen Whitehead[4], **Wei-Kung Wang**[1]

[1]JABSOM, University of Hawaii at Manoa, Honolulu, HI, United States, [2]Tropical Medicine Center, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, [3]Center for Immunization Research, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [4]Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

The four serotypes of dengue virus (DENV) are the leading cause of arboviral diseases in humans in tropical and subtropical regions. After secondary DENV infection, multitypic neutralizing (NT) antibodies (Abs) were developed not only against the previously exposed serotypes but also against the serotypes to which they have never been exposed ("non-exposed serotypes". These heterotypic NT Abs are believed to contribute to protection against subsequent infection by the non-exposed serotypes. The nature of these NT Abs remains largely unknown. To study cases of well-documented primary and secondary DENV infections, we examined sera from 10 vaccinees, who received two doses of live-attenuated vaccine in a heterotypic immunization study (Durbin et al. J Infect Dis 2011;203:327-334). Serum samples prior to and at 42 days after primary and secondary DENV immunizations were examined by Western blot analysis, virion-ELISA, modified 8M urea-ELISA, and focus reduction neutralization test (FRNT). Binding studies with virion-ELISA and IgG avidity showed stronger recognition of the primary infection serotype compared to other serotypes after secondary DENV infection, which is consistent with the "original antigenic sin". FRNT revealed multitypic NT Abs to both exposed and non-exposed serotypes. Depletion with West Nile virus antigens resulted in reduction of NT activities, suggesting that group-reactive Abs contributing to NT activities after secondary infection. Similar trend was also observed in sera from patients with secondary DENV infection. Together, these findings resonate with our recently published report of high-avidity and potently NT cross-reactive human mAbs derived from patients after secondary infection.

827

DENGUE TRANSMISSION IN A MEDIUM-SIZED CITY FROM BRAZIL AND THE LESSONS WE CAN LEARN TO AVOID IDENTICAL SCENARIOS ELSEWHERE

Adriano Mondini[1], **Aline Chimello Ferreira**[1], Francisco Chiaravalloti Neto[2]

[1]UNESP - São Paulo State University, Araraquara - São Paulo, Brazil, [2]USP - University of São Paulo, Sao Paulo - Sao Paulo, Brazil

Dengue viruses (DENV) have been a public health problem in tropical regions for decades but autochthonous transmission has now been reported in countries like USA, France and Croatia. In 2013, 2.35 million dengue cases were reported in the Americas and Brazil alone was responsible for more than 50%. The country has been presenting outbreaks for more than three decades and important lessons can be learned. Thus, in depth analysis of dengue transmission in Araraquara, a medium-sized city at the central portion of São Paulo, which is the richest state of Brazil and a critical area for DENV transmission, may clarify how random circulation of the virus may evolve to massive outbreaks. DENV has been circulating in the city since 1990s at low incidences. However, the number of cases has increased in recent years and we will be describing the scenario of five years of transmission herein. Official data on dengue reports from 2008 to 2012 were recovered from the Information System on Diseases of Compulsory Declaration. Data from 5,282 reported cases were analyzed. The majority - approximately 60% for the five-year period - was reported in people with up to six years of formal education; this trend is an indication that areas with low living standards may play an important role in DENV dispersion. Another important observation is that dengue transmission has become endemic in the city, with cases being officially reported in all months of the year, with the exception of 2009, which was atypical in the whole state of São Paulo. Severe dengue or dengue with complications was a rare event in the city. The incidence in Caucasians (78,4%) was higher than in other ethnic groups, a pattern that has been described worldwide. Females were more affected (54,5%) than males and further analysis is required to assess this figure. This is part of an ongoing project that is also focused on classical and molecular epidemiology involving the implementation of regular molecular diagnosis in the city, phylogenetic analysis of serotypes in humans and mosquitoes and spatial statistics.

828

USE OF ANTIGENIC CARTOGRAPHY TO CHARACTERIZE NEUTRALIZATION OF DIVERSE DENGUE VIRUSES IN THE MONTHS FOLLOWING PRIMARY DENGUE VIRUS INFECTION

Leah C. Katzelnick[1], Judith M. Fonville[1], Gregory D. Gromowski[2], Jose Bustos Arriaga[2], Stephen S. Whitehead[2], Derek J. Smith[1]

[1]University of Cambridge, Cambridge, United Kingdom, [2]National Institutes of Health, Bethesda, MD, United States

The four dengue virus types (DENV1-4) elicit early neutralizing antibody responses with high titers to homologous as well as heterologous DENV types, which have been observed to narrow to the homologous type in the ensuing months. However, research on changes in the neutralizing response over time often use single representatives of each DENV type, and thus the degree to which changes are due to the quantity of antibodies or the pattern of neutralization is not well understood. Here, we use antigenic cartography to describe how 17 primary-infection African green monkeys (AGM) with antisera drawn one, three and five months after inoculation neutralize a panel of 40 diverse DENV isolates assembled by the Dengue Antigenic Cartography Consortium. The neutralizing antibody response to the DENV panel over the four-month period significantly declined for eight AGMs, was stable for eight AGMs, and dramatically increased for one AGM. We made an antigenic map of the DENV panel titrated against the AGM antisera drawn at one, three and five months, as the fit of the data was comparable to maps made of each time point separately. Like antigenic maps made of each time point, the distance within and between DENV types was comparable: DENV2, DENV3, and DENV4 had similar variation within and between DENV types, while DENV1 isolates were slightly more varied between than within type. Antisera drawn one-month post-infection differed in position on the map by an average of 3.2-fold dilutions from three-month antisera (SD: 2.4-fold), while three-month antisera were only 1.6-fold dilutions from five-month antigenic positions (SD: 1.4-fold). This difference was significant, and suggested moderate changes in reactivity between one and three months, but minimal changes between three and five months. Only four AGMs shifted to the periphery of homologous antigenic cluster, suggesting increasing type-specificity. The remaining antisera moved toward the center of the antigenic map over time, thus transitioning to more cross-reactive responses. We find that only a subset of primary infection antisera become more type-specific over time, and that minimal changes in reactivity are observed between three and five

months after infection. Further, the use of all three time points enables better coordination of the DENV panel, making possible comparison of the relationships between viral genetic and antigenic differences.

829

EVIDENCE FOR THE RECENT EMERGENCE OF DENGUE IN BANGLADESH: RESULTS FROM A SEROPREVALENCE STUDY

Henrik Salje[1], Abu M. Naser[2], Mahmudur Rahman[3], M. Ziaur Rahman[2], Justin Lessler[1], Derek A. Cummings[1], Stephen P. Luby[4], Emily Gurley[2]

[1]Johns Hopkins School of Public Health, Baltimore, MD, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]IEDCR, Dhaka, Bangladesh, [4]Stanford University, Stanford, CA, United States

Dengue disease is endemic throughout Southeast Asia and has been reported throughout India. Global models of dengue incidence suggest it is widespread across Bangladesh; however, while dengue infections have been reported in Dhaka and other cities, it is unknown if the pathogen has spread to rural communities that make up the majority of the country. To address this gap we conducted a seroprevalence study of dengue in a rural district that borders India in the northwest of the country. We randomly selected 40 villages and visited randomly chosen households within each community. All household residents were asked to provide a blood sample and information about socio-demographics. Indirect PanBio IgG ELISAs were used to identify dengue-specific antibodies as a marker of past dengue infection. In total, 1497 individuals participated in the study with a median age of 26 (range 0 - 90) years. 18% of the study population had serological evidence of past infection. There was significant spatial heterogeneity with virtually no past exposure detected in the north of the district whereas communities in the south near the district capital had over 60% seropositivity, although we found no differences in seropositivity by age (p-value: 0.96). In addition, we found no difference in seropositivity by age (p-value 0.17), suggesting that all individuals had experienced a similar cumulative risk of infection characteristic of recently emergent pathogens. We used a multilevel model to identify risk factors associated with historic dengue infection. Males were 1.4 (95% confidence interval [CI] 1.0-1.9) times more likely to have been infected than females. Having other infected individuals in the household increased the probability of being seropositive by 1.3 (95% CI 1.1-1.5) times. The presence of seropositive individuals in the community (but outside the household) further increased the risk of having been infected by 1.2 (95% CI 1.1-1.3) times. These findings suggest that dengue has only recently emerged in parts of this rural area and underscore the importance of considering rural communities when assessing the burden of dengue. Household and community-specific factors appear key to determining individual risk. Further work exploring differences in the ecological suitability for the vector in this region and the flow of people from dengue endemic communities will help us further understand the observed patterns of exposure.

830

ATTITUDES TOWARD HEALTH, VECTOR CONTROL AND DENGUE FEVER IN SEVEN ENDEMIC COUNTRIES: INSIGHTS FROM ETHNOGRAPHIC RESEARCH IN BRAZIL, COLUMBIA, INDONESIA, MALAYSIA, MEXICO, PHILIPPINES AND SINGAPORE

Patricia R. Blanc[1], Katarzyna Hein-Peters[2], Stephane Auvergne[2], Thomas D. Szucs[1]

[1]ECPM Institute of Pharmaceutical Medicine, University of Basel, Basel, Switzerland, [2]Sanofi Pasteur, Lyon, France

Perception of dengue and its prevention was assessed in 84 adults from various socio-economic groups and both urban and suburban dengue-endemic areas in 7 countries. Participants aged 18-55y (76 were women), 79 of whom had children <16y, were interviewed at home in 1.5-2h, semi-structured interviews. Prior to interviews, participants prepared scrapbooks to gauge emotional aspects. Interviews were filmed and vector control measures observed. Health was a top priority for all, contributing to family wellbeing and happiness. Work, financial stability and religion contributed to family wellbeing in Asia, and education in both regions. Health prevention was associated with nutrition, hygiene and exercise, and with childhood vaccination in Latin America. Prevention was considered part of hygiene efforts. The top health concerns included: 1/ cancer, heart attacks, and meningitis, 2/ diabetes and obesity, 3/ childhood diseases, 4/ dengue, and 5/ other infectious diseases (IDs). Dengue was spontaneously mentioned in Brazil, Indonesia and Philippines only, and was perceived as a bigger threat than other IDs in all countries. Latin Americans perceived vaccination positively and favored public vaccination, while Asians expressed concerns about vaccines and favored vaccination in private clinics. Knowledge of dengue was high, with gaps around the severity of secondary infection, existence of different serotypes, differences between DF and DHF, and the dangers of self-medication with some classes of NSAIDs. Dengue was associated with negative images, e.g. blood, humidity, pain, disability, dirt, filth, death. Level of concern and intensity of preventive measures were influenced by: personal experience of dengue, time of day, rainy season, national dengue incidence, public awareness campaigns, and concerns about children. Respondents felt safe at home and more exposed outside. They were committed to dengue prevention, yet preventive measures were inadequate and mosquito repellents were often absent. These results shed light on attitudes to dengue and prevention, and may help inform public communication campaigns.

831

VIROLOGICAL TEST ALGORITHM FOR DENGUE CASES - OBSERVATIONS FROM A PHASE 2 LATIN AMERICAN DENGUE VACCINE TRIAL

Mark Boaz, Linda Starr-Spires, Seema Garg, Helene Janosczyk, Stacy Yost, Stephen W. Hildreth, Gustavo Dayan

Sanofi Pasteur, Swiftwater, PA, United States

The CYD tetravalent dengue vaccine candidate is being evaluated for protective efficacy against symptomatic dengue in phase III efficacy trials. The laboratory test algorithm to confirm dengue cases was evaluated prior to Phase III trials. During a Phase II safety and immunogenicity trial in Latin America (Clinicaltrials.gov NCT00993447) a dengue epidemic occurred in the study countries. A total of 72 suspected dengue cases were reported and assessed: virological confirmation comprised qRT-PCR methods and a commercial ELISA kit for NS1 protein (Bio-Rad). The qRT-PCR included a screening assay targeting a dengue-conserved region of the 3'-UTR (Dengue screen assay) followed by 4 individual serotype assays targeting the conserved dengue NS5 genomic region (WT dengue qRT-PCR assays). The NS1 and WT dengue qRT-PCR were the protocol endpoint assays for virological confirmation (PVC). Of the 72 suspected cases, 14 were PVC: 9 by WT dengue qRT-PCR (5 Den-1, 4 Den-3) and 5 positive by NS1 Ag ELISA only. For the 9 PCR positive cases, 8 were also positive by NS1. However, a unique pattern of dengue qRT-PCR results were observed in 5 suspected cases. In these 5 cases, all from Honduras, the dengue screen qRT-PCR assay was positive but both the WT dengue qRT-PCR and NS1 Ag ELISA were negative. To investigate, exploratory data were generated using additional molecular methods: a SYBR Green-based RT-PCR assay, sequencing assays directed at the dengue genomic regions covered by the WT dengue qRT-PCR, and a commercial dengue RT-PCR test (Simplexa™ Dengue, Focus Diagnostics). The exploratory data confirmed these additional cases as dengue. Results indicated the serotype 2 WT dengue qRT-PCR assay was unable to detect a circulating Latin American strain (DENV-2/NI/BID-V608/2006) due to a mutation in the probe-binding region of the isolate. The Simplexa™ Dengue RT-PCR test was able to detect dengue in all samples tested except one. On the basis of these results and additional evaluations, the PVC algorithm was modified for the Phase III efficacy trials and future studies.

832

EVALUATING THE UTILITY OF REACTIVE VECTOR CONTROL FOR DENGUE OUTBREAKS

Oliver J. Brady, Simon I. Hay

University of Oxford, Oxford, United Kingdom

Dengue has the highest burden of any viral vector borne disease. A major contributor to this high burden is the unpredictable timing and magnitude of dengue outbreaks that often overwhelm healthcare facilities. While some early warning systems have shown local successes, the standard response to such outbreaks remains reactive. With no effective human prevention or cure, control efforts have to focus on the mosquito population with activities such as fogging of adults or larviciding of juveniles. While reactive measures are important, it has not yet been tested whether the timeliness and effectiveness of currently available vector control interventions are appropriate for controlling dengue outbreaks. Here we use a compartmental SIR model of mosquito and human population dynamics to estimate the effects of interventions on eventual outbreak size given the time delays between dengue virus transmission and intervention implementation. In this analysis we evaluate the effects of several commonly used reactive interventions and calculate the threshold timeliness and effectiveness that would be required for vector control interventions to be more appropriately used as response measures than simply applied at random. This is the first time the time delays at various stages of transmission and subsequent intervention have been incorporated into a dengue transmission model and the resultant outcomes will be important for evaluating and supporting effective public health policies regarding dengue outbreaks.

833

SEROTYPE-SPECIFIC DENGUE NEUTRALIZING ANTIBODY RESPONSES IN FCΓR-EXPRESSING CV1 CELLS

Anthony M. Byers[1], Ryan Broder[1], Janice M. Moser[1], Mark Boaz[2], William Warren[1], Nicholas Jackson[3], Bruno Guy[3]

[1]Sanofi Pasteur, Orlando, FL, United States, [2]Sanofi Pasteur, Swiftwater, PA, United States, [3]Sanofi Pasteur, Marcy l'Etoile, France

A Phase 2b efficacy trial (CYD23; Clinicaltrials.gov NCT00842530) of the live attenuated CYD tetravalent dengue vaccine (TDV) showed clinical protection against dengue. Point estimates of vaccine efficacy differed between dengue virus (DENV) serotype and no measurable efficacy was observed against DENV2. This observation contrasted with results of a Vero cell based PRNT50 assay that showed similar levels of vaccine-induced neutralization antibodies for all four serotypes. We investigated the potential of a seroneutralization assay in FcγRIIa-expressing CV1 cells to assess both neutralization and potential enhancement of dengue infection. Parallel assays were performed in Vero and CV1-FcγRIIa cells to evaluate the neutralizing antibody response against all 4 dengue serotypes on serum samples from a naturally dengue-infected cohort and serum samples collected after three injections of CYD-TDV in a clinical trial (NCT01134263) of "dengue-naïve" subjects. Neutralizing antibody titer GMT values were 2- to 9-fold lower in CV1-FcγRIIa cells than in Vero cells with lowest relative (Vero titer/CV1 titer) decrease amongst the 4 serotypes seen for the anti-DENV2 response. While the presence of an activating FcγR led to a global decrease in neutralizing capacity, the fact that the lowest relative decrease was against DENV2 suggests that anti-CYD2 responses lead to lesser enhancement of infection relative to the other 3 serotypes. Additional CV1 studies are underway with sera from CYD-TDV vaccinees in dengue-endemic areas (NCT01187433) to determine what role, if any, differential levels of dengue pre-immunity have on the generation of neutralizing antibodies against each serotype. Results of these investigations suggest that despite the absence of clinical efficacy observed against DENV2 in the CYD23 trial, *in vitro* neutralizing antibody responses against DENV2 elicited by CYD2 in TDV vaccination were not more enhancing than responses against other serotypes.

834

MULTICENTER CLINICAL EVALUATION OF TWO ELISA AND TWO RAPID FORMAT ASSAYS FOR DIAGNOSING DENGUE

Subhamoy Pal

Henry M. Jackson Foundation, Silver Spring, MD, United States

Extensive prospective evaluations using multiple trial sites, a defensible gold-standard reference testing methodology, and quality systems that provide confidence in the study results, are required for reliable performance assessment of diagnostic tests. In this study, we evaluated the SD Bioline Dengue Duo (NS1/IgM/IgG) and the Panbio Dengue Duo (IgM/IgG) rapid diagnostic tests as well as the Panbio IgM and IgG Dengue ELISAs in a prospective, controlled, multicenter study. Paired samples were prospectively collected from 1021 individuals initially presenting on Days 0-7 at study sites in Peru, Venezuela, Cambodia, and the United States. An additional panel of 135 paired retrospective samples from Thailand was also used. A mix of primary and secondary infections and all four dengue serotypes were captured. Reference testing was performed using an algorithm involving virus isolation, IgM capture ELISA, and plaque reduction neutralization tests, in order to fully characterize the dengue status of each subject. Our primary end-points was positive and negative percent agreements of these devices against the reference methodology, but these numbers were also stratified using several factors known to influence overall accuracy including geography, days post onset of symptoms, infecting serotype, primary or secondary infections, and other demographic features. We determined that the SD Bioline Duo Cassette (NS1/IgM/IgG) had an overall sensitivity of 87% and specificity of 87% over the first 14 days post onset of symptoms. The Panbio Duo Cassette had a sensitivity of 92% and specificity of 59% during days 4-14 post onset of symptoms. This study generated reliable performance characteristics for several dengue diagnostic assays using prospectively collected specimens from both Asia and the Americas. Such results facilitate data-driven healthcare product choices for managing patient care during dengue

835

HOST RESPONSES AFTER PRIMARY DENV-2 CHALLENGE IN CYNOMOLGUS MACAQUES: IMPACT OF STRAIN, DOSE AND ROUTE OF ADMINISTRATION

Veronique Barban[1], Leslie Gosse[2], Nathalie Mantel[1], Nathalie Bosquet[2], Aymeric De Montfort[1], Christophe Charnay[1], Anke Harenberg[1], Roger Legrand[2], Florence Boudet[1]

[1]Sanofi Pasteur, Marcy-L'Etoile, France, [2]CEA, Division of Immuno-Virology, Fontenay-aux-Roses, France

Some non-human primates including Rhesus (*Macaca mulatta*) and Cynomolgus monkeys (*M. fascicularis*) are sensitive to infection by Dengue viruses and reproducively develop viremia when inoculated by SC route with 4-5 $\log_{10}$ PFU. Viremia is modest, usually several orders of magnitude below human viral load in dengue patient, and animals do not develop dengue clinical signs in this model. However classical dengue hemorrage was observed in rhesus monkeys showing high viremia after intravenous challenge with 7 $\log_{10}$ PFU (Onlamoon 2010). We report here the results of 2 challenge studies carried out in Cynomolgus macaques with aim to explore parameters associated to high viral loads. In the 1st study, 4 groups of 5 monkeys were challenged by SC route with 5.0 log10 CCID50 of 4 DENV-2 strains presenting different passages history: 2 laboratory strains (DENV-2 16681 and DENV-2 16803) and 2 low-passage isolates. Serum viremia was followed by qRT-PCR, daily from D1 to day 14, then at D28. Viral RNA was detected in all animals (except 1 in the DENV-2 16803 group) on D2 after injection for laboratory strains, and at various time points between D1 and D6 for viral isolates. Peak titers, viremia duration, and AUC were significantly lower in the DENV-2 16803 group than in other groups. The more homogeneous curves were observed in animals challenged with DENV-2 16681. In the second study, 6 groups of 5 monkeys received 5.0 or 7.0 log10 CCID50 of this virus administrated

by SC, ID or IV route. A clear dose effect was observed whichever the route of administration, with a peak viremia increase of about 1.0 log10. Reduced viremia duration and shorter time to viremia were also associated to dose increase. The IV challenge generated the highest peak titers and the most homogenous viremia curves: peak titer 7.0 ±0.2 on day 2, duration 5.0 ±0.0 days. DENV-2 neutralizing antibody titers and some blood biochemical parameters were also analyzed and will be presented. The implications of these data on the development of new DENV challenge model to measure protective efficacy of vaccine candidates will be discussed

836

COST OF DENGUE VECTOR CONTROL: SYSTEMATIC LITERATURE REVIEW

Donald S. Shepard, Yara A. Halasa, Eduardo A. Undurraga

Brandeis University, Waltham, MA, United States

As the most important vector-borne viral disease, dengue is a serious and growing global public health problem, with 3.6 billion people at risk of infection. Currently, vector control is the only prevention tool to control dengue transmission. Little is known about the cost and the effectiveness of current dengue vector control measures, which rely heavily on outdoor fumigation and use of larvacides. We performed a systematic literature review, searching for studies on the cost of dengue vector control and found 14 articles and reports. Of these, 4 examined only one specific vector control intervention, such as community mobilization or source reduction, while 10 analyzed comprehensive vector control activities. We then separated these 10 studies according to dengue severity in the study year. Of these, 3 analyzed an epidemic year and 7 a non-epidemic year, consistent with dengue epidemiology. As 2 sites were duplicated, the studies cover 8 locations: Brazil, Cuba Malaysia, Mexico, Panama, Puerto Rico, Thailand and Venezuela, representing the regions in which dengue is most heavily endemic: Latin America and the Caribbean, and South East Asia. Finally, the costs of these comprehensive programs were compared to the cost of dengue illness in the same country for the same year. Preliminary results show that, on average, comprehensive vector control cost $1.69 per capita and was 52% of the economic cost of dengue illness in these same countries ($3.28). However, this relationship varied widely among countries. For example, Cuba's per capita cost of dengue vector control activities ($3.03) was 23 times its cost of dengue illness ($0.13). On the other hand, Venezuela's cost per capita of vector control ($0.57) represented only 8% of the country's cost of dengue illness ($7.38). If half the population at risk of dengue infection received comprehensive vector control at the current average cost, the global cost would be $6.1 billion annually. Innovative vector control strategies under development include genetics-based sterile insect methods, infection of mosquitoes with Wolbachia, interior residual spraying, auto-dissemination approach (spreading of insecticides by adult mosquitoes), attractive lethal ovitraps, sticky traps, and new pesticides. If any prove more effective than current measures, billions of dollars on current vector control and dengue illness could be saved or redirected.

837

EXPERIMENTAL EVOLUTION OF WEST NILE VIRUS IN WILD-CAUGHT BIRDS

Nathan D. Grubaugh[1], Darci R. Smith[1], Angela M. Bosco-Lauth[1], Doug E. Brackney[1], Corey Rosenberg[1], Todd Felix[2], Scott Sieke[1], William C. Black IV[1], Aaron C. Brault[3], Gregory D. Ebel[1]

[1]Colorado State University, Fort Collins, CO, United States, [2]U.S. Department of Agriculture, Lakewood, CO, United States, [3]Centers for Disease Control and Prevention, Fort Collins, CO, United States

Wild birds are the most important vertebrates in the West Nile virus (WNV) transmission cycle. Several studies have demonstrated that they generally impose purifying selection on the virus, and have suggested that they may select for novel WNV variants. However, the extent to which different important avian species influence WNV at the population level is poorly understood. Therefore, we evaluated whether different wild birds have distinct impacts on WNV populations. Specifically, we serially passed clone-derived WNV five times in wild birds that and experience varying levels of mortality: American crows (*Corvus brachyrhynchos*), house sparrows (*Passer domesticus*), and American robins (*Turdus migratorius*). After passage, we measured virus replication, pathogenesis and fitness in wild birds, chickens and mosquitoes. We also determined levels of intra-host genetic diversity using next-generation sequencing. Crows infected with crow-passed WNV developed higher viremias and experienced earlier mortality compared to birds infected with the unpassed virus. Sparrows developed an earlier peak viremia with the passed compared to unpassed virus, however the mortality and viremia differences compared to the unpassaged virus were insignificant. Passage in birds resulted in the generation of viruses with increased fitness gains in the same species and chicks compared to the unpassaged virus. Additionally, the bird passaged viruses did not lead to a trade-off of decreased competitive fitness in mosquitoes, which was previously observed in our laboratory. Studies in robins are ongoing. We obtained 20,000 to 60,000x WNV sequence coverage and found that intra-host genetic diversity increases in the early crow passages followed by positive selection of potentially adaptive variants. We did not find consensus changes to the crow passaged virus, suggesting that fitness gains may be achieved through rare mutations. Collectively, these results lend insight into the role of wild birds in selecting novel WNV genotypes.

838

FLAVIVIRUS INFECTION INDUCES TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS 1 (TREM-1): IMPLICATION FOR A ROLE IN INNATE IMMUNE RESPONSES

Kelsey O. Roe[1], Jacob T. Nelson[1], Beverly Orillo[1], Sebastien Gibot[2], Saguna Verma[1]

[1]University of Hawaii at Manoa, Honolulu, HI, United States, [2]Universitaire de Nancy, Nancy, France

Innate immune responses are essential for the control of flaviviruses, including WNV, which has emerged as a significant cause of viral encephalitis in humans. However, the specific mechanisms that regulate the programming of the innate immune signaling pathways remain unclear. Activation of TREM-1 signaling via the adaptor protein DAP12 is important for inflammation and activation of antigen presenting cells, however little is known about its role in viral infections. Here, we investigated the effect of flavivirus infection on the expression of TREMs and its potential role in the production of cytokines. We show that the expression of TREM-1 was markedly increased in dengue virus-infected THP-1 cells, which correlated with peak viral titers. Similarly, WNV infection significantly increased the mRNA levels of TREM-1 in MEFs, BMDMs and BMDCs. *In vivo* characterization of TREMs in mice demonstrated a significant up regulation in the transcripts of TREM-1, -3 and -4 in the peritoneal cavity cells and brain at day 3 and 8 after WNV infection respectively. Interestingly, serum levels of soluble-TREM-1 also increased significantly at days 2-3 after infection. Further, activation of TREM-1 using an agonist antibody increased mRNA of WNV-induced cytokines such as IFN-α, TNF-α and IL-6 in MEFs, which decreased following blocking of TREM-1. The changes to IFN-α and IL-6 secretion were validated with ELISA and Luminex, respectively. Collectively, our results for the first time document the response of TREMs to flavivirus infection and indicate a novel role of TREM-1 in modulating inflammatory response to WNV. Further studies are ongoing to define role of TREM-1 in WNV disease outcome.

839

COMPARISON OF THE EFFICIENCY AND COSTS OF WEST NILE VIRUS SURVEILLANCE METHODS IN CALIFORNIA

Jessica M. Healy[1], William K. Reisen[1], Vicki L. Kramer[2], Marc Fischer[3], Nicole Lindsey[3], Roger S. Nasci[3], Paula A. Macedo[4], Gregory White[5], Richard Takahashi[6], La Khang[6], Christopher M. Barker[1]

[1]Center for Vectorborne Diseases, University of California Davis, CA, United States, [2]California Department of Public Health, Sacramento, CA, United States, [3]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [4]Sacramento-Yolo Mosquito and Vector Control District, Elk Grove, CA, United States, [5]Coachella Valley Mosquito and Vector Control District, Indio, CA, United States, [6]Kern Mosquito and Vector Control District, Bakersfield, CA, United States

Surveillance systems for West Nile virus (WNV) aim to determine the location and timing of viral amplification early enough to direct mosquito control and prevent transmission to the human population. To ensure an optimal surveillance approach, the sensitivity and timing of each component of the surveillance system must be measured against its efficiency and costs. We evaluated each of the most widely used surveillance methods for WNV (testing of mosquitoes and public-reported dead birds, and seromonitoring of sentinel chickens) using data from three vector control agencies in California for the years 2004 through 2012, encompassing the period following WNV's arrival to California. The methods were compared after standardizing spatial sampling density, frequency of sampling, and costs. At equal spatio-temporal sampling, testing mosquitoes and dead birds typically detected viral activity 2-5 weeks earlier than seromonitoring of sentinel chickens. Viral activity was detected most frequently in mosquitoes during the early season (May-June) and in sentinel chickens during peak season (July-August). Testing dead birds reported by the public was found to be the most cost-effective of the available methods in areas where corvids and other avian hosts with high disease-dependent mortality were abundant. For a given budget, testing of dead birds or mosquitoes provided the greatest early warning and return on costs during spring and early summer (1.3 and 3.5 WNV-positive samples per $1,000 spent), and serological monitoring of sentinel chickens was of most utility during summer and early fall (2.8 WNV-positive samples per $1,000) as viral activity began to wane.

840

EVIDENCE OF NEUTRALIZING ANTIBODIES TO WEST NILE AND SAINT LOUIS ENCEPHALITIS VIRUSES IN PERUVIAN HORSES

Carolina Guevara[1], Alicia Rosas[1], Miguel Quevedo[2], Jorge Mantilla[2], William Valderrama[2]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]SENASA, Lima, Peru

Arboviruses are responsible for thousands of human and animal infections worldwide. Saint Louis encephalitis virus (SLEV), West Nile virus (WNV) and Venezuelan Equine Encephalitis virus (VEEV) are zoonotic arboviruses that infect horses and are widely spread in South America. SLEV was first reported in Brazil in 1960, and then reported in human sera in Peru in 1965. Venezuela, Colombia, Argentina, and other Caribbean-bordering countries have reported bird and equine infections. Epizootics and epidemics of VEEV has been reported in many countries throughout Central and South America, including Colombia, Venezuela, Trinidad, Ecuador, Mexico, and Peru. VEEV was isolated in 1942 for first time in Peru. In order to assess the presence of neutralizing antibodies to SLEV, WNV, and VEEEV we tested 3470 horse sera samples from 25 locations in Peru. The Peruvian Animal Health Office (SENASA) collected serum samples in 2011. We screened these samples with IgG indirect ELISA at the Naval Medical Research Unit No. 6 (NAMRU-6) in Lima, Peru. Positives were confirmed by solid Plaque Reduction Neutralization Test with 80% reduction ($PRNT_{80}$). Virus neutralization titers ranged from 1/20 to 1/640. We found 19 (0.5%) positive samples for WNV, mostly from the Cajamarca Region. Fourteen (0.4%) samples were positive for VEEV and 24 (0.7%) for SLEV, mostly from Piura. The samples were equally distributed within ecologically different areas in the coast, highlands, and rainforest. The presence of the neutralizing antibodies in horses against SLEV and WNV suggest prior infections and possible continuing spread of these arboviruses universally throughout Peru. Therefore, continued epidemiologic surveillance in horses is necessary in order to protect human populations against future outbreaks and subsequently confirm the continuing circulation of these viruses.

841

CONTEXT-DEPENDENT CLEAVAGE AT THE C-PRM JUNCTION BY THE WEST NILE VIRUS NS2B/3 PROTEASE MODULATES THE EFFICIENCY OF VIRUS ASSEMBLY AND RELEASE

Laura A. VanBlargan, Kaitlin A. Davis, Kimberly A. Dowd, Theodore C. Pierson

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Flavivirus assembly is governed in part by cleavage of the single viral open reading frame orchestrated by host and viral proteases. The sites recognized by flavivirus proteases have been defined principally through the use of small peptide substrates. Prior studies have shown that it is possible to produce infectious virions composed of the structural genes of one flavivirus and a sub-genomic RNA of a distantly related virus. While the production of "pseudo-typed" virions composed of the structural genes of DENV2 (strain 16681) and a WNV replicon is quite efficient (>106 infectious units/mL), the production of virus particles incorporating the C-prM-E proteins of the DENV2 NGC strain was not possible, despite sharing 97.8% amino acid sequence identity. To understand the underlying mechanism, we constructed chimeras of the structural genes of these two DENV2 strains and identified the capsid (C) protein as the source of incompatibility. Subsequent mutagenesis studies revealed that a single C substitution, T101S, restored the ability to produce infectious virions composed of NGC C-prM-E and the WNV replicon. Residue 101 is the P1' position of the NS2B/3 viral protease cleavage site, and indeed, we found virion production capability mapped to the efficiency of C-prM cleavage. The significant impact of the T101S substitution on the efficiency of cleavage by the WNV protease is surprising in light of published biochemical studies of the requirements for cleavage. We conducted more extensive mutagenesis of the C-prM junction to further define the sequence requirements for cleavage by the WNV and DENV proteases; these studies revealed a context-dependent substrate specificity of the viral protease. Definition of the substrate specificity of the viral protease against the backdrop of the viral polyprotein may facilitate the development of new protease inhibitors and provide insight into associated patterns of drug resistance.

842

ECOLOGY OF CULEX FLAVIVIRUS DURING WEST NILE VIRUS EPIDEMIC AND INTER-EPIDEMIC YEARS IN SUBURBAN CHICAGO, USA

Christina Newman[1], Tavis Anderson[2], Gabriel Hamer[3], Bethany Krebs[4], Marilyn O'Hara Ruiz[4], Jeffrey Brawn[4], William Brown[4], Edward Walker[5], Uriel Kitron[6], Tony Goldberg[1]

[1]University of Wisconsin-Madison, Madison, WI, United States, [2]Georgia Southern University, Statesboro, GA, United States, [3]Texas A&M University, College Station, TX, United States, [4]University of Illinois-Urbana Champaign, Champaign, IL, United States, [5]Michigan State University, Lansing, MI, United States, [6]Emory University, Atlanta, GA, United States

Insect-only flaviviruses have been identified in mosquito populations throughout the world. These insect host-adapted viruses co-circulate with medically important arboviruses, but their ecology and effects on their mosquito hosts in nature are poorly understood. Culex flavivirus (CxFV) is an insect-only flavivirus found in *Culex* species mosquitoes, important

vectors of West Nile virus (WNV) in the United States. CxFV and WNV co-circulate in and co-infect *Culex* mosquitoes at a WNV "hotspot" in suburban Chicago. We previously identified a positive association between CxFV and WNV in mosquito pools collected in 2006. To further investigate the ecology of CxFV and its association with WNV, we compared the spatial and temporal distribution of CxFV in 2011, an inter-epidemic year for WNV, with its distribution in 2012, an epidemic year for WNV. The overall prevalence of WNV in mosquitoes in 2011 was 0.11% (95%CI: 0.04-0.25), whereas in 2012 it was 0.62% (95%CI: 0.42-0.90). The overall prevalence of CxFV in 2011 was 10.21% (95%CI: 9.28-11.26), whereas in 2012 it was 17.02% (95%CI: 15.37-18.86). Both viruses were significantly more prevalent in mosquito pools in 2012 than in 2011 (Wilcoxon signed rank test, WNV: V=41, p<0.02; CxFV: V=164, p<0.001). CxFV was identified at all 37 trap locations that were repeated in both years. During 2012, the trap location with the highest WNV prevalence (2.83%) was also the trap location with the highest CxFV prevalence (79.96%). Among locations positive for both viruses, a positive correlation between CxFV and WNV was observed in 2011 (t= 4.31, df= 3, p < 0.05), but not in 2012 (t = 1.61, df= 14, p > 0.1). These results demonstrate an association between WNV and CxFV on a fine spatial scale in an urban setting that may be driven by similar responses of the two viruses to common environmental drivers.

843

SPATIAL ANALYSIS AND EVALUATION OF 2014 PREDICTIONS FOR A WEST NILE VIRUS EARLY WARNING SYSTEM

Carrie A. Manore[1], Justin K. Davis[1], Rebecca C. Christofferson[2], Dawn M. Wesson[1], James M. Hyman[1], Christopher N. Mores[2]

[1]Tulane University, New Orleans, LA, United States, [2]Louisiana State University, Baton Rouge, LA, United States

Since the introduction of West Nile virus (WNV) to the USA in 1999, numbers of human cases reported to the CDC have varied from fewer than a thousand cases per year to 5,674 cases in 2012. A national model conditioned on weather and other data, including bird species distributions and human population density, has explained the large variations seen over the 2005-2013 period and a prediction was made for 2014. We found the most significant predictors for a human WNV case in a county are the mean minimum temperature in January, the deviation of this minimum temperature from the expected minimum temperature, the total population of the county, the bird population, and if the county had a case of WNV the previous year. Due to aberrant weather patterns in early 2014, the pattern of human WNV was predicted to be equally aberrant, presenting a genuine test of the model and implications for public health in the face of climate change. Predictions are compared to up-to-date case reports and locally conditioned models are examined for regions where more data are available, providing a start toward regional early warning systems.

844

AVIAN SPECIES DIVERSITY AND AMPLIFICATION OF WEST NILE VIRUS IN ATLANTA, GEORGIA

Rebecca S. Levine[1], David L. Hedeen[2], Meghan W. Hedeen[2], Gabriel L. Hamer[3], Daniel G. Mead[4], Uriel D. Kitron[1]

[1]Emory University, Atlanta, GA, United States, [2]Georgia Department of Transportation, Atlanta, GA, United States, [3]Texas A&M University, College Station, TX, United States, [4]University of Georgia, Athens, GA, United States

The dilution effect is the reduction in vector-borne disease risk that occurs through the presence of a diverse set of potential host species, some of which are relatively or completely incompetent as hosts. West Nile virus (WNV) is a mosquito-borne disease that is maintained in various avian host species. In Atlanta, Georgia, substantial WNV presence in the vector and host species has not translated into a large number of human cases. In order to determine whether a dilution effect was contributing to the reduced WNV spillover in the area, we conducted comprehensive multi-season, multi-habitat, characterization of the avian species community as well as longitudinal WNV surveillance in avian hosts and mosquito vectors in urban Atlanta between 2010 and 2011. We measured host diversity in two ways: diversity at-large and diversity as experienced by the pathogen. Regardless of how we measured avian species diversity or whether we considered host infection and vector infection as predictor variables or outcome variables, we did not detect a dilution effect. Instead, we detected an amplification effect, in which increased host diversity resulted in increased rates of infection, the first empirical evidence for this effect in a mosquito-borne system. We suggest that the observed amplification effect may primarily be driven by an over-abundance of moderately to poorly competent host species, such as Northern Cardinals, which may cause optimal hosts to be more rare and therefore to be present primarily in more species-rich areas. Other possible mechanisms driving amplification could be increased vector species richness and innate mosquito preference for certain host species over others. We encourage further research to assess the scale and prevalence of amplification effects in the WNV system, as well as the contributions of various host and vector species to its establishment.

845

GENETIC DETERMINANTS OF AVIAN PATHOGENESIS OF LINEAGE 2 WEST NILE VIRUS STRAINS

Hannah E. Romo[1], Angela Bosco-Lauth[1], Todd A. Felix[2], Richard A. Bowen[1], Gregory D. Ebel[1], Aaron C. Brault[3]

[1]Colorado State University, Fort Collins, CO, United States, [2]APHIS-United States Department of Agriculture, Denver, CO, United States, [3]Centers for Disease Control and Prevention, Fort Collins, CO, United States

In 2010, a human outbreak of a West Nile virus (WNV) lineage 2 (L2) in Greece established, for the first time, an association between L2 WNVs and extensive human disease in Europe. Sequence analysis of a L2 WNV virus (NS10) isolated from a pool of infected mosquitoes in Nea Santa, Greece during the 2010 WNV outbreak revealed an NS3-H249P mutation not previously observed in any sequenced L2 WNV strains. Interestingly, an NS3-T249P mutation has previously been demonstrated to increase viremia and virulence of a lineage 1 WNV strain in American crows (AMCRs). In order to assess the virulence potential of NS3-249P substitution in L2 WNVs, AMCRs, thought to be a key reservoir host for WNV in North America, were inoculated with the parental L2 WNV viruses, NS10 and South Africa 1989 (SA89), in addition to mutants generated containing polymorphisms at the NS3-249 site (Pro, His and Thr). The parental NS10 and SA89 strains displayed 100% and 33% mortality with average peak viremias of 9.5 and 7.5 log10 PFU/mL in AMCRs, respectively. The NS10 mutants, NS10 NS3-249H and NS10 NS3-249T, exhibited 80% mortality with peak viremias of 8.7 log10 PFU/ml sera and 20% mortality with peak viremia of 6.0 log10 PFU/mL sera, respectively. The SA89 mutant, SA89 NS3-249H, elicited 100% mortality with a peak viremia of 9.6 log10 PFU/mL and the SA89 NS3-249T resulted in 0% mortality with a peak viremia of 2.6 log10 PFU/mL sera. Viremia and mortality differences in AMCRs between the L2 WNV backbones harboring the same polymorphisms at the NS3-249 site suggest that epistatic interactions of alternative genetic elements are involved in generating the variable phenotypes. The sequence of the SA89 strain was compared with that of NS10 and a total of 16 amino acid differences were identified exclusive to the nonstructural genes. In order to understand the factors related to the emergence of a human disease associated L2 WNV in Europe, and the potential role of alternative genetic factors in epistatic maintenance of the virulence associated with the NS3-249 site, chimeras between NS10 and SA89 were generated and tested in AMCRs and House Sparrows.

846

METEOROLOGICAL CONDITIONS ASSOCIATED WITH INCREASED INCIDENCE OF WEST NILE VIRUS DISEASE IN THE UNITED STATES, 2004-2012

Micah B. Hahn[1], Andrew J. Monaghan[2], Mary J. Hayden[2], Rebecca J. Eisen[3], Mark J. Delorey[3], Nicole P. Lindsey[3], Roger S. Nasci[3], Marc Fischer[3]

[1]Centers for Disease Control and Prevention/National Center for Atmospheric Research, Fort Collins/Boulder, CO, United States, [2]National Center for Atmospheric Research, Boulder, CO, United States, [3]Centers for Disease Control and Prevention, Fort Collins, CO, United States

West Nile virus (WNV) is the leading cause of mosquito-borne disease in the United States. Annual seasonal outbreaks vary in size and location. Predicting where and when outbreaks will occur can help direct public health control efforts. Weather can impact several factors associated with WNV transmission, such as mosquito vector and zoonotic host abundance. We developed models for the continental United States to identify meteorological conditions associated with higher incidence of WNV neuroinvasive disease (WNND) from 2004-2012. We used county-level WNV surveillance data reported to CDC and meteorological data from the North American Land Data Assimilation System. Due to geographic differences in WNV transmission, we divided the United States into east and west (defined by ~100 degrees West longitude) and 10 US Environmental Protection Agency regions. Meteorological conditions were evaluated from October of the prior year through September of the given WNV season. For each US county, we calculated standardized z-scores that described annual WNND incidence, average temperature, and total precipitation compared to the mean values for 2004–2012. For WNND incidence, a z-score ≥0.5 was defined as above average. We used fixed effects models to assess independent associations between anomalies in temperature or precipitation and above average WNND incidence within each geographic area. Preliminary results showed warmer than average annual temperature was associated with above average WNND incidence nationally and in all geographic areas. Lower than average total precipitation was associated with higher disease incidence nationally but the effect varied significantly by region. These findings suggest anomalies in temperature and precipitation are associated with above average WNV disease incidence but the overall effects vary by region. Although multiple factors influence WNV transmission, readily accessible meteorological data may be used to develop predictive models to forecast geographic areas with elevated WNV disease risk prior to the coming season.

847

PREVALENCE AND RISK FACTORS OF HUMAN CORONAVIRUSES

Michael Owusu

Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana

Acute respiratory tract infections (ARI) are the leading cause of morbidity and mortality in developing countries, especially in Africa. However, information on the viral aetiological agents are scanty. We therefore conducted a cross-sectional serological study to determine the prevalence of Human Coronaviruses among individuals living in rural areas of Ghana from September 2010 to October 2013. The study areas are Kwamang in the Ashanti Region, Buoyem in the Brong Ahafo Region and Forikrom also in the Brong Ahafo Region. A total of 201 subjects were enrolled in the study. Subjects were tested for IgG antibodies to three HCoVs namely; HCoV-NL63, HCoV-OC43 and HCoV-229E. Of the 201 subjects, 97 (48.3%) were positive for all viruses. The most prevalent virus was HCoV-229E (23%; 95% CI: 17.2 - 29.3), followed by HCoV-OC43 (17%; 95% CI: 12.4 - 23.4), then HCoV-NL63 (8%, 95% CI: 4.6 - 12.6). Of all positive HCoV-NL63 subjects, those in Kwamang had the highest sero-prevalence (68.8%). In contrast, HCoV-229E (41.3%) and HCoV-OC43 (45.7%) were much higher in Forikrom compared to the other study areas. There was however no statistical difference between living in any of the study areas and being positive for HCoVs. The gender distribution for all three viruses was also similar. The median ages of those positive for HCoV-OC43 (47 years, IQR = 33 - 52.5) and HCoV-229E (40 year, IQR = 27 - 54) were higher than negative subjects. The age difference for HCoV-NL63 subjects were similar (p = 0.994). A comparison of the blood group types between subjects positive for HCoVs and those negative showed no significant statistical difference (p = 0.163). This study demonstrates the occurrence of three types of HCoVs in remote areas of Ghanaian rural populations.

848

THE LABORATORY AS A TOOL IN THE ENDGAME POLIOVIRUS ERADICATION PROGRAM IN GHANA

Miriam Eshun[1], Jacob Barnor[2]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Noguchi Memorial Institute For Medical Research, Accra, Ghana

Polioviruses and other enteroviruses cause acute flaccid paralysis, and this is of public health concern. The World Health Organization (WHO) sees the laboratory as being an important tool in the virological investigation of acute flaccid paralysis cases, therefore, the laboratory having a crucial role to play in ensuring that no viruses, especially, wild polioviruses are missed. Until 1999, the Laboratory isolated indigenous wild poliovirus outbreak infections, which led to quick interventions with oral polio vaccines.The country enjoyed close to five years of wild type poliovirus elimination until the laboratory in 2003 and then in 2008 isolated a wild type 1 imported poliovirus in the country. Since the 2008 outbreak, Ghana has been free from wild type poliovirus infections.This paper sought to look at the crucial role the laboratory continues to play in ensuring that no wild poliovirus is missed; even after Ghana, being free of wild poliovirus since 2009. A detailed analyses of data on all AFP stool specimens investigated for a period of 10 years (2004 to 2013), using Epi Info data software; as we look at the performance and progress of the laboratory in polio eradication in Ghana. A total of 4,555 AFP specimens from all ten regions of Ghana were analyzed. Ninety percent(90%) of the stool specimens were from children below the age of 15 years. Males constitute just above half (57.2%) of the total specimens. Over 80% of the specimens were received in good condition.The annual non polio enterovirus isolation rate was above 10%, which was within WHO recommended non polio enterovirus isolation rate of 10% for the laboratory. Two hundred and twenty three(223 -4.9%) of the specimens were positive for poliovirus; and 8(3.6%) of the 223 were wild-type 1 imported polioviruses.Timeliness of reporting within 14days from date of specimen receipt,annually rated not less than 98%.The laboratory over the period have consistently passed annual proficiency test for virus isolation and also for intratypic differentiation (ITD) of polioviruses.The Laboratory's ability and skills in the delivery of accurate results in a timely manner made it possible for the timely intervention when Ghana recorded the two poliovirus outbreak in 2003 and 2008. Indeed the laboratory's continuous involvement in Polio eradication through virological investigations and timely dissemination of results has brought Ghana into the reality zone of wild poliovirus eradication.

849

DEEP SEQUENCING AS A TOOL TO IDENTIFY PATHOGENS FROM POOLED RESPIRATORY SAMPLES FROM SOUTH/ SOUTHEAST ASIA

Wiriya Rutvisuttinunt

Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Emerging and re-emerging respiratory pathogens represent an increasing threat to public health. Asia continues to be the regional epicenter for the emergence of novel pathogens and the site where several pandemics have originated. Etiological determinations during outbreaks have generally relied on clinical information, occasionally accompanied by traditional molecular or serological techniques. Often, the information is inconclusive.

In 2013, the Armed Forces Research Institute of Medical Sciences (AFRIMS) identified one hundred and sixteen nasal-pharyngeal specimens collected from acute influenza-like illness (ILI) patients in several countries in South/South East Asia which were negative by conventional molecular and culture techniques but demonstrated cytopathic effect (CPE) in cell culture. Groups of 8 to 15 CPE-positive samples were organized by the geographic region from where they were initially collected. Deep sequencing was performed on each pool to generate sufficient sequence reads to allow for initial pathogen identification. A total 7.9 Gbases or 22.28 million sequence reads passed quality control with ≥30Q scores. After filtering out host and microbiome background, low abundance sequence reads were analyzed. We were able to identify various respiratory pathogens, which tended to localize in specific regions: (i) parainfluenza 3 in the Bhutan/Nepal pool, (ii) human metapneumovirus and human coxsackievirus A21 strain in the Cambodian pool, (iii) influenza A in the Philippines pool, (iv) human coxsackievirus A21 strain in the Bangkok, Thailand pool, and (v) parainfluenza 4a in the northern Thailand pool. The pools and individual samples with high viral content were confirmed by singleplex PCR, real-time PCR or conventional PCR. Overall, deep sequencing performed efficiently as an initial identification tool for viral pathogens using pooled respiratory samples of unknown etiology but capable of inducing CPE in cell culture.

850

ABOLISHMENT OF INDIVIDUAL N-GLYCOSYLATION SITES WITHIN RIFT VALLEY FEVER VIRUS GN/GC ALTERS INFECTIVITY VIA DC-SIGN

Inaia Phoenix, Terence E. Hill, Olga Lihoradova, Sabarish V. Indran, Birte Kalveram, Alexander N. Freiberg, Tetsuro Ikegami

University of Texas Medical Branch, Galveston, TX, United States

Rift Valley fever is a mosquito-transmitted zoonosis that is characterized by high rates of abortion and fetal malformations in ruminants and causes severe disease in humans. Recently, DC-SIGN (a C-type lectin receptor) has been demonstrated to be a receptor for phleboviruses, including Rift Valley fever virus (RVFV: genus *Phlebovirus*; family *Bunyaviridae*). We hypothesized that N-glycosylation of Gn/Gc plays an important role in the infection of RVFV via DC-SIGN. RVFV glycoproteins (Gn/Gc) encode 5 putative N-glycosylation sites: aa. 438 (Gn), 794 (Gc), 829 (Gc), 1035 (Gc), and 1077 (Gc), but their significance in viral infection via DC-SIGN has not been elucidated. Using a reverse genetics system, we generated recombinant MP-12, which lack one of the five potential N-glycosylation sites of Gn/Gc: N438Q (Gn); N794Q (Gc); N829Q (Gc); N1035Q (Gc); and N1077Q (Gc). To identify which sites are utilized for N-glycosylation, we immunoprecipitated [^{35}S] methionine/cysteine-labeled MP-12 or the mutants in supernatant with anti-RVFV antibody and analyzed the size of the Gn/Gc proteins by autoradiography. The Gn of N438Q, and Gc of N794Q, N829Q, N1035Q, and N1077Q migrated faster than those of parental MP-12, indicating aa.438, 794, 829, 1035, and 1077 are N-glycosylated. To test the infectivity of each mutant, we measured viral RNA copy number using digital droplet PCR with a Taqman probe specific to the MP-12 L-segment. The ratio of PFU in VeroE6 cells per RNA copy was analyzed. Then, Jurkat cells and those that express DC-SIGN were infected with the mutant viruses at the same RNA copy number, and we analyzed the number of infected cells at 6 hpi by flow cytometry (FACS). We are currently repeating the experiments to conclude the statistical differences of infectivity among the N-glycan mutants. Our findings will be useful for understanding of the pathology of RVFV and the rational design of live-attenuated vaccine candidates.

851

MOLECULAR ANALYSIS OF INFLUENZA B VIRUSES ISOLATED IN KENYA, 2012

Stephen Ocholla

U.S. Army Medical Research Unit-Kenya, Nairobi, Kenya

Introduction: Influenza B viruses belong to two evolutionary lineages (B/Victoria/2/87-like and B/Yamagata/16/88-like) that continue to co-circulate globally in the human population since 1980s. These viruses do not undergo antigenic shifts but drifts as a result of accumulation of amino acid substitutions especially in the HA1 polypeptide have been confirmed. The evolution of surface glycoproteins occurs over time due to selection pressure exerted by host's immunity. Objective: To investigate molecular evolution of influenza B viruses isolated in Kenya by sequence analysis of HA1 hemagglutinin. Methods: Nasopharyngeal specimens obtained from patients meeting WHO definition criterion for ILI were screened by real-time PCR for influenza A and B viruses. Influenza B virus positive samples were inoculated onto Mardin-Darby Canine Kidney cells and HA protein coding gene of selected isolates sequenced and analyzed. Results: Phylogenetic analysis showed all influenza B viruses isolated in Kenya clustered together with B/Brisbane/60/2008 vaccine strain and other viruses of B/Victoria/2/87-like lineage in other regions of the world. All the Kenyan isolates were characterized with D197N amino acid substitution not present in the vaccine strain. This change occurred within 190-Helix antigenic binding site. Majority of Kenyan isolates (except B/Kenya/242/2012, which had I146A) further had I146V amino acid change within the 150-Loop antigenic site, absent in the vaccine strain. Other mutations occurred stochastically in individual isolates. Most notable was isolate, B/Kenya/239/2012 which had an additional V124I amino acid change within the 120-Loop antigenic binding site. Conclusion: There was limited variation among the Kenyan isolates. Kenyan viruses matched closely with the seasons vaccine strain, B/Brisbane/60/2008 despite having mutations at antigenically significant positions in the HA1 subunit. Molecular analysis of influenza B viruses is important for early detection of strains with epidemic potential.

852

THE MOLECULAR EPIDEMIOLOGY OF NOROVIRUS IN MILITARY RECRUITS IN IQUITOS, PERU: EVIDENCE OF A NOVEL GENOTYPE

Simon Pollett[1], Sarah Ballard[2], John-Sebastian Eden[3], Giannina Luna[1], Marita Silva[1], Susan Espetia[4], Erik Reaves[1], Drake H. Tilley[1], Mark Simons[1], Rene C. Guzmán[5], Edward C. Holmes[3], Robert Gilman[2], Daniel G. Bausch[1]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Johns Hopkins School of Public Health, Baltimore, MD, United States, [3]Marie Bashir Institute for Infectious Diseases and Emerging Infections, University of Sydney, Sydney, Australia, [4]Universidad Peruana Cayetano Heredia, Lima, Peru, [5]Policlínico Militar de Chorrillos, Chorrillos, Peru

Norovirus (NoV) is a leading cause of diarrhea in Peru, yet its molecular epidemiology in this region is largely unknown. We therefore explored the genetic diversity of NoV in Peruvian military recruits in Iquitos, a Peruvian city in the Amazon basin. Stools from a random subsample of recruits from a diarrhea surveillance study in Iquitos between 2004 and 2011 were screened for NoV and genogrouped by AgPath-ID One-RT-PCR. The NoV VP1 gene was partially sequenced (330/344 bp, region C) and genotyped using the Noronet webtool. Phylogenetic trees of Iquitos sequences compared with BLAST and Noronet reference taxa were inferred using the maximum likelihood (ML) method with bootstrapping by RAxML software. 360/4234 (8.5%) participants were tested for NoV, 11.1% (40/360) were positive, including 12 for genogroup (GI), 29 for genogroup II (GII) and one GI/GII dual infection. Genotypic and further analysis was able to be performed in 49 % (20/41) of sequences. ML trees demonstrated a wide range of Peruvian GI genotypes with the majority belonging to a GI.4 clade. Spatial clustering of GI Peruvian and Brazilian taxa was noted,

although weakly supported. Genotype diversity of GII NoV in Iquitos was broad with many in GII.4 2006bDenHaag clades. One GII Iquitos sequence was untypable and appears to represent a novel genotype, with a GII.3 Tunisian sequence as the closest typable relative (86% nucleotide similarity) and strong support for clustering with near identical and also untypable Nicaraguan strains. Within most genotype clades there was temporal clustering of Peruvian and reference sequences, consistent with Peru being affected by globally circulating lineages. In conclusion, Noroviruses in the Peruvian Amazon are genetically diverse with evidence of a novel genotype, mixing with global lineages and weak regional spatial structure. Larger studies are needed to clarify the regional phylogeography of noroviruses and confirm and characterise this and perhaps other novel NoV genotypes in Peru

853

TIME TRENDS AND CORRELATES OF ROUTINE MEASLES IMMUNIZATION COVERAGE IN ABIA STATE, SOUTHEAST NIGERIA; 2007-2012

Abiodun J. Oladejo[1], Gabriele Pogensee[1], Aisha Abubakar[2], Saheed Gidado[1], Patrick Nguku[1], Godwin Okezue[3]

[1]Nigerian Field Epidemiology and Laboratory Training Program Programme, Abuja, Nigeria, [2]Ahmadu Bello University, Zaria, Kaduna, Nigeria, [3]Abia State Primary Health Care Development Agency, Abia, Nigeria

Measles is a vaccine-preventable-viral disease associated with high morbidity and mortality. The national target in Nigeria for routine measles immunization coverage is ≥80%. We conducted this study to assess the variations in annual trends of routine measles immunization coverage rates in Abia State and to identify the factors affecting coverage. A time series analysis was performed on administrative measles immunization data collected by Abia State Primary Health Care Development Agency from 2007-2012. Trends in measles immunization coverage, measles vaccine wastage, measles vaccine supply rates, measles vaccine weekly stock and immunization outreach sessions (vaccination points outside health facilities) were assessed in all 17 Local Government Areas (LGAs) and disaggregated by LGA type (urban or rural LGA). The relationship between measles vaccine wastage, supply rates, weekly stock, immunization outreach sessions, LGA type and an outcome of ≥80% measles immunization coverage was assessed and modeled using step-up logistic regression. The annual birth cohort (study population) was 121,107 in 2007, declined to 110,636 in 2009 and rose to 123,034 in 2012. Both measles immunization coverage and immunization outreach sessions increased at a linear rate ($p < 0.001$) while measles vaccine wastage rate declined linearly ($p < 0.001$). Measles vaccine weekly stock declined at a rate of 3 days stock per month till 2009, increasing afterwards ($p<0.001$). 28% of the LGAs attained ≥80% immunization coverage by 2012; achieved by 46% of urban LGAs. Immunization outreach sessions increased from 1762 in 2007 to 8595 in 2012; 48% were <25sessions/month. 58% of LGAs got ≥80% of their measles vaccine. 38% of LGAs had a vaccine wastage rate of <30%. Having <30% measles vaccine wastage (OR=2.2), ≥80% measles vaccine supply (OR=9.8), ≥25 immunization outreach sessions ($p<0.001$) and being an urban LGA (OR=2.9) was associated with the outcome. The effect of measles vaccine wastage was modified by LGA type ($p= 0.009$). These variables were significant positive predictors for ≥80% measles coverage, following modeling. Routine measles immunization coverage improved over the study period. Only a third of LGAs met the required national target; mostly urban LGAs. Public health resources should be directed at reducing vaccine wastage at service delivery, improving vaccine supply chains and increasing access to immunization, especially in rural LGAs.

854

EPIDEMIOLOGICAL CHARACTERISTICS OF ACUTE FLACCID PARALYSIS CASES IN LAGOS STATE NIGERIA, 2001 - 2011

Abimbola F. Aman-Oloniyo

Nigeria Field Epidemiology and Laboratory Program, Asokoro, Nigeria

Globally, Nigeria is one of three countries still endemic for indigenous transmission of polio virus. Acute Flaccid Paralysis (AFP) surveillance is one of four strategies recommended for polio eradication. Data from AFP surveillance guides implementation of immunization activities aimed at interrupting polio transmission. In November 2013, we conducted a study to characterize AFP cases in Lagos State, southwest Nigeria. We analyzed secondary data of AFP cases in Lagos State from 2001 to 2011. AFP was defined as recent onset of floppy weakness or paralysis in a child less than 15 years or any paralytic illness in any person in whom a clinician suspects polio. We reviewed the AFP data and performed univariate and bivariate analyses using Epi-info 3.5.4 software. Altogether, 2,896 AFP cases were reported; 1683 (58.1%) were males. Mean age was 2.9 years (+/-2.7). The most affected age group was 0-5 years (86.1%). Of all AFP reported, 25 (0.9%) did not receive any OPV (Oral Polio Vaccine); 301 (11%) received 1-2 doses. Over 80% of cases had received 3 or more OPV doses. Adequate stool specimen was collected for analysis in 2815 (97.4%) of the AFP cases. Only 30 (1%) were positive for wild polio virus (WPV). WPV type 1 (WPV1) were 23 of which 1 had no OPV and WPV3 were 7(all received OPV). Last confirmed human WPV was in year 2009. Polio virus transmission has been interrupted in Lagos state. The high immunization status would have contributed to population immunity and reduced transmission. Government should continue to strengthen and scale-up routine and supplemental immunizations with OPV.

855

EPSTEIN-BARR VIRUS IN PATIENTS WITH ACUTE FEBRILE ILLNESS IN KENYA

Clement A. Masakhwe

University of Nairobi, Kisumu, Kenya

Epstein Barr Virus (EBV) causes infectious mononucleosis and other lymphoproliferative disorders, including endemic Burkitt's lymphoma (eBL). EBV epidemiology is best known from serological studies. This study determined the EBV viremia and prevalence in patients with acute febrile illness (AFI) in Kenya. Patients with AFI were enrolled at 8 out-patient hospitals between September 2008 and April 2013. DNA was extracted from whole blood and BALF5 gene used as target for real-time PCR. Prevalence rates and viremia were determined and correlated by age, region and co-infection with malaria. Of the 2021 patients examined, EBV was detected in 588 (29%) and their viremia ranged from 52 to 7.2 x106 copies/mL (geometric mean 4345 copies/mL). Patient mean age was 5 years (range 1-80 years). Viral prevalence was highest in patients 2000 copies/mL, a cutoff considered clinically important. The <5 year olds constituted the majority (41%) in this group. Regions holoendemic for malaria had the highest prevalence compared to the hypoendemic regions. In addition, patients with EBV/malaria co-infections had higher viremia (geometric mean 5929 copies/mL) compared to those with EBV alone (3793 copies/mL, $p = 0.003$).The study demonstrates how common EBV is among patients with AFI. That malaria is an important determinant of EBV viremia, reinforcing the possibility that increased viremia in EBV/malaria co-infections could be a precursor to the development of eBL.

856

ADVERSE EVENTS FOLLOWING IMMUNIZATION WITH NEWLY INTRODUCED MEASLES-RUBELLA VACCINE IN JIRAPA DISTRICT, GHANA

Godfrey P. Konnyebal

School of Public Health, University of Ghana, Accra, Ghana

Background: Vaccines are one of the most cost effective public health interventions. Real or perceived adverse events following immunization (AEFI) can undermine the credibility of a vaccine and an immunization programme. Ghana rolled out a measles-rubella combined vaccine in a mass immunization campaign in September, 2013. We assessed the AEFI associated with the vaccine in Jirapa District to obtain base-line data and appropriately respond to public concerns on safety issues. Method: A risk interval cohort study was conducted. Three hundred and fifty children aged 9 months -14 years followed for twelve weeks. Seventy children in four age groups were selected from each of five communities in Jirapa District using modified EPI coverage guideline. Participants were observed for four weeks before vaccination then eight weeks after vaccination for adverse events in the pre and post vaccination control windows and risk window. An AEFI was defined as any medical incident, which occurred after vaccination with measles rubella vaccine. An AEFI was said to be serious if it was life-threatening and required intervention and/or hospitalization or resulted in disability/incapacity or death. Univariate and bivariate analysis were done using Epi info 3.5. P values less than 0.05 were considered statistically significant. Results: Three hundred and fifty (350) vaccinees, 51.6%(180/350) females and 48.4%(170/350) males were followed for twelve weeks. Overall incidence of adverse events following immunization was 5.1% (95% CI: 3.2-8.2%). Of these fever accounted for 66.7% (12/18), febrile convulsion 5.6% (1/18), headache 16.7% (3/18), skin rashes 5.6 (1/18), and pain at injection site 5.6 (1/18). Only two (11.1%) of the adverse events were serious. Three (16.7%) of the adverse events occurred within 24 hours after vaccination while 11 (61.1%) occurred between the first and seventh day after vaccination. Children aged 9months- 3 years were 6.6 times more likely to develop fever than children aged 10-14 years (RR=6.6, 95% CI: 0.83-52.62; P <0.04). Conclusion: The adverse events following immunization with Measles-Rubella Vaccine were few and generally mild. Continued surveillance for adverse events and investigation of serious ones are recommended.

857

MOLECULAR SIGN OF INFLUENZA A AND B VIRUSES IN CUBA DURING TWO CONSECUTIVE YEARS

Alexander Piñón, Belsy Acosta, Odalys Valdés, Amely Arencibia, Mayra Muné, Clara E. Savón, Suset I. Oropesa, Angel Goyenechea, Grehete González, Guelsys González, Bárbara Hernández, Rosmery Roque

Tropical Medicine Institute Pedro Kourí, Havana, Cuba

The work contributes to a better understanding of influenza virus circulation in Cuba, sub-tropical country located at the Caribbean area. The objective of this work was to determine circulation and molecular characterization of influenza viruses in Cuba. From January/2012-December/2013, Cuban National Influenza Centre received 7783 clinical samples of individuals presenting influenza-like illness symptoms, severe acute respiratory infection or fatal cases. Samples were tested for seasonal A(H3N2), A(H1N1)pdm09 and B influenza viruses by real-time reverse transcription-polymerase chain reaction. Nucleotide sequences from hemagglutinin HA1 region segment were obtained directly from positive cases clinical samples. Genetic distances were calculated using MEGA v.5.05. Phylogenetic tree was constructed using Mr.Bayes v.3.1.2 software. Potential N-Glycosylation sites were predicted using NetNGlyc server 1.0. Of the 1335 samples positives to influenza virus, 48% were positive to influenza A(H1N1)pdm09, 31% to influenza B and 21% to influenza A(H3N2). Year 2012 was marked by low circulation of H3N2 subtype, with only 23 detections. Sequences obtained directly from clinical samples, belong to the clade 6. In year 2013 circulation of H3N2 subtype was highest, all of them grouped into the clade 3C (3C.2 and 3C.3), related to the vaccine strain signed by lineage A/Texas/50/2012. Circulation of influenza A(H1N1)pdm09 was highest in 2013 (26,2%) respect 2012 year (8,3%). Studied sequences distributes into three distinct clades: sequences from 2012 year belong into the clade 7, sequences from January 2013 belong into the clade 7 mainly and one of them into the clade 6C together with sequences from May 2013, last sequences from November 2013 belong into the clade 6B. Influenza B viruses were detected during the two studied years, characterized by the circulation of lineage B/Victoria in 2012 (20.5% of influenza detections) and lineage B/Yamagata in 2013 (7.4% of influenza detections). Strains of B/Victoria lineage grouped with the vaccine strain B/Brisbane/60/2008, while strains of the B/Yamagata lineage belong into the clade 2 represented by the vaccine strain B/Massachusetts/02/2012. It remains to be defined if these viral variants represent an important antigenic drift that would enable viral immune evasion and/or affect influenza vaccine effectiveness.

858

EXPRESSION OF INNATE IMMUNE GENES IN HUMAN CELLS INFECTED BY BUNYAVIRUS

Jorge G. Ferreira[1], Fernanda N. Villani[2], Izabella C. Batista[1], Danilo B. Oliveira[2], Gabriel M. Almeida[2], Jaquelline G. Oliveira[1], Paulo C. Ferreira[2], **Carlos E. Calzavara-Silva**[1]

[1]CPqRR/Fiocruz-MG, Belo Horizonte, Brazil, [2]ICB/UFMG, Belo Horizonte, Brazil

The virulence of the pathogenic Bunyaviruses is directly linked to the roles of viral virulence factors and their capacity to counteract the host pathways. These viruses use cellular proteins to promote their own replication/transcription and, in response, host induces transcriptional reprogramming to activate antiviral effects. In order to verify the early steps of Apeu virus (APEV) and Tahyna virus (THAV) induced innate immune system activation, we performed TaqMan-based qPCR assays using cDNA obtained from mRNA extracted from A549 cells after 4hs or 8hs of infection with APEV, TAHV and VSV virus (ssRNA control virus), besides a mock control. We verified that APEV is recognized by TLR9, differently of TAHV, which follows VSV due to TLR7 recognition. However, APEV follows VSV decreasing TICAM1 expression after 4hs of infection. It is also possible to note an early induction of TLR pathway by VSV when compared to APEV and TAHV. TAHV and mainly VSV, but no APEV, increased expression of IRF5, notably after 8hs of infection. All the viruses were able to increase the expression of TLR3, IRF3 and 7. TRAF3 was slightly more expressed (4hs and 8hs) in cells infected by APEV, but not by VSV and TAHV. The TICAM and IRF3 expression levels were normalized after 8hs of infection. We also observed and 8-fold increase of IRF5 expression after 8hs of incubation with VSV. Also, VSV induced IFNb1 expression just after 4hs of infection, meanwhile TAHV induced IFNb1 increased levels only after 8hs of infection. At this time, IFNb1 expression levels in VSV-infected cells started to diminish, but remained higher than the other viruses. Finally, APEV, even after 8hs of infection, was unable to induce a significant increase of IFNb1 expression. We concluded that these viruses are able to triggers different recognition and intracellular signaling pathways leading to differences in the immune responses and, consequently, determining the pathogenic potential of each tested viruses.

859

CYCLICAL OUTBREAKS OF RIFT VALLEY FEVER IN EAST AFRICA: WHY THEY PERSIST AND POSSIBLE SOLUTIONS TO PREVENT OR CONTAIN ITS SPREAD

Marycelin M. Baba[1], Daniel Masiga[2], Jandouwe Villinger[2]

[1]University of Maiduguri, Maiduguri, Nigeria, [2]International Centre for Insect Physiology, Nairobi, Kenya

Rift Valley fever (RVF) caused by Rift Valley Fever virus (RVFV) is a zoonotic viral disease primarily of domesticated animals that also can cause disease in humans. RVFV belongs to the genus Phlebovirus, family Bunyaviridae and was first isolated during epizootics in Rift Valley Province, Kenya in 1930. RVF outbreaks have occurred in periodic cycles of 4-15 years in East Africa. During 2006-2007, an RVF outbreak that started in Kenya involving 684 cases (case fatality rate (CFR) = 49%), spread to Somalia (114 cases; CFR = 45%) and extended to Tanzania (309 cases; CFR = 46%). In 2008, the outbreak reoccurred in Sudan (698 cases; CFR = 31.8%) and Mozambique (412 cases, 17 deaths). The outbreaks have been associated with flooding from unusually high precipitations in many flood-prone habitats and with significant increases in vegetation cover. Flooding of the dambos (shallow depressions) usually induces the hatching of transovarially infected *Aedes* mosquito eggs that are dormant in the soil. The hatched eggs produce infected adult females which are capable of transmitting RVF virus to high population of domestic of animals and / or human (amplification hosts). Culex mosquitoes subsequently colonize these flooded dambos, feed on the amplifying hosts and produce large populations of infectious mosquitoes which efficiently transmit the virus to non-infected domestic animals and immunologically naïve humans within the environment. Due to these unique ecological and geographical factors involved in RVF transmission cycles, RVF outbreaks may be feasibly predicted and prevented. We review the epidemiological factors associated with outbreaks and their predictability, methods of estimating infection rate based on confirmed or suspected cases, and the importance of entomological/sero- surveys. Ultimately, we conclude that RVF outbreak prevention and outbreak impact mitigation requires timely implementation of appropriately phased activities during inter-epidemic, prediction and outbreak periods.

860

DEVELOPMENT OF A TISSUE CULTURE INFECTIOUS DOSE (TCID50) ASSAY AS METHOD FOR QUANTIFYING INFECTIOUS UNITS IN NON-CYTOPATHIC EFFECT CAUSING VIRUSES

Brian Kearney[1], Scott P. Olschner[1], Priscilla L. Williams[2], Matthew A. Voorhees[1], Cynthia A. Rossi[1], Randal J. Schoepp[1]

[1]United States Army Medical Research Institute for Infectious Diseases, Fort Detrick, MD, United States, [2]Ke'aki Technologies, Fort Detrick, MD, United States

For cytopathic viruses, the gold standard for quantitation of infectious viral particles is the plaque assay; however this method can yield inconsistent results due to variability in plaque quality and the subjective nature of plaque counting. For non-cytopathic viruses, plaque assays are not an option. We report the development of a novel tissue culture infectious dose at 50% assay (TCID50) which is simple to perform, can be utilized for both cytopathogenic and non-cytopathogenic viruses, and does not have the shortcomings of the plaque assay. The TCID50 assay is performed by adding virus dilutions to cells seeded in a 96 well plate. After viral replication in cells, a solution of Presto BlueTM dye (Life Technologies, Grand Island, NY) is added and incubated for 30 min at room temperature. The dye is enzymatically reduced in living cells proportional to the concentration of NADH and NADPH present, causing a color change proportional to cellular changes in metabolism. The results can be either visually read or spectrophotometrically measured. By monitoring of the colorimetric change and comparing this to the non-infected cells, infectious viral concentrations can be determined. Multiple cytopathic and non-cytopathic strains of Crimean Congo Hemorrhagic Fever (CCHF) virus were used to develop this technique. TCID50 assays were tested concurrently with plaque assays and ELISA antigen detection assays that correlated the change in color with the presence of antigen. The assay was also evaluated using Ebola virus, Marburg virus, Middle Eastern Respiratory Syndrome Coronavirus, and Lassa virus. The TCID50 assay developed is a useful tool for the quantitation of both cytopathic and non-cytopathic infectious viruses.

861

ARBOVIRUS SURVEILLANCE IN BATS IN UGANDA

Rebekah C. Kading[1], Robert Kityo[2], Eric Mossel[1], Jonathan Towner[3], Brian Amman[3], Tara Sealy[3], Teddie Nakayiki[4], Julian Kerbis[5], Betty Nalikka[2], Erin Borland[1], Jeremy Ledermann[1], Nicholas Panella[1], Amy Gilbert[3], Luke Nyakarahuka[4], Mary Crabtree[1], Sadic Waswa[2], Stuart Nichol[3], Julius Lutwama[4], Barry Miller[1]

[1]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [2]Makerere University, Kampala, Uganda, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Uganda Virus Research Institute, Entebbe, Uganda, [5]The Field Museum, Chicago, IL, United States

Arboviruses including Rift Valley fever (RVFV), Yellow fever (YFV), West Nile (WNV), Chikungunya (CHIKV) and Zika (ZIKV) viruses have been isolated or detected serologically from various East African bats, however the role of bats in arbovirus transmission cycles is poorly understood. The aim of this study was to investigate the exposure history of Uganda bats to arboviruses as well as attempt virus isolation from bat tissues. Blood, tissues, or both were obtained from 1067 bats from Uganda between 2009 and 2013. Liver/spleen samples were mechanically homogenized in tissue culture media and virus isolation was performed on Vero cells. Virus isolates were identified by either RT-PCR using virus group-specific primers, or next generation sequencing. Serum samples were tested for specific neutralizing antibodies against WNV, YFV, Dengue 2 (DENV2) virus, CHIKV, O'nyong-nyong virus (ONNV), Babanki virus (BABV), ZIKV and RVFV by plaque reduction neutralization test. *Rousettus aegyptiacus* from Maramagambo forest in western Uganda had specific neutralizing antibodies against CHIKV (2/303), ONNV (32/303), YFV (3/303) and WNV (1/303). *R. aegyptiacus* from Mt. Elgon in eastern Uganda also contained neutralizing antibodies against YFV (1/45). *Epomophorus labiatus* from the Entebbe/Kampala area demonstrated specific neutralizing antibodies against BABV (3/52), DENV2 (1/52), and WNV (2/52). DENV2 antibodies were also present in *Chaerephon pumila* (3/123) and *Mops condylura* (1/36) captured around Entebbe, and *Nycteris* spp. (2/10) from Mt. Elgon. One *C. pumila* also had a high neutralizing titer against ONNV. Virus isolates to date include Entebbe bat virus (*Flaviviridae: Flavivirus*) from *C. pumila* in Entebbe. Testing is still in progress and complete results will be presented. Serological and virological evidence suggest that multiple species of fruit and insectivorous bats from Uganda are exposed to and are potential amplification hosts for arboviruses.

862

OPTIMIZATION OF A RIBOSOME PROFILING-BASED PIPELINE TO MEASURE GLOBAL CHANGES IN GENE EXPRESSION IN RESPONSE TO VIRAL INFECTION

Diana S. Juarez[1], Antón Vila-Sanjurjo[2], Michael Torres[1], Mariana Leguia[1]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]University of A Coruña, A Coruña, Spain

Ribosome profiling is a new, powerful technique that enables direct measurement of protein expression at the whole cell level. The technique is based on the deep sequencing of ribosome footprints generated during nuclease digestion of extracted polisomes. During digestion, individual ribosomes protect discrete 30bp fragments (referred to as footprints) that reflect the various positions of ribosomes along all actively translated

mRNAs. Aggregation of footprint sequence data generates all the information needed to build a comprehensive understanding of how global gene expression may delineate particular phenotypes. Ribosome profiling was first developed by Ingolia et al. to run on the Illumina platform. We have adapted the original ribosome profiling strategy to run on the Ion Proton platform, and have optimized it to monitor virus-induced changes in gene-expression profiles. The main modification to the workflow includes a complete re-design of primers and adapters to include the A and P1 sequences required for Ion Proton workflows. Final library lengths were about 151pb (as opposed to 175pb for Illumina libraries) and 13pM of each library were amplified by emulsion PCR. Once amplified, libraries were sequenced using a PIv2 chip. PI chips contain 165M (million) wells. Assuming a 60-70% bead deposition it is expected that runs will generate 99-115M reads. We have achieved deposition rates of up to 93%. We routinely obtain runs of around 145M reads, which after initial quality control processing (elimination of clonal beads, etc) result in runs of about 105M usable reads. These then enter bioinformatics pipelines that make use of Botwie, Cutapad, Tophat and Bioconductor in order to specifically filter process the data and perform differential analyses of expression. To date, we have successfully adapted ribosome profiling for the study of dengue-induced changes in gene expression profiles. Further studies of a variety of infectious diseases caused by various pathogens, including bacteria and parasites, will also be possible provided minor modifications to the already established ribosome-profiling pipeline.

863

DISTRIBUTION OF INFLUENZA ANTI-VIRAL RESISTANCE IN SOUTHEAST ASIA IN 2012-13

Stefan Fernandez, Chonticha Klungthong, Butsaya Thaisomboonsuk, Prinyada Rodpradit, Duangrat Mongkolsirichaikul, In-Kyu Yoon

Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

The Department of Virology at AFRIMS conducts influenza surveillance in SE Asia. Over 400 acute respiratory specimens collected from Thailand, Bhutan, Nepal, Cambodia and the Philippines in 2012 and 2013 and found positive for influenza virus by RT-PCR were selected for genotypic anti-viral analyses by pyrosequencing. Of these, close to 200 were further analyzed using a functional cell-free neuraminidase inhibition assay to determine their oseltamivir Inhibitory Concentration (IC50). Pyrosequencing analyses found a wide-spread prevalence of genome markers associated with resistance to M2-inhibitor adamantane derivatives in pdmH1N1 and H3N2 specimens. Resistance to M2 inhibitors, associated with the S31N mutation, was present in 100% of 93 pdmH1N1 and 155 H3N2 specimens tested. However, pyrosequencing showed wide-spread susceptibility to neuraminidase inhibitors (NAI) among influenza A specimens, with 98.9% of all tested pdmH1N1 lacking the NAI resistance-associated H275Y marker. Only one pdmH1N1 specimen was found to carry the H275Y marker. Of the H3N2 specimens tested, 78% were found to be susceptible to NAI. One H3N2 specimen had the E119V NAI resistant marker. Thirty-three H3N2 specimens had indeterminate results since they showed mixed populations at the D151N mutation. Two of these H3N2 specimens also showed the E119V mutation with varying distribution. There were no NAI-associated R292K or N294S mutations among the tested H3N2 specimens. NAI-associated resistance markers were more common among the 168 influenza B specimens tested, with nearly 20% displaying the E117A and R374K resistance-associated mutation at varying degrees. Phenotypic testing of NAI resistance to oseltamivir carboxylate showed widespread susceptibility to oseltamivir, most of which correlated with lack of genotypic NAI resistance-associated markers. However, several influenza A and B specimens showed reduced susceptibility to oseltamivir (several-fold higher IC50 than negative controls), some of which did so despite lacking NAI resistance-associated genotypic markers.

864

COMPARISON OF MICROSCOPY, NESTED AND REAL-TIME PCR-BASED ASSAYS WITH HIGH-THROUGHPUT POOLED SAMPLES FOR SCREENING ASYMPTOMATIC MALARIA CARRIERS FROM ENDEMIC AREAS OF MYANMAR

Bo Wang[1], Soe-Soe Han[2], Cho Cho[2], Jin-Hee Han[1], Yang Cheng[1], Seong-Kyun Lee[1], Gawrie N. L Galappaththy[3], Thimasarn Krongthong[3], Myat Thu Soe[3], Htet Wai Oo[3], Myat Phone Kyaw[2], Eun-Taek Han[1]

[1]Department of Medical Environmental Biology and Tropical Medicine, Kangwon National University, Chuncheon, Republic of Korea, [2]Department of Medical Research (Lower Myanmar), Yangon, Myanmar, [3]World Health Organization, Yangon, Myanmar

Asymptomatic infection is an important obstacle for controlling disease in malaria-endemic countries. Because asymptomatic carriers do not seek treatment for their infection, asymptomatic carriers can have high levels of gametocytes and constitute a reservoir available for new infection. Herein, we employed a sample pooling/PCR-based molecular detection strategy for screening malaria infection in residents from endemic areas of Myanmar. Blood samples (n = 1,552) were collected from residents in three malaria-endemic areas (Kayin State, Bago and Tanintharyi regions) of Myanmar. Two nested PCR and real-time PCR assays showed that asymptomatic infection was detected in about 1.0%-9.4% of residents from surveyed areas. The sensitivities of the two nested PCR and real-time PCR techniques were higher than that of microscopy examination (100% vs. 26.4% sensitivity; kappa value = 0.2-0.5). Among the three regions, parasite-positive samples were highly detected in subjects from the Bago and Tanintharyi regions. Active surveillance of residents from regions of intense malaria transmission would reduce the risk of morbidity and mitigate transmission to the population in these endemic areas. Our data demonstrate that PCR-based molecular techniques rather than microscopy are more efficient for nationwide surveillance of malaria in endemic countries.

865

AWARENESS OF EXISTENCE OF MALARIA DIAGNOSTIC SERVICES AND PATTERN OF PRE-HOSPITAL TREATMENT, MAKARFI, NIGERIA

Olufemi O. Ajumobi[1], Godwin Ntadom[1], Yetunde Oke[2], Nnenna Ezeigwe[1]

[1]National Malaria Elimination Programme, Abuja, Nigeria, [2]President's Malaria Initiative - Malaria Action Program for States, Abuja, Nigeria

Malaria is the leading cause of childhood mortality in Nigeria. Averagely, children <5years (U5) are prone to three episodes annually. In 2005, the national malaria policy recommended Artemisinin-based combination therapy (ACT) due to established resistance to Chloroquine (CQ). It provided for the presumptive treatment of suspected malaria cases in U5. In 2011, the policy was revised to ensure parasite-based diagnosis before treatment of malaria irrespective of age. However, treatment remains largely presumptive. We conducted a hospital-based cross-sectional study in a low malaria prevalence setting to determine factors associated with awareness of existence of malaria diagnostic services (MDS) among caregivers of febrile U5 (FU5) and pattern of pre-hospital treatment practices for FU5. We interviewed consecutively selected caregivers of 295 FU5, attending Makarfi General Hospital, Kaduna state, Nigeria; from December 2010 to August 2011. We included all eligible FU5 without rash. Information on factors influencing awareness of MDS and pre-hospital treatment (PHT) was collected. We examined the Giemsa-stained blood smear of FU5 for malaria. Fifteen (5.1%) caregivers have ever heard about MDS. Eleven (3.7%) caregivers were ever offered MDS by physicians. Being formally educated (Prevalence Odds ratio (POR): 0.05, 95% Confidence Interval (CI): 0.01-0.20), living <5km from a health facility (POR: 4.21, CI: 1.39- 12.55), being a government staff (POR: 9.18, CI: 1.74- 39.93) and ever being offered MDS (POR: 35.09, CI

10.13-134.00) were positively associated with awareness of MDS. Overall, 201(67.9%) children had received any PHT, 121 children (41.0%) at patent medicine stores. Of the 31(10.5%) FU5 diagnosed with malaria and 264 (89.5%) without malaria diagnosis, 13 (41.9%) and 65 (24.6%) have had PHT with CQ respectively. Awareness of MDS remains low. Treatment of FU5 against malaria is predominantly inappropriate within the community despite widespread deployment of affordable ACTs. There is a need to sensitise caregivers and health staff on use of ACTs and adherence to confirmatory malaria diagnosis.

866

IMPROVING MALARIA DIAGNOSIS THROUGH MICROSCOPY COMPETENCY ASSESSMENT PROGRAMS IN RESOURCE LIMITED SETTINGS

Bosco B. Agaba[1], Emmanuel Yamo[2], John Nyamuni[3], Elizabeth Streat[4], Moses Kamya[5], Charles Katurebe[6], Susann Nasir[7], Belay Kassahum[8], Bryan Kapella[9], Umaru Sekabira[10], Hope Ninsiima[11], Alex Ojaku[10]

[1]Department of Disease Control, Malaria Control Program, Kampala, Uganda, [2]Africa Medical Research Foundation, Nairobi, Kenya, [3]Division of Malaria Control, Nairobi, Kenya, [4]Malaria Consortium, Kampala, Uganda, [5]Makerere University Medical School, Kampala, Uganda, [6]World Health Organization, Kampala, Uganda, [7]President's Malaria Initiative, Centers for Disease Control and Prevention, Kampala, Uganda, [8]President's Malaria Initiative, Kampala, Uganda, [9]Centers for Disease Control and Prevention, Kampala, Uganda, [10]Infectious Diseases Institute, Kampala, Uganda, [11]Mulago National Hospital, Kampala, Uganda

Malaria remains a major public health problem in Uganda. Early case detection is fundamental for the reduction of mortality and morbidity. Following the WHO recommendation for Parasitological confirmation of suspected cases, Uganda adopted this guideline with scale-up of Microscopy and Rapid Diagnostic Tests. Although microscopy-based diagnosis remains the standard, its quality is frequently inadequate to ensure good treatment outcomes and accurate epidemiological and surveillance data. Our aim in this program was to identify expert blood smear readers through WHO competency assessment scheme that could be utilized to improve Microcopy capacity in Uganda. A total of 33 experienced microscopists working as trainers and performing routine blood smear examination were selected to participate in the competency assessment. Participants were subjected to read standardized blood slides from the WHO slide bank under an "examination" environment. Scoring and grading was done for parasite detection, species identification and counting in accordance with WHO guidelines. Performance data was analyzed to generate sensitivity, specificity, level of agreement and if there was improvement at significant level of 5%.Participants were aged 20-50 years with 5 to over 10 years of experience in malaria microscopy. The mean score was 79%, 32% and 19% for parasite detection, species identification and parasite counting respectively in the baseline pretest and scores were 83%, 52% and 27% for parasite detection, species identification and parasite counting respectively in the final assessment. Performance improved significantly between pre-assessment baseline and final assessment 79% to 83 %, p=0.006 for parasite detection**,** 43% to 65%, p=0.003 for species identification and 28% to 27%, p=0.648 for parasite quantification. The Mean sensitivity at baseline and final assessment were 80% and 88.4%, p=0.314 and specificity 67.5% and 87%, p=0.817 respectively. Based on results obtained in this assessment, performance for parasite counting and species identification was below the WHO recommended levels of >50% and 90% respectively. Performance in parasite detection was better for all participants. In light of these results, we recommend that competency assessment schemes be conducted for all persons involved in microscopy training, reference expert slide readers and those involved in clinical trials and therapeutic efficacy trials.

867

C-REACTIVE PROTEIN IN DIAGNOSIS OF MALARIA IN HYPERENDEMIC RURAL AREA OF LAKE VICTORIA IN UGANDA AND TANZANIA

Gertruda Mikolasova[1], Barbora Silharova[2], Zuzana Kuranova[2], M. Franekova[3], Jose Suvada[2], Lenka Vojtasova[2], **Vladimir Krcmery**[1]

[1]Laboratory of Molecular Microbiology, Bratislava, Slovakia, [2]John Paul II Clinic, Buikwe, Buikwe, Uganda, [3]Kibara Hospital, Kibara, United Republic of Tanzania

There is increasing discussion on the role of C-reactive protein (CRP) in differentiation of malaria from other (bacterial) infections especially in regions with hyperendemic malaria. The aim of this study was to assess if CRP is confirmative to the classic microscopic diagnosis of malaria and if it helps in differentiating malaria from other infections. Altogether, 68 patients with positive blood smear for malaria from two rural hospitals (Kibara, Tanzania and Buikwe, Uganda) close to Lake Victoria in hyperendemic malaria region were assessed for CRP levels during acute phase of infections. All of the 68 patients had CRP levels measured within 48 hours of onset of symptoms when seeking medical advice. Their levels were within 8-184 mg/l. Only 7 patients (10,3%) had CRP values >100 (high level of CRP patients - HLCP) and 2 (2,9%) had even more than 200 mg/l, with suspicion of additional bacterial super-infection (e.g. meningitis). However, 33 cases (48,5%) had values below the reference level (8-10 mg/l). According to our results, CRP values can neither predict, nor exclude malaria in 51% of all microscopically positive cases which had CRP less then reference level (< 8 mg/l). Given this, also negative CRP does not exclude acute falciparum malaria, however positive CRP with levels >100 mg/l may suggest cerebral malaria or severe bacterial infection. Therefore, the yield of CRP detection in hyperendemic malaria region of great lakes in sub-Saharan Africa remains to be low.

868

LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) - EVALUATION OF A NEW KIT FOR DETECTION OF ASYMPTOMATIC LOW-DENSITY MALARIA INFECTIONS IN ZANZIBAR

Berit Aydin-Schmidt[1], WeiPing Xu[1], Iveth J. Gonzalez[2], Jackie Cook[1], Mwiniyi I. Msellem[3], Abdullah S. Ali[4], Anders Björkman[1], Andreas Mårtensson[1]

[1]Karolinska Institutet, Stockholm, Sweden, [2]Foundation for Innovative New Diagnostics, Geneva, Switzerland, [3]Zanzibar Malaria Elimination Programme, Zanzibar, United Republic of Tanzania, [4]Ministry of Health, Zanzibar, Zanzibar, United Republic of Tanzania

Loop mediated isothermal amplification (LAMP) provides an opportunity for improved, field-friendly detection of malaria infections in low-endemic settings but data on its accuracy for detection of asymptomatic low-density parasitemias in pre-elimination settings are lacking. We therefore evaluated the performance of a commercial LoopampTM MALARIA Pan/Pf detection kit (Eiken Chem., Japan) compared with PCR using DNA extracted from dried blood spots from 465 asymptomatic individuals in Zanzibar. All samples were analysed both for Pan (all *Plasmodium* species) and Pf (*P. falciparum*) specifically. The amplification was performed at 65° and results were interpreted after 40 minutes in a real-time turbidometer. A total of 49 (10.5%) and 38 (8.2%) samples were Pan and Pf-LAMP positive, respectively, whereas 54 (11.6%) were positive by PCR, i.e. 33 *P. falciparum* and 13 P. malariae mono-infections and 8 mixed *P. falciparum*/P. malariae infections (mean parasite density 10/µL, range 0-4972). The sensitivity of Pan-LAMP for *P. falciparum* mono-infections (33) was 97% (95%CI 84.2-99.9) and Pf-LAMP for all *P. falciparum* infections (33+8) was 92.7% (95%CI 80.1-98.5), respectively. The sensitivity of Pan-LAMP for P. malariae detection was 76.9% (95%CI 46.2-95) (range 0-5 p/µL). The corresponding specificities were 100% for both Pan and Pf-LAMP. The LoopampTM MALARIA Pan/Pf kit was further evaluated in a field pilot study of 1026 asymptomatic individuals in three malaria hot

spot villages in Zanzibar. Screening was done with Pan-LAMP and Rapid Diagnostic Test (RDT). LAMP was performed using a simple DNA extraction method (boil and spin) followed by LAMP reaction in a heat block and results interpreted under UV-light. LAMP detected 18 (1.8%) and RDT 10 (1,0%) infections. LAMP results were ready within two hours and positive individuals received treatment the same day. In conclusion, the LAMP kit revealed high diagnostic accuracy for detection of asymptomatic low-density parasitemias and performed well under field conditions and detected 80% more parasite carriers than RDT.

869

ARE EXIT INTERVIEWS RELIABLE? ANALYSIS OF THE HAWTHORNE EFFECT IN A STUDY OF ADHERENCE TO MALARIA TREATMENT GUIDELINES IN TANZANIA

Baptiste Leurent, Hugh Reyburn, David Schellenberg

London School of Hygiene & Tropical Medicine, London, United Kingdom

Interviewing patients exiting health facilities is a commonly used way to assess consultation practices. It is however unclear if health professionals change their practices when they are aware of such interviews taking place, possibly paying more attention to follow recommended practices. This so-called "Hawthorne effect" could have important consequences for interpreting research and for monitoring program performance, but has rarely been assessed. A cluster-randomised trial of interventions to improve adherence to national guidelines for the use of anti-malarial drugs was conducted in Tanzania. As part of the evaluation, patient interviews were conducted outside participating health care facilities on two randomly-selected days per week for a one year period. Health workers in these facilities were also routinely documenting each consultation in their ledgers. A possible Hawthorne effect was investigated by comparing routine data recorded in ledgers on days when exit interviews were conducted with data from days when no exit interviews were conducted. Routine data were collected in 34 facilities on over 38,000 consultations. No statistically significant differences were found on survey versus non-survey days on any of three pre-specified primary outcomes, after adjusting for geographic region and season. The odds of having a rapid diagnostic test (RDT) result was 7% higher on survey days (Odds Ratio 95%CI: 0.94-1.21, p=0.31), 11% lower for prescribing an anti-malarial drug to a RDT negative patient (0.70-1.14, p=0.36), and 10% lower for prescribing anti-malarial without an RDT result (0.71-1.14, p=0.38). We found no strong evidence of a Hawthorne effect in a study using exit surveys of primary care clinics with data collected from locally trained assistants. This is likely to support such methods in other studies.

870

OVER-DIAGNOSIS OF MALARIA USING A RAPID DIAGNOSTIC TEST IN A HIGH MALARIA TRANSMISSION SETTING IN UGANDA

Denise Njama-Meya[1], Pascal Magnussen[2], Anthony K. Mbonye[3], Kristian Schultz Hansen[4], Charles Karamagi[1]

[1]Clinical Epidemiology Unit, College of Health Sciences, Makerere University, Kampala, Uganda, [2]Unit for Parasitology and Aquatic Diseases, Institute for Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, [3]Uganda Ministry of Health, Health Services Commission, Department of Community Health, Kampala, Uganda, [4]Health Policy Unit, London School of Hygiene & Tropical Medicine, London, United Kingdom

The introduction of rapid diagnostic tests (RDTs) has provided a means for improving the diagnosis of malaria so as to minimize overuse of treatment and thereby delay development of resistance to ACTs. RDTs based on *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) have been rolled out across Uganda and several types of RDTs are now available, both in the public and private healthcare sectors. However, important questions remain as to whether all approved RDTs perform well compared to microscopy. The accuracy of SD Bioline Malaria Ag P.F (HRP2/PLDH) RDT was assessed in an area of high malaria transmission in Uganda. The study was conducted at Malaba Health Centre where children aged 0-12 years with a history of fever/ axillary temperature ≥37.5°C in the past 48 hours were recruited. Those with recent antimalarial use were excluded. A total of 217 febrile children were tested using the RDT and the results compared to microscopy as the gold standard. Patients were treated on the basis of the RDT results alone and follow up was done on day 3 and subsequently at 7-day intervals for 28 days. Ninety-four of the 217 patients tested had a positive blood smear for asexual forms of *P. falciparum*. An additional 45 patients tested RDT positive and received antimalarial treatment. Malaria Ag P.F (HRP2/PLDH) RDT had an overall sensitivity of 97%. However the specificity was significantly low at 63%. A negative predictive value (NPV) of 97% and a positive predictive value (PPV) of 68% were observed. A proportion of positive HRP2-based test results that were categorized as false-positive when compared with microscopy may have been due to the presence of subpatent parasitemia and thus PCR testing is being carried out. Reports on specificity of RDTs should be interpreted with caution as there may be wide variations in these measurements depending upon endemicity, season and the age group of patients studied. As RDTs become increasingly available there is a need in Uganda to recognize that 'one size does not fit all'.

871

POSITIVE CONTROL WELLS (PCW) FOR MALARIA RAPID DIAGNOSTIC TESTS (RDT): TRAINING EFFECTIVENESS, IMPACT ON RDT USE AND HEALTH WORKER PERCEPTIONS IN LAO PDR AND UGANDA

Heidi Hopkins[1], Mayfong Mayxay[2], Daniel Kyabayinze[3], Koukeo Phommasone[2], Elizabeth Streat[4], John Baptist Bwanika[4], René Umlauf[5], Simon Peter Kibira[6], Bbaale Ndawula[7], Iveth J. Gonzalez[1], Paul Newton[2], David Bell[8], Jane Cunningham[9]

[1]Foundation for Innovative New Diagnostics, Geneva, Switzerland, [2]Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit, Vientiane, Lao People's Democratic Republic, [3]Foundation for Innovative New Diagnostics, Kampala, Uganda, [4]Malaria Consortium, Kampala, Uganda, [5]University of Bayreuth, Bayreuth, Germany, [6]Makerere University, Kampala, Uganda, [7]Independent Consultant, Kampala, Uganda, [8]Intellectual Ventures Lab, Seattle, WA, United States, [9]World Health Organization, Geneva, Switzerland

Malaria rapid diagnostic tests (RDT) are widely used in health facilities and in community-based care settings in endemic countries. To maintain health worker (HW) and patient confidence in RDT and to optimize their utility, RDT must have consistently reliable results; tools to assess the quality of malaria RDT at the point-of-care are unavailable. Prototype positive control wells (PCW), plastic tubes containing critical concentrations of lyophilized recombinant antigens (HRP2, pLDH, aldolase) that are reconstituted with water, have been developed for HWs to test RDT stocks at their health facilities, to ensure RDT validity and accuracy. HWs routinely using RDTs in Lao PDR (n=269) and Uganda (n=289) underwent standardized half-day training on the use of PCWs; >70% were village health volunteers. After training, HWs were supplied with PCWs for 6 months, and recorded frequency and reason for PCW use and action taken. HW competence in PCW use was measured immediately after training and 3 and 6 months later. Data on RDT use during the study period were extracted from HW logbooks in control and intervention areas. Focus group discussions and interviews were conducted to capture HW preferences for PCW implementation as well as feasibility, acceptability and value of use. Initial analysis shows that on strict observation immediately following training, 241 (90%) participants in Lao and 244 (84%) in Uganda performed all critical PCW steps correctly; performance was generally maintained after 6 months. Most common errors were failing to fill the water dropper provided exactly to the measured mark, and failing to transfer exactly one drop of PCW solution to the RDT well. Overall, ≥91% of participants could correctly identify 'good' and 'bad' RDT and ≥89% could report appropriate action. 784 PCW were reportedly used during the study period in Lao PDR and 1679 in Uganda. The most common reasons cited for performing

PCW during routine work were receiving a new stock of RDT, and wanting to check on RDT stock quality. Initial field reports of negative RDT with PCW were not confirmed upon repeat testing. Data on RDT usage and adherence to RDT results will be available in May 2014. PCW training was effective and in general, PCW appear to improve HW confidence in RDT results.

872

PREVALENCE OF ASYMPTOMATIC MALARIA AND GLUCOSE 6 PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY IN WESTERN PROVINCE, SOLOMON ISLANDS

Diego F. Echeverri-Garcia[1], Nicholas A. Deason[1], Tanya L. Russell[2], Thomas R. Burkot[2], Frank H. Collins[3], Robert D. Cooper[4], Hugo Bugoro[5], Hedrick Reuben[5], Alby Bobogare[5], Nigel W. Beebe[6], Neil F. Lobo[1]

[1]Eck Institute for Global Health, University of Notre Dame, Notre Dame, IN, United States, [2]Faculty of Medicine, Health and Molecular Sciences, Queensland Tropical Health Alliance, James Cook University, Queensland, Australia, [3]Australian Army Malaria Institute, Enoggera, Australia, [4]Ministry of Health, Solomon Islands, Honiara, Solomon Islands, [5]University of Queensland, Queensland, Australia

The Solomon Islands has experienced a significant decrease in malaria cases over the last decade. In Central and Temotu Provinces, the estimated malaria prevalence by microscopy is up to 5% while PCR-based diagnostics have indicated local prevalences between 4 and 40%. In Temotu Province, more than 84% of the malaria-positive cases were asymptomatic, with *Plasmodium vivax* being the predominant species. The prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency in Isabel and Guadalcanal Provinces were up to 20.3%. Malaria prevalence and G6PD deficiency in other provinces is not described in the literature. The objective of this study is to determine the prevalence of malaria and G6PD deficiency in Western Province, Solomon Islands, toward baseline data for malaria intervention studies and characterizations of the province for the Solomon Islands' malaria elimination program. A total of 3,837 blood spots on filter paper were collected from 19 selected villages located in five island regions of Western Province between August and October 2013. G6PD deficiency was assessed with a NADPH-fluorescent-based diagnostic kit. A total of 2.4% (range 0-6.4% by village) and 9.3% (range 1.9-24% by village) of the population have the deficient and intermediate G6PD phenotype. The samples were then screened for malaria parasites based on amplification of the 18S rRNA gene using a direct PCR approach (sensitivity of 1.6 parasites/ul). Based on successful genus-specific PCR, the average malaria prevalence was 16% (range 5.13-44% by village). In contrast, when *Plasmodium* species-specific primers were used, the total prevalence was 5% (range 0-13% by village), with *P. vivax* accounting for 95% of cases, and 99% of malaria-positive subjects being asymptomatic. Genus-specific positives that did not amplify with species-specific primers are being sequenced to identify species. We will discuss our results in the context of the performance of malaria diagnosis PCR in areas with extremely low parasitemia and implications for the malaria elimination program in the islands.

873

ASSESSMENT OF A THREE-BAND (HRP-2/PLDH) RAPID DIAGNOSTIC TEST FOR THE IDENTIFICATION OF SEVERE MALARIA AT A PERIPHERAL HEALTH FACILITY IN WESTERN UGANDA

Ross Boyce[1], Raquel Reyes[1], Moses Ntaro[2], Edgar Mulogo[2], Michael Matte[2], Mark Siedner[1]

[1]Massachusetts General Hospital, Boston, MA, United States, [2]Mbarara University of Science and Technology, Mbarara, Uganda

The use of rapid diagnostic tests (RDTs) for the diagnosis of malaria has rapidly proliferated at peripheral health facilities in resource-limited settings where there is often no laboratory capability. In this observational study, we examined the utility of a three-band, HRP-2/*pan*-pLDH (SD Bioline FK60) RDT as a tool for the early identification of patients at risk for severe malaria. A total of 1,509 patients underwent RDTs with 637 (42%) positive for malaria. 326 (21.6%) exhibited a single HRP-2 band, 307 (20.3%) exhibited both HRP-2 and pLDH bands, while only 4 (0.3%) exhibited a single pLDH band. The rate of three-band positive results was twice as high among patients <15 years compared those ≥15 years of age (26.3% vs. 13.2%, χ^2=39.9, p<0.001). Notably, the absolute number and proportion of three-band positive RDTs declined over the study period with the transition from the dry to rainy season. The trend of declining three-band positivity in the setting of relatively stable HRP-2 positivity was significant (OR 0.51, 95% CI 0.39 to 0.66). The vast majority (92.1%) of smears from patients with three-band positive RDT results demonstrated *Plasmodium falciparum* mono-infections. The mean parasite density was approximately 42,000/µl (95% CI 6,827 to 77,349) for HRP-2 positive RDTs and 72,300/µl (95% CI 54,859 to 89,727) for three-band positive RDTs (p=0.072). The mean hemoglobin (Hb) was 8.9g/dL (95% CI 8.3 to 9.3) in patients with a negative RDT, 7.3g/dL (95% CI 6.6 to 8.1) in patients with a HRP-2 positive RDT, and 6.3g/dL (95% CI 5.6 to 7.1) in patients with a three-band positive RDTs. The difference in Hb levels between HRP-2 and three-band positive RDTs, however, was not significant (p=0.17). These results require further investigation, but suggest that a HRP-2/pLDH RDT may help identify patients with higher parasite densities and more severe anemia, both risk factors for severe malaria.

874

MALARIA IN PREGNANT WOMEN LIVING IN AREAS OF LOW TRANSMISSION OF THE SOUTHEAST BRAZILIAN COAST: MOLECULAR DIAGNOSIS AND HUMORAL IMMUNITY PROFILE

Angelica D. Hristov[1], Maria Carmen A. Sanchez[2], José Jarbas B. Ferreira[3], Giselle F. Lima[1], Juliana Inoue[1], Maria de Jesus Costa-Nascimento[4], Arianni R. Sanchez[2], Eduardo M. Ramos-Sanchez[2], Silvia M. Di Santi[1]

[1]Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, [2]Instituto de Medicina Tropical da Universidade de São Paulo, São Paulo, Brazil, [3]Secretaria de Saúde de Juquitiba, São Paulo, Brazil, [4]Superintendência de Controle de Endemias, São Paulo, Brazil

Pregnant women and children are the main groups under risk of acquiring malaria worldwide. Although *Plasmodium falciparum* in pregnant women has been widely addressed in the literature, the interaction of this cohort with *P. vivax* and *P. malariae* was poorly explored to date and requires a more comprehensive approach. In Brazil, 99% of the infections occur in the Amazon Region. Although malaria is not considered endemic outside this region, autochthonous cases are registered in areas covered by the Atlantic Forest biome. Studies related to malaria in these low transmission areas have acquired scientific and epidemiological relevance, since they suggest continued transmission and potential outbreaks. The Southeast of Brazilian coast has been focus of studies on the occurrence of *Plasmodium*, with reports of asymptomatic cases. In this region, where the transmission of *P. vivax* was established from several studies, our group detected for the first time the occurrence of *P. malariae*, using molecular tools. Data on the occurrence of the disease or presence of antiplasmodial antibodies in pregnant women living in this area of low endemicity had not been described previously. This study monitored quarterly the circulation of *Plasmodium* in pregnant women attended in five health facilities located in Juquitiba, State of São Paulo. We performed diagnosis by thick blood film and sensitive molecular protocols for parasite gDNA detection, as well immunological assays in order to investigate humoral immune parameters. For the first time *P. vivax* and *P. malariae* were detected in pregnant women living in this low endemicity area, with positivity (95% CI) of 5.6% (1.7-9.0). It was possible to detect the two species through sensitive molecular tools, once the cases were asymptomatic. We also found a high prevalence of IgG antibodies showing a significant exposure of this

population to *Plasmodium*, with 44.0% (35.6-52.7) for ELISA-*Pv* and 18.4% (12.6-26.1) for IFA-*Pm*. In regions with a similar profile presented in this study, the diagnosis of malaria might be indicated in prenatal care.

875

A QUANTITATIVE NANOPARTICLE-BASED HISTIDINE-RICH PROTEIN 2 ASSAY FOR THE MALARIA PARASITE *PLASMODIUM FALCIPARUM*

Yagahira E. Castro[1], Chloe Kim[2], Robert H. Gilman[3], David J. Sullivan[4], Peter C. Searson[2]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Institute for Nanobiotechnology, Johns Hopkins University, Baltimore, MD, United States, [3]Department of International Health, Johns Hopkins University, Bloomberg School of Hygiene and Public Health, Baltimore, MD, United States, [4]Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States

Diagnosis of severe malaria is particularly important in highly endemic regions since most of the patients are positive for parasitemia. Accurate diagnosis is increasingly important to avoid overprescribing antimalarial drugs, minimize drug resistance, and minimize costs. Microscope does not reflect the pathogenic-sequestered parasite burden. HRP2 levels associated with severe malaria are typically greater than 100 ng mL^{-1}. Rapid diagnostic tests (RDTs) are qualitative and because of that they cannot be used for diagnosis of severe malaria. Here we report on a magnetic bead - quantum dot (MBQD) assay for measurement of levels of HRP2 antigen. The assay is relatively straightforward with magnetic beads for capture and concentration of the target protein, and quantum dots for efficient quantitative detection. Magnetic beads containing surface epoxy groups were coupled to a mouse IgG monoclonal antibody anti-HRP2 (clone 3A4). Human urine samples spiked with HRP2 were incubated with antibody-bead conjugates and then analyzed by Western Blot. Western Blot shows detection of captured protein down to a concentration of 5 ng mL-1. Without concentration, the detection limit was about 100 ng mL-1, suggesting a protein concentration of about 20-fold. To demonstrate the complete sandwich assay, magnetic beads conjugated with antibody were incubated with different amounts of HRP2 spiked in serum samples. After magnetic isolation and washing, the magnetic beads were resuspended and incubated with Quantum Dots 525 coupled to the same monoclonal antibody. Using this assay, we were able to detect HRP-2 concentrations in serum as low as 1 ng per test. The correlation between intensity and HRP-2 concentration is linear at higher concentrations with a slope of 1.0 (from 1 ng to 1000 ng). Here we have demonstrated an assay for capture, concentration, and quantitative detection of HRP-2 using magnetic beads and quantum dots that can be easily adapted for point-of-care diagnostics for classification of severity of malaria caused by *Plasmodium falciparum*.

876

ADVANCED QUANTITATIVE MICROSCOPY FOR *PLASMODIUM FALCIPARUM* DIAGNOSIS DURING PFSPZ CHALLENGE AND OTHER CONTROLLED HUMAN MALARIA INFECTION STUDIES: RESULTS OF AN AFRICAN TRAINING WORKSHOP

Awalludin Sutamihardja[1], Ken Awuondo[2], Salim Abdullah[3], Stephen L. Hoffman[4], Bernards Ogutu[5], **Peter F. Billingsley**[6]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Kenya Institute of Medical Research, Nairobi, Kenya, [3]Ifakara Health Institute, Bagamoyo, United Republic of Tanzania, [4]Sanaria Inc., Rockville, MD, United States, [5]Centre for Research in Therapeutic Sciences, Strathmore University, Nairobi, Kenya, [6]Sanaria Inc., Rockville, MD, United States

Sanaria has developed aseptic, purified, cryopreserved infectious *Plasmodium falciparum* (Pf) sporozoites (SPZ) called PfSPZ Challenge as a tool for Controlled Human Malaria Infections (CHMI) to study protective efficacy of anti-malarial drugs and vaccines, to allow refinement of the method of administration of the highly protective PfSPZ Vaccine, and to study innate and acquired immunity to Pf. A critical component of the CHMI studies with PfSPZ Challenge is the diagnosis of malaria parasites in the blood. Diagnosis needs to be highly sensitive in order to detect parasites before the onset of major clinical symptoms, and needs to be highly specific in order to prevent misdiagnosis (i.e. false positive results), which could alter the outcome of the study. False positive results must be avoided in PfSPZ studies where misdiagnosis after vaccination would have potential safety implications and where misdiagnosis after CHMI would alter the estimates of protective efficacy. The European and Developing Countries Clinical Trials Partnership has funded a 7 country African consortium of institutions working with PfSPZ Challenge to optimize CHMI studies in Africa. Technical staff from across the network were hosted by the Ifakara Health Institute in Bagamoyo, Tanzania, for a 1 week course to develop expert level technical expertise in Advanced Quantitative Microscopy for rapid, sensitive, and specific diagnosis of Pf. Here we describe the results of the intensive training sessions and the subsequent establishment of a quantitative thick smear microscopy certification center at KEMRI, Nairobi, Kenya.T

877

STIMULATING A PRIVATE SECTOR MARKET FOR MALARIA RAPID DIAGNOSTIC TESTS (RDTS): BASELINE RESULTS FROM KENYA, MADAGASCAR AND TANZANIA

Stephen Poyer[1], Stephanie Dolan[1], Purity Njagi[1], Rhoune Ochako[2], Terry Muchoki[2], Jacky Raharinjatovo[3], Rova Ratsimandisa[3], Daniel Michael[3], Rachel Thompson[1], Kavita Misra[1], Furaha Kabuye[4], Nikki Charman[1]

[1]Population Services International, Nairobi, Kenya, [2]Population Services Kenya, Nairobi, Kenya, [3]Population Services International, Antananarivo, Madagascar, [4]Population Services International, Dar es salaam, United Republic of Tanzania

Key baseline results and formative insights from a multi-country project to stimulate the creation of a private sector market for malaria Rapid Diagnostic Tests (RDTs) will be presented and discussed. Since 2010 the WHO has recommended confirmatory diagnostic testing for suspected malaria, followed by treatment with ACT for positive cases. However, in the private sector, where over 40% of the population in endemic countries seeks care and treatment for febrile illness, RDTs are either non-existent or no cheaper than ACT. The UNITAID Private Sector RDT Project aims to increase both access to and demand for quality-assured RDTs, while improving private providers' fever case management skills and implementing a public-private roadmap that will guide policy and regulation. The Project is led by Population Services International, together with its collaborating implementers, Foundation for Innovative New Diagnostics, Malaria Consortium and the World Health Organization; it is being implemented in Kenya, Madagascar, Nigeria, Tanzania (mainland) and Uganda. In 2011, RDTs were available in fewer than 1 in 10 private health facilities in Kenya and Nigeria, and in Kenya the median price for a test was approximately $1.00, much more expensive than (subsidised) ACT treatments for children ($0.46). Although RDTs were available in over 90% of public facilities in Madagascar, in 2011 only 9% of private doctors and clinics had tests available. Within this context, results will be presented from the 2013 household survey of over 1,300 fever cases on the Kenyan coast, and exit interviews among private-sector fever patients in Kenya, Madagascar and Tanzania (results due July 2014). Operational insights from qualitative research with participating providers will also be shared. The project will contribute to the ongoing discussion on how best to scale-up access to affordable, quality-assured RDTs offered by trained and supervised providers who are incentivized to correctly manage febrile illness.

878

MALARIA TESTING AND TREATMENT IN TANZANIA AFTER INTRODUCTION OF RAPID DIAGNOSTIC TESTS IN ACCREDITED DRUG DISPENSING OUTLETS

Kathleen M. Maloney, Abigail Ward, Bonnie Krenz, Caitlin Dolkart, Arnaud Le Menach, Justin Cohen

Clinton Health Access Initiative, Boston, MA, United States

In Tanzania, the private sector provides 30% of all treatment for febrile illness to children under 5 years receiving it, but the availability of diagnosis is more limited than in the public sector. The reliance on presumptive malaria treatment in this sector means that many patients receive an incorrect diagnosis and inappropriate treatment. One challenge is that drug outlets comprise 70% of the private sector, but are not allowed to sell or perform malaria tests. This study assessed how patient testing and medication-purchasing behaviors evolved following a pilot study that allowed Accredited Drug Dispensing Outlets (ADDOs) in two districts to sell and perform malaria rapid diagnostic tests (RDTs). A baseline survey was completed in March 2013 to assess testing practices, RDT availability, and medication sales in ADDOs in Morogoro Region. In May 2013, dispensers in 270 intervention ADDOs were trained on how to properly stock and perform RDTs, and given access low cost RDTs. Dispensers from 91 control ADDOs were not given access to the RDTs. Over the next year, ADDO patient register books were used to evaluate the proportion of febrile patients that received an RDT and an artemisinin combination therapy (ACT). Surveys found RDTs were available in 77.5%-87.0% of ADDOs in the intervention region following training. Of 12,730 patients that sought treatment for fever or malaria in the accredited ADDOs during the nine months following training, 79.4% (95% CI: 78.7%-80.2%) elected to purchase an RDT. Of those for whom an RDT test result was recorded (n=9,872), 57.0% (95% CI: 56%-58%) tested positive, and 79.1% (95% CI: 77.9%-80.1%) of those who tested positive received an ACT. Only 3.1% (95% CI: 2.5%-3.7%) of those who tested negative purchased an ACT. In this study, introducing RDTs in ADDOs resulted in high availability and uptake of testing, as well as adherence to test results. The study highlights the potential for improving appropriate use of anti-malarials and preventing overtreatment with ACTs by placing RDTs in ADDOs and training staff in their use.

879

EVALUATION OF THE EFFECTIVENESS OF A TRAINING PROGRAM FOR SCHOOL TEACHERS IN PERFORMING AND INTERPRETING MALARIA RAPID DIAGNOSTIC TESTS SAFELY AND ACCURATELY IN ZOMBA, MALAWI

Stefan Witek-McManus[1], Austin Mtali[2], Doreen Ali[3], John H. Sande[3], Don Mathanga[4], Reuben Mwenda[5], Charles Mazinga[6], Tiyese Chimuna[7], Natalie Roschnik[8], Katherine Halliday[1], Simon Brooker[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Save the Children International, Zomba, Malawi, [3]National Malaria Control Program, Lilongwe, Malawi, [4]Malaria Alert Centre, College of Medicine, Blantyre, Malawi, [5]Health Technical Support Services-Diagnostics, Lilongwe, Malawi, [6]Ministry of Education, Science and Technology, Lilongwe, Malawi, [7]Save the Children International, Lilongwe, Malawi, [8]Save the Children International, Washington, DC, United States

With increasing levels of attendance, schools present a pragmatic opportunity to improve the access of school children to timely diagnosis and treatment of malaria, increasingly recognised as a major health problem for this age group. The expanded use of malaria rapid diagnostic tests (mRDTs) by community health workers has led to an interest in whether teachers can provide similar services for school children. We investigated the ability of school teachers to constitute safe, accurate and acceptable providers of malaria diagnosis and treatment using mRDTs and artemisinin-based combination therapies (ACTs) following training, as well as the retention of these skills during implementation of a school-based malaria case management programme. A comprehensive, skill-focused, pilot training was conducted in Zomba District, Malawi. Teachers were trained in the use of first aid kits including instruction on the principles and use of mRDTs by facilitators from the Ministry of Health. The four-day training workshop consisted of theoretical and practical sessions, with manuals and job aids adapted from a range of developed materials pre-tested with community medicine distributers and health surveillance assistants. Feedback from this pilot was used to design the full seven-day training workshop conducted prior to implementation of the intervention in schools. We present results on both the effectiveness of the pilot and full training workshops, in relation to increased knowledge and skill sets, and the retention of these through pre and post evaluation questionnaires, and checklist evaluations. Additionally, we report on the acceptability to teachers of carrying out such a role, and their confidence in providing this service, assessed through focus group discussions. To our knowledge, this is the first study in which teachers have been trained to use mRDTs. The results provide important evidence on the feasibility of using teachers to diagnose malaria using mRDTs in terms of safety, accuracy and confidence and to make appropriate treatment decisions based on the results.

880

MALARIA DIAGNOSTICS QUALITY IMPROVEMENT AND ASSURANCE PROGRAM FOR TANZANIAN MILITARY HEALTH FACILITIES

George Amoo[1], Awalludin Sutamihardja[2], Colin Ohrt[3], David Schnabel[3], Charles E. Mwanziva[4], Sarah Chiduo[2], Saidi Mgata[2], Lucky Temu[2], Lalaine Anova[3], Mark Hickman[3], Akili Kalinga[5], Adam S. Mwabulanga[4], Jack Balsinger[3], Kimberly Brown[1], Amanda Felton[1]

[1]Amethyst Technologies, LLC, Baltimore, MD, United States, [2]Walter Reed Program – Tanzania, Dar es Salaam, United Republic of Tanzania, [3]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [4]Tanzania Peoples Defense Forces, Dar es Salaam, United Republic of Tanzania, [5]National Institute for Medical Research, Tukuyu, United Republic of Tanzania

Malaria is responsible for over 100 million reported cases annually and 1-2 million deaths, especially in children. Within Tanzania, malaria diagnostics continues to be challenging, especially in resource-challenged settings. Amethyst Technologies LLC (ATL) partnered with the US Army, Tanzania People's Defense Force (TPDF), and Tanzania National Service Program (JKT) to develop and implement a malaria diagnostics quality assurance program to support the Tanzanian military's malaria measurement and control efforts at 21 TPDF/JKT training camps throughout Tanzania in 2011-2013. The approach of the strategy was to 1) develop Quality Improvement tools, which included assessment checklists (for evaluating camp health facilities), standardized laboratory SOPs, and a malaria diagnostic training course, 2) conduct baseline assessments of the 21 camps using the QI tools and provide malaria diagnostic training at all sites, 3) develop a QI and lab strengthening plan based on individual site assessments, 4) provide feedback to the health facilities and execute QI plans, and 5) execute a quality management plan to provide on-going quality improvement of malaria diagnostics. The QI assessment checklists evaluated sixteen quality criteria, including laboratory safety, human resources, personnel training, supply/stock management, results recording, results reporting, implementing of quality assurance procedures, external quality assessment (EQA), electrical supply, and staining capabilities (thick or thin smears). The quality management plan involved continuing quarterly re-assessment visits using QI tools, performing quality assurance of malaria diagnostics (by collecting malaria blood smears for cross-checking by expert microscopists), and supporting corrective actions. An overall improvement in the diagnostic services performed and strong increases in initial site assessment scores were observed, though sustaining the quality improvements proved challenging. We will present data captured from the baseline assessments and reassessments, showcase areas of success and challenges, demonstrate the utility of the QI tools, and discuss the sustainability of the program.

881

MALARIA MICROSCOPY QUALITY ASSURANCE AND SLIDE CROSSCHECKING AT RUVU JKT LABORATORY OF TANZANIA

George Amoo[1], Awalludin Sutamihardja[1], Colin Ohrt[2], Charles Mwanziva[3], Seif Shekalage[4]

[1]Amethyst Technologies LLC, Baltimore, MD, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Tanzania People Defense Forces LLC, Dar es Salaam, United Republic of Tanzania, [4]Ifakara Health Institute, Bagamoyo, United Republic of Tanzania

The purpose of this exercise was to provide malaria microscopy External Quality Assurance (EQA) at JKT Ruvu where Walter Reed Program - Tanzania (WRP-T) is conducting malaria attack rate study. This EQA consist of 3 elements of (1) On-site Evaluation to assess the performance of the laboratory against the available SOPs, (2) Proficiency testing, and (3) Slide cross-checking.This exercise was also to assess the effect of microscopy error on estimating the malaria attack rate. Overall the performace at JKT Ruvu is satisfactory on the parameter of documentation, accommodation, electrical power supply, laboratory safety, equipment, stain reagents, and training. However the performance of blood film preparation and staining need to be improved as well as re-estimating the workload. Of 583 slides that had been randomly selected, 28 (5%) were judged to be unreadable, 67 (51%) were readable with difficulty due to artefacts, 22 (4%) were of poor quality due to poor preparation, 136 (23%) had poor staining quality, and 330 (57%) were in good quality and easily readable. The technician serving as microscopist at the study site was categorized as expert malaria microscopist for reading *Plasmodium falciparum*. The sensitivity and specificity scores of the microscopist were 90% and 95% respectively. The agreement of initial results between microscopist and expert confirmation is 99.14%. Agreement on positive slides was 95% (76/80), and for negative slides 99.8% (502/503). Those disagreements are due to poor preparation and workload. In conclusion, the commonest cause of inaccurate results was the quality of the slides, correction of which is likely to be achievable within existing Standard Operating Procedures on site. An expert malaria microscopist may report inaccurate results when the workload is too high.The laboratory workload should be measured, recorded and monitored. Microscopy error (false positives, false negatives, and species identification errors) may mis-lead estimation of malaria attack rate results.

882

CLINICAL PERFORMANCE EVALUATION OF THE FYODOR URINE MALARIA TEST (UMT)

Wellington A. Oyibo[1], Nnenna Ezeigwe[2], Godwin Ntadom[2], William Brieger[3], Wendy O'Meara[4], Anne Derrick[5], Bao Lige[5], Oladosu Oladipo[1], Eddy C. Agbo[5]

[1]ANDI Centre of Excellence for Malaria Diagnosis, International Malaria Microscopy Training and RDT QA Programme and World Health Organization/TDR/FIND Malaria Specimen Bank Site, College of Medicine, University of Lagos, Lagos, Nigeria, [2]National Malaria Control Program, Federal Ministry of Health, Abuja, Nigeria, [3]Johns Hopkins University School of Public Health, Baltimore, MD, United States, [4]Duke University School of Medicine, Durham, NC, United States, [5]Fyodor Corporation, Baltimore, MD, United States

Effective case management of malaria requires prompt diagnosis and treatment within 24 hours. Despite current policy guidelines that mandate confirmed parasitological diagnosis before treatment, access to diagnostic testing remains low in sub-Saharan Africa. Today, malaria diagnosis is only by blood-tests (microscopy and rapid diagnostic tests, RDTs), which are invasive, multistep and therefore relatively complex to perform, require technical expertise, and not available in most public and private sector healthcare settings where more than 65% of the population seek care. Here, we report the results of a multicenter pivotal clinical trial of Fyodor Urine Malaria Test (UMT) - a simple (one-step, no blood, no reagents, no equipment) dipstick test that detects *Plasmodium falciparum* parasite proteins shed in the urine of febrile malaria patients. A total of 1,893 participants (≥2 years) with fever (axillary temperature ≥37.5°C) or history of fever in the last 48 hours were enrolled at 6 primary healthcare centers in rural and suburban communities in Lagos State, Nigeria, over a 7-month period that covered both rainy and dry seasons. Matched patient urine and fingerprick blood samples were tested using the UMT, Binax NOW (Inverness) (HRP-2/pLDH) test, and microscopy. A total of 358 participants (18.9%) had confirmed malaria by microscopy; Fyodor UMT, 450 (23.8%); Binax NOW (pLDH), 386 (20.4%) and Binax NOW RDT (HRP-2), 731 (38.6%). Statistical data analyses to determine test performance characteristics are ongoing and will be made available within a month. The UMT has the potential of expanding access to malaria diagnosis especially in settings where blood test is not possible.

883

MALARIA INFECTION IN CHILDREN AGED 0 TO 5 YEARS IN THE KASSENA NANKANA DISTRICT, NORTHEASTERN GHANA. A GROUP BASED TRAJECTORY ANALYSIS

Carlos Canelo-Aybar[1], Frank Atuguba[2], Kwadwo Koram[3], Timothy Awine[2], Abraham Odoru[2], Victor Asoala[2], Thomas Anyorigiya[2], Abraham Hodgson[2], Francis Nkrumah[3], David Fryauff[4]

[1]Peruvian National Institute of Health, Lima, Peru, [2]Navrongo Health Research Centre, Navrongo, Ghana, [3]Noguchi Memorial Institute of Medical Research, Accra, Ghana, [4]Naval Medical Research Center, Silver Spring, MD, United States

Information about progression of *P.falciparum* risk infection in children over time is limited. This study aimed to assess the pattern of infection events over time in a cohort of children under five years, and to explore the association to maternal factors such as education, ethnicity or preventive care behavior during pregnancy. A cohort study analysis of health surveys with on-site temperature, thick blood smear and hemoglobin measurements. Subjects were from Kassena-Nankana District of northern Ghana, who were previously enrolled at birth into a five-year prospective study. Assessment visits were done twice a year in high and low seasonal transmission periods. A Based Group Trajectory Analysis approached was used to allow identification of sub-groups over time, and a multinomial logistic regression to assess risk factors. A total of 2,107 children were recruited at birth, 4,892 events of infection were identified. Individuals belongs to three patterns of infection, a low-risk group "LoR" (47.5%) which had a prevalence of infection <20% over the whole observation, a intermediate risk "InR", ascending group (44.0%) which reached to 50%-60% of prevalence in the 3-4 years of age, and a high risk group "HiR" (8.5%) with a rapid ascending pattern reaching over 80% of prevalence by the third year of age. The risk of infection increased with less years of mother education especially in those under elementary school (InR OR 1.70: HiR: OR 3.10); and decrease with: each additional antenatal care visit (InR: OR 0.94; HiR: OR 0.80), use of bed net during pregnancy (InR: OR 0.69: HiR: OR 0.60), and receiving antimalarial drugs during pregnancy for the HiR group (OR 0.59). Populations are not homogenous in patterns of infection over time, subgroups can be identified based in social characteristics which may allow preventive health intervention in those individuals.

884

ECONOMIC EVALUATION OF INTERVENTIONS TO IMPROVE HEALTH WORKERS' PRACTICE IN DIAGNOSING AND TREATING UNCOMPLICATED MALARIA IN CAMEROON

Virginia Wiseman[1], Lindsay Mangham-Jefferies[1], Olivia Achonduh[2], Thomas Drake[1], Bonnie Cundill[1], Obinna Onwujekwe[3], Wilfred Mbacham[2]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]University of Yaoundé I, Yaounde, Cameroon, [3]University of Nigeria, Enugu, Nigeria

Malaria rapid diagnostic tests (RDTs) are a valid alternative to malaria testing with microscopy and are recommended for testing of febrile patients before prescribing an antimalarial. There is the need for interventions to support the uptake of RDTs by health workers. This study evaluates the cost-effectiveness of introducing RDTs with basic or enhanced training in health facilities where microscopy was available, compared to current practice. A three-arm cluster randomized trial was conducted in 46 facilities in Centre and North-west Cameroon. Basic training had a practical session on RDTs and lectures on malaria treatment guidelines. Enhanced training included small-group activities designed to change health workers' practice and reduce consumption of antimalarials among test-negative patients. The primary outcome was the proportion of febrile patients correctly treated: febrile patients should be tested for malaria, artemisinin combination therapy should be prescribed for confirmed cases, and no antimalarial should be prescribed for patients who are test-negative. Individual patient data were obtained from facility records and an exit survey. Costs were estimated from a societal perspective using project reports and patient exit data. Results showed that the incremental cost per febrile patient correctly treated was $8.40 for basic and $3.71 for enhanced arms. Upon scale-up it was estimated RDTs with enhanced training would save $0.75 per additional febrile patient correctly treated. Introducing RDTs with enhanced training was more cost-effective than RDTs with basic training, when each was compared to current practice.

885

IMPROVED TARGETING OF ANTIMALARIAL TREATMENT IN COMMUNITY-BASED MANAGEMENT OF MALARIA: EVIDENCE FROM CLUSTER-RANDOMIZED TRIALS IN UGANDA

Richard Ndyomugyenyi[1], Pascal Magnussen[2], Kristian S. Hansen[3], Sham Lal[4], Clare I. Chandler[4], Anthony K. Mbonye[5], **Sian E. Clarke**[4]

[1]Ministry of Health, Kampala, Uganda, [2]Centre for Medical Research, University of Copenhagen, Copenhagen, Denmark, [3]ACT Consortium, London School of Hygiene & Tropical Medicine, London, United Kingdom, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom, [5]Department of Community Health, Ministry of Health, Kampala, Uganda

Universal access to diagnostic testing for malaria is now recommended by WHO, to encompass all levels of health care, including community-based treatment programmes. Rapid diagnostic tests (RDTs) provide a simple means of confirming malaria diagnosis in locations lacking electricity and qualified staff, however data on the impact of diagnostic testing on treatment and referral practices by community health workers remains limited. A cluster-randomised trial to evaluate the impact and cost-effectiveness of RDTs in community case management was conducted in two areas with contrasting malaria transmission in Rukungiri District, Uganda. A total of 120 communities [379 community medicine distributors (CMDs)], were randomised to training either in use of RDTs or presumptive diagnosis of malaria. All CMDs were trained on how to give antimalarial treatment with ACTs, rectal artesunate pre-referral treatment, and when to refer. Supporting interventions included activities to raise community awareness, and close support supervision to CMDs for the first six months of implementation, after which supervision was scaled back to mimic levels typically seen in health systems in rural Africa. Nonetheless, adherence to RDT results by CMDs remained high, with over 95% of ACT treatments given being consistent with the RDT test results. When treatment decisions by providers were validated by expert microscopy on a reference blood slide collected at the time of consultation, the proportion of patients receiving appropriately targeted treatment was significantly higher in villages where community health workers used RDTs, compared to presumptive treatment: 79% vs 31% ($p<0.001$) and 90% vs 8% ($p<0.001$) in the high and low transmission areas respectively. Data on the impact of RDTs on referral practices will also be presented. In conclusion, diagnostic testing with RDTs in community case management can reduce over-diagnosis and substantially increase the proportion of patients receiving appropriately targeted malaria treatment.

886

DISPENSER PERFORMANCE ADMINISTERING MALARIA RAPID DIAGNOSTIC TESTS IN THE PRIVATE RETAIL SECTOR OF TANZANIA

Abigail Ward[1], Bonnie Krenz[2], Kathleen Maloney[1], Caitlin F. Dolkart[3], Arnaud Le Menach[1], Justin M. Cohen[1], Esther Mtumbuka[2], Sigsbert Mkude[4]

[1]Clinton Health Access Initiative, Boston, MA, United States, [2]Clinton Health Access Initiative, Dar es Salaam, United Republic of Tanzania, [3]Clinton Health Access Initiative, Nairobi, Kenya, [4]Tanzania National Malaria Control Program, Dar es Salaam, United Republic of Tanzania

In 2010, the World Health Organization (WHO) recommended that all suspected malaria cases be tested. In Tanzania, the low confirmatory diagnosis rate (approximately 25% for children under 5) means that many febrile cases receive clinical diagnoses and potentially inappropriate treatment. While approximately 30% of febrile cases seek treatment in the private sector, the informal shops comprising 70% of this sector are not allowed to stock or perform malaria tests. We evaluated whether the gap in accessibility to testing could be addressed by allowing Accredited Drug Dispensing Outlet (ADDO) shop dispensers to sell and perform malaria rapid diagnostic tests (RDTs). Dispensers from 270 ADDOs were trained on RDT administration and safety in Morogoro Region in May 2013. If they passed the training, they were certified and allowed to purchase RDTs at a negotiated low or subsidized price. Certified dispensers were monitored quarterly from May 2013 to 2014. RDT safety, administration, and interpretation were assessed using a list of indicators adapted from the checklist published by the WHO in "Universal Access to Malaria Diagnostic Testing: an Operational Manual". Data on ADDOs' safety and hygiene were also collected. RDTs were stocked in 87% of enrolled shops at the first and second visits and 78% of shops at the third visit ($p=0.006$ for change in stocking across surveys). At least 83% of dispensers performed an RDT correctly on all of the 17 indicators. More than 98% of dispensers correctly interpreted results at each visit. Regarding ADDO's safety and hygiene practices, over 95% of ADDOs kept the area around the shop clean and free of used RDT products across surveys. However, the fraction performing the test in a private area decreased between the first and third visits from 31% to 19% ($p<0.001$ for change across surveys). Following training, ADDO dispensers competently stocked and safely administered RDTs, demonstrating that placing RDTs in certified private shops may be a feasible solution to increase malaria diagnostic access.

887

FATTY ACID SYNTHESIS AND PYRUVATE METABOLISM PATHWAYS REMAIN ACTIVE IN DIHYDROARTEMISININ INDUCED DORMANT RING STAGES OF *PLASMODIUM FALCIPARUM*

Nanhua Chen[1], Alexis N. LaCrue[2], Franka Teuscher[1], Norman C. Waters[3], Michelle L. Gatton[4], Dennis E. Kyle[2], **Qin Cheng**[1]

[1]Australian Army Malaria Institute, Brisbane, Australia, [2]University of South Florida, Tampa, FL, United States, [3]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [4]Queensland University of Technology, Brisbane, Australia

Artemisinin (ART) based combination therapy (ACT) is used as the first line treatment of uncomplicated falciparum malaria worldwide. However, despite high potency and rapid action there is a high rate of recrudescence associated with ART monotherapy and recrudescence is not uncommon even when ACT is used. This is independent of the recent observation of ART resistance. ART induced ring stage dormancy and recovery has been implicated as possible cause of recrudescence; however, little is known about the characteristics of dormant parasites including whether dormant parasites are metabolically active. We investigated the transcription of 12 genes encoding key enzymes in various metabolic pathways in *Plasmodium falciparum* during dihydroartemisinin (DHA) induced dormancy and recovery. Transcription analysis showed an immediate down regulation for 10 genes following exposure to DHA, but continued transcription of 2 genes in apicoplast and mitochondria. Transcription of several additional genes in apicoplast and mitochondria, particularly genes encoding enzymes in pyruvate metabolism and fatty acid synthesis pathways, were also maintained. Additions of inhibitors for biotin acetyl CoA carbozylase and enoyl-acyl carrier reductase of the fatty acid synthesis pathways delayed the recovery of dormant parasites by 6 and 4 days, respectively following DHA treatment. Our results demonstrate most metabolism is down regulated in DHA induced dormant parasites. In contrast fatty acid and pyruvate metabolic pathways remain active. These findings highlight new targets to interrupt recovery of parasites from ART-induced dormancy and to reduce the rate of recrudescence following ART treatment.

888

EFFECT OF TRANSMISSION SETTING ON THE SULFADOXINE-PYRIMETHAMINE RESISTANT HAPLOTYPES AND SELECTIVE SWEEP CHARACTERISTICS IN MALAWI

Elena M. Artimovich[1], Atupele Kapito-tembo[2], Karl Seyde[3], Paul Pensulo[2], Sudhaunshu Joshi[2], Sarah Brown[4], Osward Nyirenda[2], Don Mathanga[2], Terrie Taylor[3], Miriam K. Laufer[1], Shannon Takala-Harrison[1]

[1]University of Maryland Baltimore, Baltimore, MD, United States, [2]Malaria Alert Centre, University of Malawi College of Medicine, Blantyre, Malawi, [3]Blantyre Malaria Project, University of Malawi College of Medicine, Blantyre, Malawi, [4]University of Maryland Baltimore County, Baltimore, MD, United States

The decline of antimalarial resistant parasites, and the re- expansion of drug sensitive parasites, after the removal of drug pressure, raises the possibility that previously abandoned drugs might once again find clinical utility. Here we investigate the role of transmission setting on the dynamics of sulfadoxine-pyrimethamine (SP) resistance alleles and the characteristics of their associated selective sweeps. High transmission settings are associated with more clinically immune hosts who may serve as a reservoir for drug-sensitive parasites. High transmission intensity leads to higher parasite recombination, with greater genetic diversity. Samples from patients who presented to health care facilities and were found to have malaria were collected from three transmission sites in Malawi: urban (Ndirande), rural-high transmission (Chikwawa), and rural-low transmission (Thyolo). Pyrosequencing was used to determine the predominant haplotypes of dhfr and dhps in each infection. A ~96% prevalence of dhfr 51I/59R/108N, and ~96% prevalence of dhps 437G/540E was found at all three sites. There was no significant difference in haplotype prevalence found between any of the transmission settings, regardless of season. A single SP-sensitive parasite was found in Thyolo. Neutral and flanking microsatellite analysis was used to calculate expected heterozygosity (He) and estimate diversity ratios between the three sites. At markers flanking dhfr and dhps significant differences in He were found between Thyolo and Chikwawa and between Thyolo and Ndirande, however no significant difference in diversity ratios was found between Ndirande and Chikwawa for either dhfr or dhps. Our data indicate that the differences in transmission between these sites, given the high level of SP resistance, were not sufficient to effect change in haplotype prevalence. Differences in sweep characteristics between Thyolo and the other transmission settings will be pursued in future analyses..

889

CHANGES IN OCCURRENCE OF DRUG RESISTANCE POLYMORPHISMS TO SULPHADOXINE - PYRIMETHAMINE IN THE *PLASMODIUM FALCIPARUM* IN SOUTHERN ZAMBIA

Saidon Mbambara, Mwiche Siame, Sungano Mharakurwa

Macha Research Trust, Choma, Zambia

Malaria remains one of the major global diseases affecting humans and drug resistance is a major concern for effective treatment. In Zambia, a change from Sulphadoxine Pyrimethamine to artemether lumefantrine as first line treatment for malaria was made in 2004 due to evidence of increasing levels of resistant parasites. However, it is still used for intermittent preventative treatment in pregnancy. Mutations in the dhfr and dhps genes are associated with resistance to pyrimethamine and sulphadoxine. *Plasmodium falciparum* antifolate drug resistance polymorphisms are detected using nested PCR and restriction enzyme digestion. This study aimed to determine and compare the prevelance of dhfr and dhps polymorphisms in a low endemic area at two time points 2008-2009 and 2012-2013. Finger-pricked blood samples (Dried Blood Spots) were collected on filter paper from 993 consenting participants from communities in Macha, Choma District between 2008 and 2009, and 1303 consenting participants between 2012 and 2013. Parasite DNA was extracted and a nested PCR run on these samples from which the positives were genotyped for dhfr and dhps mutations. Restriction enzyme digestion was done on the positives and restriction fragments analysed by gel electrophoresis and visualized under UV transillumination. The number of individuals with *P. falciparum* resistance mutations in the dhfr gene and dhps was 11 for 2008 and 2009 and 5 for 2012 and 2013 respectively. Malaria infections in Southern Zambia have declined due to combined interventions and efforts. The prevalence of mutations in the dhfr and dhps genes has decreased from 2009 to 2013, resistance alleles are still present in the general population. The impact of resistant parasites on the efficacy of SP for IPTp needs to be assessed.

890

FORWARD GENETIC CHEMICAL PROFILING OF PIGGYBAC MUTANTS OF *PLASMODIUM FALCIPARUM* REVEALS NEW DRUG TARGETS AND INSIGHTS INTO MECHANISMS OF RESISTANCE TO ARTEMISININ

Anupam Pradhan[1], Geoffrey Siwo[2], Naresh Singh[1], Brian Martens[1], Bharath Balu[1], Asako Tan[2], Katrina A. Button-Simons[2], Michael T. Ferdig[2], John H. Adams[1], **Dennis E. Kyle**[1]

[1]University of South Florida, Tampa, FL, United States, [2]University of Notre Dame, South Bend, IN, United States

The emergence and spread of *Plasmodium falciparum* multidrug resistance highlights the urgency to discover new targets and chemical scaffolds with reduced potential for emergence of resistance. Achievement of this goal will be enhanced by developing a better understanding of mechanisms of action and resistance of available drugs and inhibitors on vulnerable metabolic pathways. In this study we conducted a novel forward genetics, chemical profiling screen of 80 *P. falciparum* piggyBac (pB) mutants to

identify, validate and prioritize anti-malarial drugs, targets, and resistance mechanisms. Mutants carrying a single transposon insertion were profiled for altered responses to standard antimalarial drugs and inhibitors of known metabolic pathways. The results of the screen provide proof of concept and yield new insights into mechanism of action and resistance. For example we found that drugs targeting the same pathway have a significantly higher connectivity to each other than to drugs that inhibit other pathways. In addition we made novel observations about important drug resistance mechanisms. One of the mutants profiled in our study contains an insertion in the intergenic region between the kelch protein 13 (K13) gene implicated in artemisinin resistance (PF3D7_1343700) and a gene encoding a conserved protein of unknown function (PF3D7_134800). This mutant exhibited 2-7 fold increased susceptibility to artemisinin drugs and was one of 10 pB mutants that based upon hierarchical cluster analysis had similar enhanced responses to artemisinin and other inhibitors. We demonstrate that K13 and 3 other genes (PF3D7_0727100, PF3D7_0619800 and PF3D7_1126100) in its chemogenetic cluster are tightly linked in co-expression networks. This implies that these genes are functionally related to K13 and mediate *in vitro* artemisinin response. Our data demonstrate that chemical-genetic profiles can reveal unexpected drug relationships and connect them to gene functions, including hypothetical genes in the malaria parasite.

891

NOVEL K13 PROPELLER (KELCH PROPELLER) MUTATIONS IN BANGLADESHI *PLASMODIUM FALCIPARUM* CLINICAL ISOLATES

Abu N. Mohon[1], Mohammad S. Alam[2], Asongna Folefoc[1], Rashidul Haque[2], Dylan R. Pillai[1]

[1]University of Calgary, Calgary, AB, Canada, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

The K13 propeller protein of *Plasmodium falciparum* is associated with artemisinin resistance. K13 propeller is a kelch motif containing protein, related to KEAP1 protein of its human host. Its functional role in artemesinin resistance is unknown. On the other hand, few clinical studies carried out in Bangladesh have not concluded any case of artemisinin resistance. In the current study, *Plasmodium falciparum* positive blood samples (n=238) were collected from seven endemic districts of Khagrachari (n=131), Rangamati (n=3), Cox's Bazar (n=68), Bandarban (n=14), Mymensingh (n=2), Netrokona (n=9) and Moulvibazar (n=11) in Bangladesh. K13 was bidirectionally sequenced. ClustalW and Jalview analysis have revealed five different mutations present in these clinical isolates. Two of these were synonymous mutations originating from Mymansingh and Rangamati. A578S mutation was found in two different samples collected from Khagrachari district and W470C and Y604H were found in Rangamati. Mutations observed in this study are different to those reported from Cambodia. W470C and Y604H mutation are located in the highly conserved β-sheet structure. These mutations may alter the integrity of the sheet. A578S is located in the loop structure close to the C580Y mutation - the major one in Cambodia associated with treatment failure and resistance. We report several novel mutations in Bangladesh in the K13 gene associated with artemisinin resistance. Further clinical studies are required to confirm any relationship to delayed parasite clearance

892

RAPID TURNOVER OF *PVMDR1* HAPLOTYPES IN *PLASMODIUM VIVAX* POPULATIONS

Larissa Rodrigues-Gomes, Natatia K. Oliveira, Suelen R. Lima, Aline Lavigne, Patricia Brasil, Claudio T. Daniel-Ribeiro, Maria de Fátima Ferreira-da-Cruz

Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

Plasmodium vivax is the most widely distributed malaria parasite and causes a serious public health burden. The emergence of chloroquine (CQ) *P. vivax* resistance - the first line treatment in most of the world - represents a hurdle to malaria control. Due to difficulties to *in vitro P. vivax* culture systems, a data base of the mutations in target genes could help to set a baseline for CQ drug-resistance surveillance. For this purpose, 97 *P.vivax* blood samples collected from patients attending for PCR diagnosis at the Brazilian Ministry of Health Extra-Amazonian Malaria Reference Laboratory, between 2010 and 2013, were direct DNA sequencing from PCR products containing *pvmdr1* Y976F and F1076L SNPs. We observed that, between 2010 and 2012, the great majority of the 64 samples tested presented a single mutation, showing the FF (62 / 97%) or the FL (1 / 1.5%) profiles, while only one sample presented the FL double mutant (1.5%). Interestingly, this pattern of haplotypes inversely changed when samples collected in 2013, where analyzed: in this case all the samples presented double mutants showing the FL profile, and single mutations were not detected anymore. Thus, this first report showing the turnover of *pvmdr1* haplotypes in *P. vivax* parasites could supply a baseline to monitor *P. vivax* CQ-resistance. The turnover of *P. vivax* parasites may reflect the introduction of new parasites carrying *pvmdr1* alleles associated to drug-resistance by mosquitoes. To explain this finding additional studies on molecular epidemiology are required.

893

EFFICACY OF ARTEMETHER-LUMEFANTRINE AND AMODIAQUINE-ARTESUNATE FOR TREATMENT OF UNCOMPLICATED MALARIA IN CHILDREN: RANDOMIZED CLINICAL TRIAL AT THREE SITES IN UGANDA

Yeka Adoke[1], Ruth Kigozi[2], Myers Lugemwa[3], Peter Okui[3], Kassahun Belay[4], Bryan K. Kapella[5], Michelle Chang[5], Steven S. Yoon[5], Sarah G. Staedke[6], Charles Katureebe[7], Philip J. Rosenthal[8], Grant Dorsey[8], Moses R. Kamya[9]

[1]Makerere University School of Public Health, Kampala, Uganda, [2]Infectious Diseases Research Collaboration Uganda, Kampala, Uganda, [3]Uganda Ministry of Health, Kampala, Uganda, [4]United States Agency for International Development, Kampala, Uganda, [5]Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States, [6]London School of Hygiene & Tropical Medicine, London, United Kingdom, [7]World Health Organization, Kampala, Uganda, [8]University of California San Francisco, CA, United States, [9]Department of Medicine, Makerere University College of Health Sciences, Kampala, Uganda

Plasmodium falciparum resistance to artemisinin derivatives is emerging in Southeast Asia, and resistance to artemisinin partner drugs may be increasing. In Uganda, artemether-lumefantrine (AL) is the first-line therapy and artesunate-amodiaquine (AS/AQ) the alternative for the treatment of uncomplicated malaria. We are conducting a randomized, single-blinded trial comparing AL and AS/AQ to treat uncomplicated falciparum malaria at 3 sites in Uganda with different transmission intensities and a range of malaria intervention coverage. A total of 600 children aged 6-59 months will be enrolled (100 per treatment arm per site), randomized to treatment with AL or AS/AQ, and followed for 28 days. The primary outcome is the risk of treatment failure unadjusted and adjusted by genotyping at day 28. Recruitment and follow-up have been completed at two sites (Apac and Mubende), and are ongoing at the third site. No serious adverse events have been reported to date. Preliminary results show no early treatment failures. The uncorrected 28-day risk of treatment failure was significantly lower for children treated with AS/AQ than for those treated with AL at Apac (15.0% vs 31.2%; p = 0.008) and Mubende (33.6% vs 53.7%; p = 0.003). Two recrudescences were observed in Apac in the AL treatment arm compared to none in the AS/AQ arm (corrected: 2.1% vs. 0%; p = 0.16). Parasite clearance was rapid at Apac (37.0 vs. 37.6 hours; p = 0.74) and Mubende (42.6 vs. 39.4 hours; p = 0.06) in AS/AQ and AL treatment arms, respectively. Our results show that the corrected treatment success rates were not different and were very high for both AS/QA and AL. However, AS/AQ appeared to have a better prophylactic effect.

894

PLASMODIUM FALCIPARUM GENOTYPES FROM GAMBIAN CHILDREN WHO FAILED TREATMENT WITH ARTEMETHER-LUMEFANTRINE; RECRUDESCENCE OR RE-INFECTIONS?

Joseph Okebe[1], Fatima Elfitouri[2], Haddy Nyang[1], Alfred Ngwa[1], Umberto D'Alessandro[1]

[1]Medical Research Council Unit, Banjul, Gambia, [2]University of Manchester, Manchester, United Kingdom

The growing risk of resistance to artemisinin derivatives calls for regular monitoring of the efficacy of artemisinin-based combination therapy locally used in endemic countries. Artemether-lumefantrine, the first line ACT in the Gambia shows high but variable efficacy in sites across the country. We conducted a 28-day follow up study to investigate the consistency of this variability in public health facilities in the country. DNA was extracted from filter paper blood spots of eight participants with treatment failures (by microscopy) using the QIAmp DNA blood kit (Qiagen) with DNA concentrations determined on the nanodrop. Each isolate was genotyped by PCR amplification and analysis of MSP1 and MSP2 gene loci and amplified products separated by capillary electrophoresis. Band sizes were determined against a 50-800bp DNA ladder. Isolates were further analysed in duplicates in two sequential assays by MSP1 & MSP2 amplification with Dye labelled primers and fragment analysis employing the GenScan1200*LIZ* size marker for size calling. Fragment size analysis was done with GeneMarker software (Sofgenetic). PCR band and fragment sizes were compared between consecutive PCR runs and scored according to WHO/WWARN prescriptions for distinguishing recrudescence from re-infection. This is based on the presence of at least one PCR band with identical size between samples from different time points and band sizes scored to be similar within a sensitivity margin of ±5 basepairs. Five of the eight samples analysed showed presence of identical-size PCR fragments for MSP1 and MSP2 either by Qiaxcel capillary electrophoresis, Fluorescent based fragment analysis or both. Two samples did not yield *Plasmodium* specific amplification products for the Post Day 0 samples and hence remain undetermined, and one sample was likely a re-infection as all loci analysed between Day 0 and subsequent timepoints were different. Further analysis will help verify the genetic identities of the five samples that indicated possible treatment failure.

895

THERAPEUTIC EFFICACY OF DIHYDROARTEMISININ-PIPERAQUINE FOR TREATING UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA AND K13-PROPELLER POLYMORPHISMS ALONG THE CHINA-MYANMAR BORDER

Fang Huang[1], Shannon Takala-Harrison[1], Christopher G. Jacob[2], Matthew Adams[1], Hui Liu[3], Henglin Yang[3], Linhua Tang[4], Xiaonong Zhou[4], Pascal Ringwald[5], Christopher V. Plowe[1]

[1]Howard Hughes Medical Institute/Center for Vaccine Development/ University of Maryland School of Medicine, Baltimore, MD, United States, [2]University of Maryland School of Medicine, Baltimore, MD, United States, [3]Yunnan Institute of Parasitic Diseases, Puer, China, [4]National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai, China, [5]World Health Organization, Geneva, Switzerland

The World Health Organization (WHO) recommends Artemisinin-based Combination Therapy (ACT) for treating uncomplicated *Plasmodium falciparum* malaria, but resistance to artemisinin derivatives in Southeast Asia threatens malaria control and elimination activities worldwide. Mutations within a *P. falciparum* kelch protein, K13, are associated with both *in vitro* and clinical measures of artemisinin-resistant malaria. Validation and mapping of this marker throughout the malaria endemic world will be helpful to track the emergence and spread of artemisinin resistance. Yunnan, China's only province with endemic *P. falciparum* malaria transmission, borders Myanmar, Vietnam and Laos, and is the key focus of the national malaria elimination program. Patients from two sites (Yingjiang and Tengchong) bordering Myanmar received antimalarial treatment with either dihydroartemisinin-piperaquine or artesunate by directly observed therapy as part of therapeutic efficacy studies conducted from 2009 until the present. After a 28 or 42-day follow-up, treatment efficacy was estimated according to the WHO protocol for assessing and monitoring antimalarial drug efficacy. The *P. falciparum* K13 gene was sequenced in samples collected from approximately400 patients including almost 120 clinical trial participants, using Sanger sequencing. The prevalence of K13 mutations was estimated by study site and year. Linear regression was used to assess the association between K13 mutations and parasite clearance half-life, while adjusting for confounding variables. Haplotype networks of SNPs surrounding the K13 gene were used to assess origins of K13 mutations. Preliminary results indicate a high prevalence (34%) of the K13 F446I mutation, which was significantly associated with the presence of parasitemia 72 hours after treatment. These results suggest that K13 mutations are responsible for artemisinin resistance in Yunnan Province, China, although the predominant K13 mutation is different than in other areas of Southeast Asia, suggesting independent emergence rather than spread of resistance.

896

A BETWEEN-HOSTS AND WITHIN-HOST COMBINED MODELING FRAMEWORK FOR THE EVOLUTION OF RESISTANCE TO ANTIMALARIAL DRUGS

Mathieu Legros, Sebastian Bonhoeffer

ETH Zurich, Zurich, Switzerland

The emergence and spread of drug resistance in *Plasmodium* parasites has long been and is still a significant problem for chemotherapeutic approaches to malaria control. Evolution of drug resistance in mosquito-borne parasites is a complex process, influenced by transmission dynamics between hosts and vectors as well as within-hosts competition among parasite strains. We present here a theoretical model of the evolution of drug resistance that combines both between-host and within-hosts scales, aiming to understand how the dynamics at each level and the interaction between scales influences drug resistance evolution. Our model combines an epidemiological model of malaria transmission between hosts and vectors and a within-host model of the course of infection for multiple competing strains. The latter includes the effects of immunity, treatment and cost of resistance. The epidemiological level can reflect various epidemiological settings and transmission intensities. The model shows that in high transmission environments, where co-circulation of sensitive and resistant strains is more frequent, resistant strains are less likely to spread, particularly when within-host costs of resistance are high. We illustrate how treatment impacts the spread of resistance, and show a general trade-off between disease prevalence reduction and resistance management. We show however that treatment coverage has a stronger impact on disease prevalence, whereas treatment efficacy primarily affects resistance control. We conclude therefore that a primary focus on coverage over efficacy would have the strongest impact on disease control while minimizing selection for resistance. More generally we underline the importance of modeling the evolution of drug resistance across biological scales for a better understanding of the evolutionary dynamics in a variety of eco-epidemiological settings, providing valuable insights for both disease control and drug resistance management.

897

EFFICACY, SAFETY AND PHARMACOKINETICS OF ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN HIV-POSITIVE ADULTS RECEIVING FIRST-LINE ANTIRETROVIRALS IN MUHEZA, TANZANIA

Lasse S. Vestergaard[1], Nyagonde Nyagonde[2], Trinh Duong[3], Bonnie Cundill[3], Filbert Francis[2], Karen Barnes[4], Michael Alifrangis[1], Martha Lemnge[2], Ib Bygbjerg[1]

[1]University of Copenhagen, Copenhagen, Denmark, [2]National Institute for Medical Research, Tanga, United Republic of Tanzania, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom, [4]University of Cape Town, Cape Town, South Africa

Although there is concern of drug interactions between artemisinin-based combination treatment (ACTs) for malaria and antiretrovirals (ARVs) for treating HIV/AIDS, very limited information is available about the clinical importance of such interactions. The aim of this study was to examine the efficacy and safety of artemethemter-lumefantrine (AL), the most commonly used ACT, for the treatment of uncomplicated falciparum-malaria in HIV-positive adults recieving first-line ARVs in Tanzania. The InterACT Study was conducted from July 2009 to September 2012 at Muheza District Hospital in northern Tanzania. HIV-positive adults (>15 years of age) receiving either nevirapine- or efavirenz-based ARVs were enrolled and followed-up for 42 days using WHO standard protocols. Three additional groups of patients were included for comparison: 1) HIV-positive malaria patients not receiving ARVs but treated with AL, 2) HIV-negative malaria patients treated with AL, and 3) HIV-positive patients receiving ARVs but without malaria. Blood levels of lumefantrine were measured on day 7 in all patients receiving AL. A total of 17,269 patients were screened for malaria, amongst whom 385 HIV-positive patients with confirmed malaria were enrolled into the study and followed-up successfully for 42 days. The therapeutic efficacy of AL after parasite PCR-correction was 99% in HIV-positive patients receiving ARVs (total n=193; 106 on nevirapine, 87 on efavirenz), 100% in HIV-positive patients not on ARVs (n=43) and 98% in HIV-negative patients (n=149). Rates of malaria re-infection within 42 days of AL treatment was low and did not differ significantly between the groups. Mild adverse events were commonly recorded in all four patient groups. Severe adverse events were more commonly observed in HIV-positive versus HIV-negative patients, regardless of receiving ARV treatment or not. Day 7 levels of lumefantrine were found to be elevated in patients receiving nevirapine, but were reduced in patients receiving efavirenz. Our results confirm the presence of drug interactions between AL and nevirapine- and efavirenz-based ARVs; however, these interactions were not found to be clinically significant. Our findings thus support the current treatment guidelines for malaria and HIV co-infection in adults.

898

VALIDATION OF A TOOL FOR PREDICTING ANTI-MALARIAL DRUG MECHANISM OF ACTION

Katrina A. Button-Simons[1], Geoffrey Siwo[1], Elizabeth Heilmann[1], Min Zhang[2], John H. Adams[2], Michael T. Ferdig[1]

[1]Eck Institute for Global Health, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, United States, [2]Department of Global Health, University of South Florida, Tampa, FL, United States

Malaria mortality has decreased over the last decade, but these gains are threatened by the emergence of drug resistance to artemisinin in South East Asia. There remains an urgent need to develop new classes of effective anti-malarial drugs. High-throughput screens have identified many drugs and drug-like compounds with anti-malarial activity. However, the mechanisms of action of these potential new drugs as well as for many established anti-malarial drugs is unknown or poorly understood. Systems biology tools that could predict the mechanisms of action of candidate drugs would be valuable for prioritizing drugs for further development. Work in our lab indicates that drug perturbations of *Plasmodium falciparum* provoke discernible transcriptional signals. These signals contain information about the pathway(s) that a drug targets and can be used to relate drugs by the extent to which their targets overlap. To validate and expand on our capacity to detect subtle drug-specific response signatures in the face of broad biological and experimental variation, transcription profiles were generated for parasites in which the purported pathway targets are genetically disrupted by *piggyBac* transposon insertions. Transcriptional profiles of a drug-perturbed parent line were compared to lines carrying a genetic perturbation of a related target. We find that genes that are differentially expressed in each genetic perturbation relative to the wild-type control are significantly enriched for biological pathways related to the perturbed genes. Transcriptional signals obtained from drug perturbations of a given pathway overlap with signals observed in genetic perturbations of the same pathway, indicating that a genome wide effort to catalog and relate drug target(s) by their transcriptional response profiles is feasible.

899

REFINING A TOOL TO PREDICT ANTIMALARIAL DRUG MECHANISMS OF ACTION

Elizabeth S. Heilmann, Roger S. Smith, Katrina A. Button-Simons, Geoffrey H. Siwo, Michael T. Ferdig

Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, United States

High-throughput screens have identified many drugs and small molecules with antimalarial activity, usually with no knowledge of their molecular targets. Cost effective prioritization of these potential drugs would be valuable to avoid pathways that have already developed drug resistance and to highlight compounds with novel mechanisms of action. We measured gene expression profiles in *Plasmodium falciparum* to construct a transcriptional response database for 31 drug perturbations. New drugs of interest can be queried against these gene expression patterns, identifying shared targets by similar response signatures. So far, successfully identifying drug-specific response signals has required that each gene's value be normalized across many drug perturbations from the same experiment to compute a drug's specific 'response index' from myriad other experimental and biological sources of transcription variation. However, this is a cumbersome task, and we are therefore exploring modifications to our approach that minimize experimental complexity while still filtering out nonspecific responses. We find that conventional, one drug vs control experiments with replication do not allow for normalization of nonspecific drug responses; consequently, we developed new protocols that can leverage existing data to specify a generalized stress response signature to be used as a standard normalization with each candidate drug. Deeper analysis of the 31 drug response gene expression profiles identified a small subset of drugs with highly diverse mechanisms of action. Normalization with this panel allowed removal of nonspecific culture and perturbation stress and accurate prediction of drug mechanism of action. By optimizing this analysis, we hope to build a standardized community web-based search tool for predicting drug mechanism of action.

900

PREVALENCE OF THE DHFR AND DHPS MUTATIONS AMONG PREGNANT WOMEN IN RURAL BURKINA FASO SEVEN YEARS AFTER THE INTRODUCTION OF INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE

Marc C. Tahita[1], Halidou Tinto[1], Robert Fitzhenry[2], Chantal Van Overmeir[2], Adama Kazienga[1], Diadier A. Diallo[3], Anna Rosanas[2], Jean-Pierre Van Geertruyden[4], Annette Erhart[2], Umberto D'Alessandro[5]

[1]Clinical Research Unit of Nanoro, Ouagadougou, Burkina Faso, [2]Institute of Tropical Medicine, Antwerp, Belgium, [3]London School of Hygiene & Tropical Medicine, United Kingdom (now at PATH Malaria Vaccine Initiative), Dakar, Senegal, [4]International Health Unit, University of Antwerp, Antwerp, Belgium, [5]Disease Control and Elimination Theme, Medical Research Council Unit, Fajara, Gambia

The spread of drug resistance is one of the major challenges for malaria control in endemic areas. Intermittent Preventive Treatment of malaria in pregnancy (IPTp) with Sulfadoxine-pyrimethamine (SP) is currently recommended by the World Health Organization for preventing the adverse effects of malaria during pregnancy in mothers and their offsprings. In order to assess the evolution of SP resistance in Burkina Faso, we analyzed the prevalence of dihydropteroate synthase (dhps) and dihydrofolate reductase (dhfr) mutations among pregnant women in samples collected in 2010 and compared our results with retrospective data from 2003. Asymptomatic and symptomatic pregnant women attending Antenal Clinics (ANC) in Nanoro District were invited to participate in the study. All enrolled women were interviewed and examined clinically before taking a blood smear for microscopy examination and a filter paper blood sample for genotyping. Mutations at codons 51, 59, 108 and 164 of the Pfdhfr gene, and at codons 437 and 540 of the Pfdhps gene were examined using PCR-RFLP. Logistic regression was used to calculate odds ratios and confidence intervals for the comparison between 2003 and 2010. The dhfr and dhps genes were successfully genotyped in most samples, respectively 99.6% (255/256) and 90.2% (231/256). The dhfr C59R mutation was the most prevalent (61.2%=156/255) followed by the S108N mutation (55.7%=142/255). No isolate had the I164L mutation. For the Pfdhps gene more than one third of the samples had the A437G mutation (34.2%=79/231), while none carried the K540E mutation. The dhfr double and triple mutations were found in 36.5% and 11.4% of the isolates, respectively. Compared to 2003, the prevalence of the A437G mutation was significantly lower [OR=0.1 (0.1-0.3)]), and double and triple dhfr mutations were slightly less frequent in 2010 than in 2003 (13.2 and 16.8%). Mutations in the Pfdhfr and Pfdhps genes associated with resistance to SP were relatively common among pregnant women in the study area. Nevertheless, the prevalence of the triple dhfr mutation was very low suggesting that SP may be still efficacious for IPTp.

901

CHARACTERIZATION OF GENETIC POLYMORPHISMS RELATED TO ANTIMALARIAL PHENOTYPES IN *PLASMODIUM FALCIPARUM* SAMPLES COLLECTED IN BRAZIL

Juliana Inoue[1], Dinora Lopes[2], Marta Machado[2], Pedro Cravo[3], Giselle F. Lima[1], Angélica D. Hristov[1], Maria J. Costa-Nascimento[4], Aluisio A. Segurado[1], Marcos Boulos[1], Virgílio Rosário[2], Silvia M. Di Santi[1]

[1]Universidade de São Paulo, Sao Paulo, Brazil, [2]Universidade Nova de Lisboa, Lisbon, Portugal, [3]Universidade Federal de Goiás, Goias, Brazil, [4]Superintendência de Controle de Endemias, Sao Paulo, Brazil

Plasmodium falciparum has shown a major ability to evolve resistance to nearly all antimalarials. Monitoring candidate mutations of drug resistance and clarifying their role in treatment outcome may contribute to optimizing malaria control measures. This study aimed to analyze resistance-associated genes over a twenty-seven year period. *P. falciparum* samples collected from 1984 to 2011 from patients enrolled at Brazilian health facilities were assessed. The infections were mainly from South American and African countries. All South American samples harbored the 76T mutation in the *pfcrt* gene, in agreement with their chloroquine-resistant *in vitro* response; African samples presented the wild type and mutant alleles. With regards to the *pfmdr1* gene, the emergence of the 86Y mutant was observed in the last decade in South American samples, whilst African samples presented both mutant and wild type alleles. Analysis of the 1246 codon revealed a mutant frequency of 100% in South American samples and the wild type variant in African samples. As to the *pfdhfr* gene, 100% of Brazilian samples presented the 51I and 108N mutants. The 59R mutant was not observed in the period of 1980-1990 but occurred in 22.6% of samples from 2000-2010. The 437G mutant of the *pfdhps* gene was observed in 100% of Brazilian samples in all decades and the 540E mutant decreased in the period 2000-2010 when compared to 1980-1990. In relation to artemisinin (ART) resistance candidate mutations, DNA sequence analysis of the *pfATPase6* gene showed previously described mutations. Two novel mutations were observed in the *pfAP2-μ* gene. A new molecular marker for ART resistance (Kelch-13 propeller) is being sequenced in several isolates. No association between the polymorphisms studied and *in vivo* or *in vitro* responses to mefloquine, quinine and ART derivatives was observed. This study established the genetic profile of *P. falciparum* regarding resistance-associated mutations over twenty-seven years, when the parasite was exposed to the selective pressure of several therapeutic schemes adopted in Brazil.

902

PARASITE CLEARANCE TIME AND *IN VITRO* SENSITIVITY OF *PLASMODIUM FALCIPARUM* TO ARTEMETHER-LUMEFANTRINE IN SOTUBA, MALI

Souleymane Dama, Issaka Sagara, Ogobara Doumbo, Abdoulaye Djimde

University of Sciences Techniques and Technology of Bamako, Bamako, Mali

In Mali, previous studies show high frequency (30-40%) of recurrent parasite after treatment with artemether-lumefantrine. We hypothesize that recurent parasites after treatment have a lower sensitivity to artemether-lumefantrine. We conducted a longitudinal study with 250 field isolates. *In vitro* field isolates sensitivity to Artemether and Lumefantrine was assessed using hypoxanthine isotopic test. A total of 250 *P. falciparum* isolates were successfully cultured *in vitro* and the sensitivities of 25% are available to date. All of the isolates tested *in vitro* were 100% sensitive to Artemether and Lumefantrine. The mean IC_{50} values are 3.08 nM and 3.54 nM for Artemether and Lumefantrine, respectively. Malian field *Plasmodium falciparum* isolates are sensitive *in vitro* to Artemether and lumefantrine.

903

THE POLYMORPHISMS IN KELCH AND FALCIPAIN-2 ASSOCIATED WITH ARTEMISININ RESISTANCE ARE NOT PREVALENT IN *PLASMODIUM FALCIPARUM* ISOLATED FROM UGANDAN CHILDREN

Melissa D. Conrad[1], Victor Bigira[2], James Kapisi[2], Mary Muhindo[2], Moses R. Kamya[3], Grant Dorsey[1], Philip J. Rosenthal[1]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Infectious Disease Research Collaboration, Kampala, Uganda, [3]Makerere University College of Health Sciences, Kampala, Uganda

Artemisinin resistance, manifested as delayed clearance of *Plasmodium falciparum* following treatment with artemisinins, has emerged in Southeast Asia. SNPs in the *PF3D7_1343700* kelch propeller (K13) domain were recently associated with artemisinin resistance after *in vitro* resistance selection and in clinical isolates, providing a molecular marker to monitor the spread of resistance. The cysteine protease falcipain-2

(FP2; *PF3D7_1115700*) contributes to artemisinin action via hemoglobin degradation, and it also was mutated after *in vitro* selection with artemisinin. Although delayed parasite clearance after artemisinin therapy has not yet been noted and artemisinin-based combination therapy remains highly efficacious in Uganda, it was important to characterize the diversity of these genes. We therefore sequenced the K13-propeller domain and FP2 genes in 104 samples collected in 2011-2012 from Ugandan children with malaria emerging after recent exposure to ACTs for treatment (within 28 days of treatment with artemether/lumefantrine) or chemoprevention (monthly DHA/piperaquine). Using 3D7 as the reference genome, we identified polymorphisms resulting in 5 amino acid substitutions in the K13 gene, none of which were among the markers of resistance seen in Asian isolates. No single SNP was found in more than 2 isolates. For FP2, we identified polymorphisms resulting in amino acid substitutions at 29 loci, 17 in the pro and 12 in the mature domain of the protease; these did not include the SNP reported after *in vitro* selection for resistance. The prevalence of K13 and FP2 polymorphisms did not increase over time, and no SNPs were associated with malaria episodes in which parasite clearance was relatively delayed (persistence ≥2 days after the onset of treatment). These results indicate that the K13-propeller and FP2 coding polymorphisms associated with artemisinin resistance are not prevalent in Uganda. Thus, we see no evidence of artemisinin resistance in Ugandan parasites at present, but continued surveillance for resistance-mediating genotypes is warranted.

904

RELATIONSHIPS BETWEEN K13-PROPELLER ALLELES AND ARTEMISININ SUSCEPTIBILITY IN CAMBODIAN AND SENEGALESE *PLASMODIUM FALCIPARUM* ISOLATES

Angana Mukherjee[1], Rachel Daniels[2], Chanaki Amaratunga[3], Charles J. Woodrow[4], Elizabeth Ashley[5], Rupam Tripura[5], Arjen Dondorp[6], Nicholas White[6], Chea Nguon[7], Daouda Ndiaye[8], Dyann F. Wirth[9], Rick M. Fairhurst[3], Sarah Volkman[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Broad Institute, Boston, MA, United States, [3]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [4]Centre for Tropical Medicine, Oxford, United Kingdom, [5]Faculty of Tropical Medicine, Bangkok, Thailand, [6]Mahidol Oxford Tropical Research Unit, Bangkok, Thailand, [7]The National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [8]Universite Cheikh Anta Diop, Dakar, Senegal, [9]Harvard School of Public Health, Boston, MA, United States

Plasmodium falciparum resistance to artemisinin and its derivatives (ART) has emerged in Southeast Asia. ART resistance manifests *in vivo* as a long parasite clearance half-life after ART monotherapy or ART-based combination therapy (ACT), and *in vitro* as increased survival of young ring-stage parasites in the ring-stage survival assay ($RSA^{0\text{-}3h}$). In Cambodia, several mutations in the PF3D7_1343700 kelch propeller domain (K13-propeller) were recently associated with ART resistance *in vitro* and *in vivo* (Ariey *et al*., 2014). To investigate the role of these and other K13-propeller mutations in ART resistance, and to screen for emerging ART resistance in Africa, we have initiated studies of Cambodian and Senegalese parasite isolates. In collaboration with the Tracking Resistance to Artemisinins Collaboration (TRAC), we are presently adapting Western Cambodian isolates (half-life range, 1.7 – 11.8 h) to *in-vitro* culture, PCR re-sequencing their K13-propeller domains, and testing them in the $RSA^{0\text{-}3h}$ as reported previously. Preliminary data from this study have identified parasites carrying the C580Y, Y493H, R539T, I543T, and D584V mutations, and have associated these K13-propeller mutations with long half-life and elevated % survival values in the $RSA^{0\text{-}3h}$. Among Senegalese samples, we have identified a parasite isolate carrying a novel K13-propeller V637I mutation which has not yet been observed in Southeast Asia. We are now developing genotyping and sequencing assays to screen for these and other K13-propeller mutations in Africa.

905

TRENDS IN ANTIMALARIAL MEDICINE AND MALARIA DIAGNOSTIC AVAILABILITY IN CAMBODIA BETWEEN 2009 AND 2013

Kevin Duff[1], Sochea Phok[2], Henrietta Allen[2], Siv Sovannaroth[3], Vamsi Vasireddy[4], Megan Littrell[5]

[1]Population Services International, Nairobi, Kenya, [2]Population Services Khmer, Phom Penh, Cambodia, [3]CNM, Cambodia National Center for Parasitology, Entomology and Malaria Control, Phom Penh, Cambodia, [4]Independent Consultant, Chicago, IL, United States, [5]Population Services International, Washington, DC, United States

The advancement of artemisinin resistance (AR) in the Greater Mekong Subregion threatens the international community's ability to combat malaria. Ensuring consistent and accurate diagnostic testing, better access to artemisinin-based combination therapy (ACT), and removal of oral artemisinin monotherapy (oAMT) are key activities to contain AR. As such, a thorough understanding of the anti-malarial market is a pre-condition for guiding containment efforts. In 2009, 2011 and 2013, ACTwatch conducted nationally representative outlet surveys in Cambodia. Data from the 2013 survey is currently being analyzed and will be presented at the ASTMH conference. Preliminary results indicate that among outlets stocking antimalarials, nearly all public sector outlets had ACT available and very few had OAMT available in 2009 (ACT: 96%, OAMT: 2%), 2011 (ACT: 97%, OAMT: 0%) and 2013 (ACT: 99%, OAMT: 0%). In comparison, antimalarial availability has been less stable in private sector outlets stocking antimalarials, with an increase in ACT availability (63% to 83%) and a decrease in OAMT availability (20 to <1%) between 2009 and 2013. The percentage of outlets stocking rapid diagnostic tests (RDTs) has increased by 20% in public sector outlets (75% to 95%) and 26% in private sector outlets (37% to 63%) between 2009 and 2013. Logistic regression will be used to compare the rate of change for antimalarial and malaria diagnostic availability between outlet types, urban/rural status and artemisinin tolerance zones. Additional analysis will be performed to identify factors associated with changes in ACT, OAMT and RDT availability in private sector outlets in order to better understand the improved private sector market profile. Several interventions are being implemented in Cambodia to contain artemisinin resistance. The current study demonstrates increasing ACT and decreasing OAMT availability in the private sector, suggesting that the regulation of antimalarial medicine sales and other AR containment efforts may have been successful in improving the antimalarial market profile.

906

POOLED SEQUENCING OF MALARIA PARASITES FOR IDENTIFICATION OF DRUG RESISTANCE GENES

Ian H. Cheeseman[1], Marina McDew-White[1], Rose McGready[2], Elizabeth Ashley[3], Aung Pyae Phyo[2], Francois Nosten[2], Tim J. Anderson[1]

[1]Texas Biomedical Research Institute, San Antonio, TX, United States, [2]Shoklo Malaria Research Unit, Mae Sot, Thailand, [3]University of Oxford, Oxford, United Kingdom

The rapid spread of drug resistance genes through malaria parasite populations distorts the allele frequencies of flanking mutations. Such "selective sweeps" can be rapidly uncovered by pooled population sequencing, but this approach has yet to be applied to malaria parasites. We explored the potential of pooled sequencing to swiftly and economically identify selective sweeps due to emerging artemisinin (ART) resistance in a South-East Asian malaria parasite population. ART resistance is defined by slow parasite clearance from the blood of ART-treated patients and mutations in the kelch gene (chr. 13) have been strongly implicated to play a role. We constructed triplicate pools of 70 slow-clearing (resistant) and 70 fast-clearing (sensitive) infections collected from the Thai-Myanmar border and sequenced these to high (~150-fold) read depth in an Illumina HiSeq lane. Allele frequency estimates from pools

showed almost perfect correlation (Lin's concordance = 0.98) with allele frequencies at 93 SNPs measured directly from individual infections, giving us confidence in the accuracy of this approach. By mapping genome-wide divergence (FST) between pools of drug resistant and drug sensitive parasites we identified three large (>150kb) regions (on chrs. 11, 13 and 14) and 18 smaller candidate genome regions. To identify individual genes within these genome regions we re-sequenced an additional 38 individual parasite genomes (22 slow and 16 fast-clearing) and performed rare variant association tests. These confirmed kelch as a major molecular marker for ART resistance (p=6.03x10-6), and provide suggestive associations for the involvement of several other genes. This two-tier approach is powerful because pooled sequencing rapidly narrows down genome regions of interest, while targeted rare variant association testing within these regions can pinpoint the genetic basis of resistance.

907

SEQUENTIAL EVOLUTION OF DRUG RESISTANCE: EVIDENCE THAT EPIGENETIC REGULATION PRECEDES GENETIC ADAPTATION IN THE ACQUISITION OF HALOFUGINONE RESISTANCE

Jonathan D. Herman[1], Daniel P. R[2], Ulf Ribacke[3], Amy Deik[4], Eli Moss[4], Michael Desai[2], Clary C. Clish[4], Ralph Mazitschek[5], Dyann F. Wirth[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Harvard University, Cambridge, MA, United States, [3]Uppsala University, Uppsala, Sweden, [4]Broad Institute, Cambridge, MA, United States, [5]Massachusetts General Hospital, Center for Systems Biology, Boston, MA, United States

Understanding mechanisms of drug resistance is essential for the design of future antimalarial therapies and rational design of drug combinations. To understand the multi-step nature of drug resistance acquisition in *Plasmodium falciparum*, we performed metagenomic sequencing of six time points in two *in vitro* drug resistance selections over 60 generations. For these experiments we used the *P. falciparum* cytoplasmic prolyl tRNA synthetase (cPRS) inhibitor halofuginone. We found that non-genetic adaptation to halofuginone precedes mutation or amplification of the target cPRS gene. Part of this non-genetic adaptation occurs through regulation of cellular amino acid homeostasis. Upon exposure to halofuginone, *P. falciparum* increases cytosolic proline and overexpresses PSAC determinants cytoadherence-linked asexual gene (clag) 2 and clag 3.2. Using an allelic exchange approach, we found that both cPRS halofuginone resistance mutations HFGRI (L482H) and HFGRII (L482F) only confer halofuginone resistance when clag genes are also overexpressed. Furthermore, by tracking the evolution of two drug resistance selections by whole genome sequencing, we demonstrate that the cPRS locus accounts for the majority of genetic adaptation to halofuginone in *P. falciparum*. Thus, we provide evidence for a three-step model of multi-locus evolution of drug resistance via genetic and non-genetic adaptations in *P. falciparum*: first, non-genetic adaptation predominates and permits later acquisition of target-site mutations; second, either target-site mutations or amplifications develop; and third, wild type target-site amplifications take over and out compete less fit target-site mutations. This sequential model of drug resistance evolution has greater implications for malaria drug-resistance surveillance and combination drug development.

908

A COLLECTION OF CLONED PARASITES FROM *PLASMODIUM FALCIPARUM* INFECTIONS SHOWING SLOW OR FAST CLEARANCE FOLLOWING ARTEMISININ TREATMENT

Marina McDew-White[1], Lisa Checkley[2], Shalini Nair[1], Rose McGready[3], Elizabeth Ashley[4], Aung Pyae Phyo[3], François Nosten[3], Michael Ferdig[2], Tim Anderson[1]

[1]Texas Biomedical Research Institute, San Antonio, TX, United States, [2]Eck Institute for Global Health, Department of Biology, University of Notre Dame, South Bend, IN, United States, [3]Shoklo Malaria Research Unit, Mae Sot, Thailand, [4]Centre for Tropical Medicine, Nuffield Department of Medicine, Oxford, United Kingdom

A collection of laboratory adapted parasites with carefully defined clearance rates following artemisinin (ART) treatment would provide a valuable resource for understanding the mechanism of ART-resistance, the role of kelch, the involvement of other loci and for understanding the costs of resistance. We therefore dilution-cloned 45 infections from hyper parasitaemia patients visiting clinics run by the Shoklo Malaria Research Unit on the Thailand-Myanmar border (2008-12) that showed either very slow (T1/2 > 5) or very fast clearance (T1/2 < 3), determined by 6 hourly measures of parasite density following ART-treatment. The clones isolated were genotyped using 93 polymorphic SNPs to verify their matches to the original infections. Clones identical to the original infection were obtained from 33/45 infections. These 33 genotype-verified parasite clones from a single location, and from the extremes of the clearance rate distribution, will be made available to the research community.

909

RESISTANCE MARKER PREVALENCE IN *PLASMODIUM FALCIPARUM* IN RESPONSE TO RECENT INTRODUCTION OF AL ANTIMALARIAL DRUG

Elizabeth A. Hemming, Eugenia Lo, Guiyun Yan

University of California Irvine, Irvine, CA, United States

Due to increasing drug resistance in the malaria parasite *Plasmodium falciparum*, chloroquine (CQ) was replaced by sulphadoxine-pyrimethamine (SP) in 1998 as first-line treatment for uncomplicated malaria in Kenya. However, after less than a decade following the policy changes, artemisinin-based combination therapies (ACTs) have replaced SP as the most effective anti-malarial option due to widespread SP-resistance. In 2006, the new malaria policy implemented artemether-lumefantrine (AL) as the first-line treatment for uncomplicated malaria and quinine for complicated and severe malaria. Monitoring and understanding the genetic factors underpinning drug resistance is imperative to maintaining effective antimalarial drug administration policies. This study aims to examine the resistance marker prevalence of *P. falciparum* and determine if there is a transition toward widespread *P. falciparum* resistance to the recent introduction of AL as compared to previously used drugs in Western Kenya. Sequences of five candidate genes were compared among *P. falciparum* samples to examine targeted mutations associated with resistance. They are (1) the chloroquine resistant transporter (crt) and multidrug resistance 1 (mdr1) genes for CQ/quinine resistance; (2) the dihydropteroate synthase (dhps) and dihydrofolate reductase (dhfr) genes for SP resistance; and (3) the sarco/endoplasmic reticulum Ca2+-ATPase (atp6) gene for AL resistance. Two comparisons were made to investigate the level of selective pressure on the various resistance genes. The first was between high and low transmission areas. In areas of high malaria transmission, we expect that selective pressure could be greater in parasite populations due to frequent use of drugs. This would be reflected by a higher frequency of the selected codon as compared to parasite populations in low transmission areas. The second compared different resistance genes based on parasite samples from the same area. This comparison allows us to infer the degree of resistance based on genetic

information with respect to different antimalarial drugs. Despite its recent implementation in Western Kenya, AL has already demonstrated reduced efficiency in treating falciparum-malaria in other parts of East Africa.

910

PERSISTENT RESISTANCE TO DISCONTINUED ANTIMALARIAL AGENTS IN *PLASMODIUM FALCIPARUM* CLONES OF SOUTHWEST INDIA

John White[1], Anjali Mascarenhas[2], Ligia Pereira[2], Jayashri Walke[2], Rhiaz Basha Shaik[2], Ambika Sharma[2], Jennifer Guler[3], Devaraja Mudeppa[1], Ashwani Kumar[4], Neena Valecha[5], Jennifer Maki[1], Nagesh Dubhashi[2], Edwin Gomes[2], Laura Chery[1], Pradipsinh Rathod[1]

[1]University of Washington, Seattle, WA, United States, [2]Goa Medical College Hospital, Bambolim, Goa, India, [3]University of Virginia, Charlotte, VA, United States, [4]National Institute of Medical Research, Panaji, Goa, India, [5]National Institute of Medical Research, New Delhi, India

Malaria control in India comes with significant challenges, including variations in accuracy of differential diagnosis, the frequent co-occurrence of *Plasmodium falciparum* and *P. vivax*, varying drug-treatment preferences for the different species in different localities, and migrations of people across the sub-continent. The impact of this, both on the emergence as well as persistence of drug resistance in this environment, is of interest. As a part of the US NIH International Center of Excellence for Malaria Research (ICEMR) program activities, we have collected and culture-adapted malaria parasites at Goa Medical College, Bambolim, Goa. This is a tertiary care center with patients presenting uncomplicated as well as severe malaria. This site is of particular interest because Goa stopped using chloroquine for *P. falciparum* treatment, as national policy favored artemesinin-derived combinations about seven years ago. Five years ago, Goa stopped using the nationally recommended first line artesunate-pyrimethamine-sulphadoxin combinations to treat *P. falciparum* in favor of artesunate-mefloquine. Given the well-known fitness costs of chloroquine-resistance and fansidar-resistance, we expected emergence of sensitivity to these traditional antimalarials. Our studies from 2012-2014, based on DNA sequencing of drug-resistance markers as well as direct phenotypic assays of freshly cultured parasites, point to persistent and frequent occurrence of chloroquine and fansidar resistance. This suggests that there must be unintended but sustained drug pressure from old antimalarials on modern parasite populations in this locality. So far, resistance is not seen to mefloquine and artesunate, the preferred drug combination in Goa. Similar background surveys are planned across multiple ICEMR sites across India, with patient parasite samples collected over a period of five years.

911

MONITORING ANTIMALARIAL DRUG RESISTANT PARASITES IN WESTERN KENYA

Naomi W. Lucchi[1], Sheila Akinyi[1], Ira Goldman[1], Kephas Otieno[2], Philip Onyona[2], John W. Barnwell[1], Ya Ping Shi[1], Simon Kariuki[2], Venkatachalam Udhayakumar[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya

Artemether-lumefantrine was implemented as first-line therapy for treatment of uncomplicated *Plasmodium falciparum* malaria in Kenya in late 2006. Amodiaquine-artesunate is registered as an alternative treatment. *In vitro* drug sensitivity assay and molecular surveillance for markers associated with resistance are useful surrogates to monitor trends in the susceptibility to *P. falciparum* parasites to anti-malarial drugs. We report findings from a study conducted in hospitals in western Kenya between 2010 and 2013. A total of 204 samples were enrolled. *In vitro* sensitivity to chloroquine (CQ), mefloquine (MQ), amodiaquine (AQ), artesunate (ART) and dihydroartemisinin (DHA) was determined. Samples were also genotyped for single nucleotide polymorphisms (SNPs) associated with CQ, MQ, AQ, and sulphadoxine-pyrimethamine (SP) resistance. No significant changes in the mean IC_{50} response to ART or DHA were observed during this study period. A significant decline in the prevalence of the CQ resistant CVIET genotype and the *Pfmdr-1* 86Y mutation (both associated with CQ and AQ resistance), was observed between 2010 and 2013. A decrease in the mean IC_{50} to these drugs was also observed. No increase in the copy numbers of the *Pfmdr-1*gene and mean MQ $IC_{50,}$ both associated with MQ resistance, was observed. On the contrary, the prevalence of parasites with the combined *Pfdhfr/Pfdhps* quintuple mutation (51I/ 59R/108N and 437G/540E), associated with SP resistance, was above 90%. The rare 164L and 581G mutations were observed in 4/204 and 3/204 samples, respectively and a 2.3% increase in the prevalence of the previously described 436H mutation was recorded during this study period. The prevalence of the *Pfdhps* 436H/ 437G/ 540E genotype is on the rise in this region of East Africa. Further microsatellite analysis around the *Pfdhps* gene demonstrates that it is under selection. In conclusion, the *in vitro* drug sensitivity pattern for ART and DHA is consistent with *in vivo* drug trials data conducted by other groups in this region indicating that artemisinin-based combination therapies are efficacious in western Kenya. CQ sensitive strains are increasing, likely due to decreased use of CQ. High prevalence of SP resistant quintuple mutations in this population is an important concern, because SP remains the only recommended drug for intermittent preventive treatment in pregnant women.

912

EVALUATION OF DIHYDROARTEMISININ-PIPERAQUINE WITH AND WITHOUT SINGLE-DOSE PRIMAQUINE: AN OPEN-LABEL RANDOMIZED, CONTROLLED TRIAL IN ANLONG VENG, CAMBODIA

Michele Spring[1], Chanthap Lon[2], Jessica Manning[1], Pattaraporn Vanachayangkul[1], Suwanna Chaorattanakawee[1], Panita Gosi[1], Ratawan Ubalee[1], Soklyda Chann[2], Sok Somethy[2], Rathvichet Bun[2], Sut Thang Phann[2], Montri Arsanok[1], Mali Ittiverakul[1], Nillawan Buathong[1], Taing Davy[2], Nareth Kong[2], Thay Kheng Heng[2], Worachet Kuntawunginn[1], Prom Satharath[3], Char Meng Chuor[4], Jessica Lin[5], Charlotte Lanteri[1], David Saunders[1]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Armed Forces Research Institute of Medical Sciences, Phnom Penh, Cambodia, [3]Royal Cambodian Armed Forces, Phnom Penh, Cambodia, [4]National Center for Malaria, Entomology and Parasitology, Phnom Penh, Cambodia, [5]University of North Carolina, Chapel Hill, NC, United States

Evidence of dihydroartemisinin-piperaquine (DP) resistance has recently emerged outside of western Cambodia. We are currently evaluating the safety and therapeutic efficacy of DP for uncomplicated *Plasmodium falciparum* or mixed malaria infections, as well as its transmission blocking potential in combination with single dose primaquine (PQ) in northern Cambodia. Up to 150 volunteers, including those with mild-moderate G6PD deficiency, are being enrolled in a randomized, open-label clinical trial of 3 consecutive daily oral doses of 120/960mg of DP with or without a single 45mg dose of PQ. Transmission blocking is assessed with a mosquito membrane-feeding assay while cardiac safety is evaluated with serial electrocardiograms and time-matched drug levels. In the 107 patients completing follow-up to date, the 42-day per protocol PCR-unadjusted failure rate was slightly higher (45%) that the previously reported PCR-adjusted rate of (36%) among the first 50 volunteers. Mean piperaquine drug levels in the terminal elimination phase were lower than mean *in vitro* parasite piperaquine IC50 among treatment failures. Mean piperaquine QT-interval prolongation was lower (428ms) than those we previously reported with a compressed 2-day course of therapy (455ms) at Cmax (4 hours after the last dose). Gametocytemia among P.f.-infected patients was uncommon, though limited data suggests that 45mg single-dose primaquine was effective in preventing transmission based on oocyst counts. DHA-piperaquine is rapidly failing in Northern Cambodia, though single dose primaquine (45mg) may help to halt the spread of resistant parasites and should be implemented where adequate safety assessment is

feasible. The standard 3 day course of DP appears to carry a lower cardiac safety risk than a 2 day course and is consistent with previously reported safety data.

913

DIHYDROARTEMISININ-PIPERAQUINE TREATMENT FAILURES IN *PLASMODIUM FALCIPARUM* IN NORTHERN CAMBODIA ARE NOT MEDIATED BY KNOWN ACT RESISTANCE MARKERS

Jessica T. Lin[1], Charlotte A. Lanteri[2], Sujata Balasubramanian[1], Panita Gosi[2], Eric Barnett[1], Michele Spring[2], Suwanna Chaorattanakawee[2], Jessica Manning[2], Char Meng Chuor[3], Chanthap Lon[2], Jonathan J. Juliano[1], David L. Saunders[2]

[1]University of North Carolina, Chapel Hill, NC, United States, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia

Dihydroartemsinin-piperaquine (DP), one of the last remaining effective drugs for multidrug resistant *Plasmodium falciparum,* has been the first line artemisinin combination therapy (ACT) in Cambodia since 2012. However, clinical trials in northern Cambodia in 2010 and 2013 show rising rates of DP failure, reaching a 42-day PCR-corrected recrudescence rate greater than 40%. With nearly 60% of patients remaining parasitemic at 72 hours and increasing piperaquine IC_{50}s, treatment failures are likely due to decreased susceptibility to both artemisinin and the partner drug. We are investigating whether these failures are associated with polymorphisms in known or candidate molecular markers of antimalarial resistance. Isolates from approximately 140 Cambodian patients with *P. falciparum* infections treated with DP are being analyzed. Preliminary analysis of over half of the isolates shows fixation of the CVIET haplotype at *pfcrt*, near fixation of the 184F mutant (96%) in *pfmdr1*, and wild type alleles only at codons 86, 1034, 1042, and 1246 of *pfmdr1*. Increased *Pfmdr1* copy number (CN) is present in 14% of isolates and is associated with greater *in vitro* susceptibility to piperaquine (mean PPQ IC50 18.3nM vs. 36.7nM in samples with CN>1 and CN≤1, respectively, p=0.02). Sequencing of the recently described kelch propeller gene (K13) associated with artemisinin resistance reveals that, as in western Cambodia, mutant K13 alleles are highly prevalent, with only 7.5% showing the wild type allele. Finally, we measured CN of the Xr5 locus, where a duplication on Chr 5 has been associated with *in vitro* piperaquine resistance in drug-pressured parasites. Using digital droplet PCR, we found no increase in CN at four genes within the Xr5 domain. In sum, none of the molecular markers analyzed correlate with DP treatment failure in these patients. These findings suggest that escalating ACT resistance in northern Cambodia is incompletely described by current molecular markers. Research to elucidate as yet undiscovered mechanisms of DP resistance are urgently needed for molecular surveillance worldwide.

914

RESIDUAL MALARIA INFECTIONS IN PRE-ELIMINATION SCENARIOS: HOW DOES DETECTABILITY RELATE TO PREVALENCE?

Olivier J. Briet, Thomas A. Smith

Swiss Tropical and Public Health Institute, Basel, Switzerland

Recent empirical and modeling studies have implicitly made conflicting claims about the relationship between *Plasmodium falciparum* malaria parasite densities in humans and transmission intensities, and about the consequences for the importance of sensitivity of diagnostic methods to detect blood stage parasitemia. Evidence that there are disproportionate numbers of sub-patent infections at low transmission appears to be at variance with suggestions that parasites are more likely to lead to clinical attacks at low levels of naturally acquired immunity. An existing mathematical model, parameterized using parasite densities from malaria therapy patients, was used to simulate a successful program to reduce *P. falciparum* transmission. The relationships between prevalence and parasite densities were further investigated in field data. The simulations suggested that after halting transmission, the proportion of infections detectable by standard diagnostic tests will initially drop, but then increase as acquired immunity wanes. In field data the relationship between parasite densities and prevalence of patent parasitemia can vary over short distances in time and space, reflecting transmission heterogeneity and seasonality. In pre-elimination transmission landscapes, cases are indicative of local active transmission. Conversely, areas with a disproportionate number of sub-patent infections are likely to represent sink areas where these lingering old infections have little vector contact, and contribute little to transmission. Hence, detection of these infections is of little relevance for elimination programs. The use of more sensitive diagnostic methods that lower the limit of detection of asexual blood stage parasitemia would not address the key limitations of mass screening and treating as a strategy for long-term reduction in malaria transmission. These limitations are the short term impact of the intervention, and inefficiency owing to lack of targeting in space and time of infections that are particularly likely to transmit.

915

COSTS OF ELIMINATING MALARIA AND THE IMPACT OF THE GLOBAL FUND IN 34 COUNTRIES

Brittany Zelman[1], Anthony Kiszewski[2], Chris Cotter[1], Jenny Liu[1]

[1]University of California San Francisco Global Health Group, San Francisco, CA, United States, [2]Bentley University, Waltham, MA, United States

International financing for malaria increased more than 18-fold between 2000 and 2011; the largest source came from The Global Fund to Fight AIDS, Tuberculosis and Malaria (Global Fund). Countries have made great progress, but achieving elimination requires sustained financial resources to interrupt transmission and prevent reintroduction. Since 2011, global financing for malaria has declined, fueling concerns that progress in reducing malaria may be impeded, especially for the 34 malaria-eliminating countries that face a particular risk of malaria resurgence if programs are disrupted. This study aims to 1) assess past total and Global Fund funding to the 34 malaria-eliminating countries, and 2) estimate future funding needs to achieve malaria elimination and prevent reintroduction through 2030 in the 34 countries. Historical funding is assessed against trends in country-level malaria annual parasite incidences (APIs) and income per capita. Following Kizewski et al. (2007), program costs to eliminate malaria and prevent reintroduction through 2030 are estimated using a deterministic model. The cost parameters are tailored to a package of interventions aimed at malaria elimination and prevention of reintroduction. The majority of Global Fund-supported countries experiencing increases in total funding from 2005 to 2010 coincided with reductions in malaria APIs and also overall GNI per capita average annual growth. The total amount of projected funding needed to achieve elimination and prevent reintroduction through 2030 is approximately US$8.5 billion, or about $1.84 per person at risk per year (PPY) (ranging from $2.51 PPY in 2014 to $1.43 PPY in 2030). Although external donor funding, particularly from the Global Fund, has been key for many malaria-eliminating countries, sustained and sufficient financing is critical for furthering global malaria elimination. Projected estimates of costs through elimination should help countries identify funding gaps and mobilize resources to obtain adequate financing to achieve their goals.

916

A CASE STUDY OF MALARIA ELIMINATION IN THE ECUADOR-PERÚ BORDER REGION

Lyndsay K. Krisher[1], Mariano Ambuludi[2], Ana Arichabala[3], Efraín Beltrán[2], Alvaro Calle[4], Jesse Krisher[1], Patricia Navarrete[3], Tania Ordoñez[3], Mark Polhemus[1], Fernando Quintana[5], Rosemary Rochford[1], Mercy Silva[6], Marcia Veintimilla[4], Anna Stewart Ibarra[1]

[1]Center for Global Health and Translational Science, State University of New York Upstate Medical University, Syracuse, NY, United States, [2]National Service for the Control of Vector-Borne Diseases, Ministry of Health, Machala, Ecuador, [3]Mabel Estupiñan Health Center, Ministry of Health, Machala, Ecuador, [4]Brisas del Mar Health Center, Ministry of Health, Machala, Ecuador, [5]Ministry of Health, Tumbes, Peru, [6]Teofilo Davila Hospital, Ministry of Health, Machala, Ecuador

In recent years malaria has been successfully controlled in the Ecuador-Peru coastal border region. The incidence of disease declined from a high of 2,117 per 100,000 in 1999 in El Oro Province, of southern Ecuador, to 0 as of 2011. The rate declined concomitantly in Piura Province of northern Peru during this same period. Our aim is to document this control effort and identify the best practices and lessons learned that are broadly applicable to malaria control and other vector borne diseases. We conducted a proximal outcome evaluation of the robust elimination program in Ecuador, using El Oro Province as a case study. We conducted semi-structured group discussions with public health experts who played central roles in the elimination effort, reviewed epidemiological records by the Ministry of Health, and reviewed national policy documents to produce a detailed timeline of events, a list of the crucial programmatic and external factors of success and the barriers faced, and to identify the important lessons learned. We found that the binational Ecuador-Peru collaboration was the most important component of the elimination program. This unique relationship created a trusting, open environment, allowing for flexibility, rapid response, innovation and resilience in times of crisis, and ultimately a sustainable control program. Strong community involvement, an extensive microscopy network and horizontal, intersectoral collaborations at the local level were also identified as key programmatic strategies. The results of this study provide key principles of a successful malaria control program that can be looked to by other nations and regions currently working to control malaria infection. These principles should be disseminated to the next generation of public health professionals in the region and serve as a guide to ongoing and future control efforts of other emerging vector borne diseases in this region and elsewhere.

917

TOWARDS MALARIA ELIMINATION IN MPUMALANGA, SOUTH AFRICA: A METAPOPULATION MODELING APPROACH

Sheetal P. Silal[1], Francesca Little[1], Karen I. Barnes[1], Lisa J. White[2]

[1]University of Cape Town, Rondebosch, South Africa, [2]Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand

Mpumalanga in South Africa is committed to eliminating malaria by 2018 and efforts are increasing beyond that necessary for malaria control. Malaria elimination strategies may be unsuccessful if they focus only on the biology of vector and parasite, and ignore the mobility patterns of humans, particularly in Mpumalanga where the majority of infections are imported. A metapopulation model is developed to assess the impact of proposed elimination-focused policy interventions in Mpumalanga. This is the first study designed for this purpose in Mpumalanga. A stochastic non-linear ordinary differential equation model fitted to Mpumalanga and Maputo (Mozambique) malaria data, is used to predict the impact of the scale-up of vector control, mass drug administration, a focused mass screen and treat campaign and foreign source reduction. Scaling up vector control is predicted to lead to a minimal reduction in local infections and mass drug administration and screening and treating at the Mpumalanga-Maputo border is predicted to be impacting but short-lived. Source reduction in Maputo is predicted to generate large reductions in local infections through stemming the flow of imported infections. The model also predicts that if malaria were to be eliminated in Maputo, malaria would also be eliminated in Mpumalanga, highlighting the need for and importance of regional collaboration. To eliminate malaria by 2018, the government of South Africa will need to design and implement an elimination strategy tailored for a country with a high level of imported infections. A regionally focused strategy may stand a better chance at achieving elimination in Mpumalanga and South Africa compared to a nationally focused one in the face of high visitation rates from neighbours in higher transmission areas.

918

ACTIVE CASE DETECTION OF MALARIA AND URINARY SCHISTOSOMIASIS IN PUPILS OF KOTTO BAROMBI, SOUTHWEST CAMEROON USING THE CYSCOPE® FLUORESCENCE MICROSCOPE

Leopold G. Lehman[1], Godlove Wepnje[2], Helen Kimbi[2], Judith Anchang-Kimbi[2], Bate Ayukenchengamba[2], Conica Njabi[2], Larissa Kouodjip Nono[3], Hervé Nyabeyeu Nyabeyeu[1], Calvin Tonga[1]

[1]University of Douala, Douala, Cameroon, [2]University of Buea, Buea, Cameroon, [3]University of Yaounde 1, Yaounde, Cameroon

Malaria and urinary schistosomiasis (U.S.) remain important public health issues in Kotto Barombi, South-west Cameroon even though the village benefits from free distribution of treatment of both diseases by health authorities. Accurate diagnosis and prompt treatment of malaria and U.S. are necessary for their elimination. Novel tools for rapid and mass diagnosis of malaria and other parasitic infections have recently been developed. This study was aimed at assessing their use in the field to determine the prevalence of malaria and U.S. in Kotto Barombi. Blood and urine samples were collected from 94 boys and 122 girls aged 3 to 14 years. Malaria and U.S. were diagnosed with a rapid test using pre-stained slides for fluorescence microscopy (CyScope®, Partec GmbH, Goerlitz, Germany). Furthermore, Malaria parasite detection and speciation were done using Giemsa-stained blood films. Lugol direct examination was performed for U.S. Performance characteristics of CyScope® for malaria and U.S. were determined. Overall prevalence of malaria was 19.0% and 41.2% for light and fluorescence microscopy respectively (X2=15.33, p-value=0.00019). Malaria prevalence and density were similar in the different age groups, sexes, socio-economic class (SEC) and nutritional class. Overall prevalence of anaemia was 18.5%. Sensitivity was 68.3% and specificity was 64.9% for malaria. Overall prevalence of U.S. was 43.4% and 48.5% for light and fluorescence microscopy respectively. Many cases of co-infection (19.9%) were recorded. Mean intensity of US was 8.1±27.02. Prevalence and intensity of U.S. were significantly higher in the Kotto Barombi Island (78.3%, 33/42) than mainland (33.8%, 52/154). U.S. prevalence was similar in the different age groups, sexes and SEC. Sensitivity of the test was 90.6% and specificity was 83.8%. The CyScope® could be a good tool for active case detection of both diseases especially in areas that lack electricity since it can be battery operated. Drastic measures need to be taken for the elimination of these diseases.

919

IDENTIFICATION OF THE MOST EFFICIENT INTERVENTION PACKAGES ACROSS DIFFERENT EPIDEMIOLOGICAL STRATA: AN APPLICATION TO *PLASMODIUM FALCIPARUM* MALARIA TRANSMISSION AND MORBIDITY IN AFRICA

Patrick Walker, Jamie Griffin, Neil Ferguson, Azra Ghani

MRC Centre for Outbreak Analysis and Modelling, London, United Kingdom

Reducing the burden of major endemic infectious diseases is a global priority, but financial constraints require available resources to be allocated rationally to maximise impact. This poses particular challenges

for infections such as malaria, where transmission intensity exhibits a high level of spatiotemporal variability. Here we incorporate financial data into a mathematical model of malaria transmission to evaluate the most efficient (minimum cost) combinations of interventions to achieve disease and transmission milestones across sub-Saharan Africa. We make the optimization problem computationally tractable by classifying each geographic location into a finite set of epidemiological strata according to the average intensity of transmission, pattern of seasonality in transmission and vector species mix. We show that the optimal set of interventions can vary substantially depending on whether disease reduction or elimination of infection is the primary goal. Our analysis indicates that malaria elimination is possible using existing interventions in 64% of the area of sub-Saharan Africa in which P.falciparum malaria is endemic, representing 63% of the population at risk, but that the optimal combination of interventions required shows substantial geographic variation at a range of spatial resolutions. By considering how the degree of this variation differs at different administrative scales, we show the extent to which the additional complexities of implementing more localised control strategies are offset by the fewer resources they require to achieve elimination. Our results provide a rational framework for the global health community to consider the feasibility, cost and resource requirements of different targets for malaria control over the coming decades.

920

MATING COMPETITIVENESS OF STERILE MALE *ANOPHELES COLUZZII* IN SEMI-FIELD CAGES

Hamidou Maiga[1], David Damiens[2], K. Roch Dabiré[1], Abdoulaye Diabaté[1], Fréderic Tripet[3], Gilles R. Jeremy[4]

[1]IRSS/Centre Muraz, Bobo-Dioulasso, Burkina Faso, [2]Insect Pest Control Laboratories, IAEA/FAO Laboratories, Vienna, Austria, [3]Keele University, Staffordshire, Keele, Staffordshire, United Kingdom, [4]Insect Pest Control Laboratories, IAEA/FAO Laboratories, Austria, Vienna, Austria

The renewed interest in the development of control strategies using sterile insects raises hopes of being able to control the disease by cutting down the high reproductive rate of mosquitoes. Specifically, investigations into factors that account for male mating competitiveness are critical to the development of genetic control strategies. In this study, we assessed the effects of partial irradiation with 75 Gy on *Anopheles coluzzii* sexual competitiveness when allowed to mate in different ratios of sterile/fertile males for 2 nights in field cages. Moreover, to determine the dynamics of this competition, competitiveness was compared between males allowed 1 night vs 2 nights of contact with females. Sterilized (S) and fertile (U) males between 4 and 6 days of age were released in field cages (1.70mx1.70mx1.70m) with females (F) of similar age and left for 2 nights at the following ratios (S:U:F): (100:0:100) (100:100:100) (300:100:100) (500:100:100) (0:100:100). Each treatment was replicated 3 times. Competitiveness was determined by assessing the hatching rate of eggs laid en masse and the insemination rate, determined by dissecting recaptured females. An additional experiment with a ratio of (500:100:100) has been done with a mating period of either 1 or 2 nights. For the first experiment, the egg hatching rate was significantly affected by the release ratio and we thus observed that the Fried competitiveness index of sterile males was between 0.29 and 0.55. A similar insemination rate was recorded after 2 nights of contact in experiment 1, while significant difference was observed in the (S:U:F) (100:0:100) ratio between the males left to mate for 1 and 2 nights. However, a similar hatching rate was observed when mating occurred for 1 or 2 nights. The results suggest a release ratio of at least 2 sterile males for 1 fertile male and that *An. coluzzii* mating competitiveness experiments in field cages should be run for 1 instead of 2 nights.

921

EVALUATION OF THE NATIONAL MALARIA SURVEILLANCE SYSTEM OF BHUTAN, 2006-2012: A CASE STUDY

Nicole West[1], Sonam Gyeltshen[2], Singye Dupke[2], Kaveh Khoshnood[1], Sonam Tashi[2], Amanda Durante[3], Sunil Parikh[1]

[1]Yale School of Public Health, New Haven, CT, United States, [2]Vector-Borne Disease Control Program, Gelephu, Bhutan, [3]Department of Health, New Haven, CT, United States

Once a leading cause of morbidity and mortality in Bhutan, malaria has drastically reduced following aggressive control efforts over the last two decades. Bhutan is now embarking on malaria elimination. We examine the role of surveillance in Bhutan's pivot to elimination, assessing the ability of the current malaria surveillance system to meet the objectives of the Bhutan Vector-borne Disease Control Programme (VDCP), highlighting the priorities of the surveillance system as the nation transitions into an era of elimination, and identifying areas that require attention for this goal to be achieved. An evaluation of the national malaria surveillance system of Bhutan from 2006 to 2012 was conducted using the Center for Disease Control guidelines for evaluating public health surveillance systems. In addition, a formal assessment of blood slide quality assurance and control in 2011 was performed and data will be presented. From 2006-2012, *Plasmodium vivax* accounted for one half to two thirds of infections, depending on the district location. The national malaria surveillance system in Bhutan was found to be reasonably flexible, representative, simple, and stable. The quality of data produced is good, but its usefulness and interpretative insight could be improved by the computation of additional assessment measures. Timeliness could be improved by telephone reporting and increased health worker training and accountability is needed going forward. Nationally, non-residents comprised between 6.2% (in 2010) and 22.6% (in 2012) of all cases. Thus, more rigorous case identification and investigation will facilitate targeted interventions, while more attention is required to address re-introductions of infections through migrant workers and cross-border prevention initiatives in the coming years. Currently, the malaria surveillance system of Bhutan generates data that is useful and of good quality, but the pivot to elimination will require system function enhancement focus and intensify malaria prevention efforts.

922

SHIFTING THE BURDEN OR EXPANDING ACCESS TO CARE? ASSESSING MALARIA INCIDENCE TRENDS FOLLOWING SCALE-UP OF COMMUNITY HEALTH WORKER MALARIA CASE MANAGEMENT IN SOUTHERN PROVINCE, ZAMBIA

David Larsen[1], Zunda Chisha[2], Tokozile Ngwenya-Kangombe[2], Sydney Kaweme[2], Sanford Cheelo[2], Busiku Hamainza[3], Mulakwa Kamuliwo[3], John Miller[4], Daniel J. Bridges[2], Anna Winters[2]

[1]Syracuse University, Syracuse, NY, United States, [2]Akros, Lusaka, Zambia, [3]Zambia National Malaria Control Center, Lusaka, Zambia, [4]PATH-MACEPA, Lusaka, Zambia

Malaria infections constitute a large proportion of outpatient services in malaria endemic areas. Health centers are often understaffed, and the frequency of malaria infections contributes to a decreased quality of care for non-malaria illnesses. Health centers in rural areas can be difficult to access; distance traveled to a health center is often cited as a reason why individuals do not seek treatment for an illness. Zambia has trained community health workers to diagnose and treat malaria using rapid diagnostic tests and artemether-lumefantrine. Community health workers (CHW) operate out of their home or a nearby health post located within the community. Has this extension of malaria services shifted the burden of care from the health center to the community health worker, or has it simply expanded the access to care within the community? Improvements in the reporting of malaria at health centers and from CHWs provide robust health facility data. We used random effects regression to estimate trends in the outcome of outpatient attendance and the outcome of

health facility malaria incidence before and after the scale-up of malaria diagnostic and treatment services at the community level in Southern Province, Zambia. We controlled for environmental drivers of malaria incidence using remotely sensed variables, and adjusted for temporal autocorrelation. After accounting for environmental factors, implementing CHW case management of malaria was associated with an 8.2% (95% CI = 4.1% - 12.2%) reduction of outpatient attendance at the health center. When combining the additional malaria cases actively and passively identified through CHW case management with monthly confirmed health center incidence, implementation of CHW case management was associated with a 45.5% increase in monthly confirmed malaria incidence (95% CI = 33.5% - 58.6%). The implementation of CHW case management has both shifted the burden of outpatient care away from the health center into the communities as well as expanded the access to malaria treatment.

923

REDUCING MALARIA AMONG MIGRANTS AND MOBILE WORKERS IN THE GREATER MEKONG SUB-REGION BY BROADENING OPPORTUNITIES FOR MALARIA SERVICES AND PREVENTION

Soy Ty Kheang

University Research Co. LLC, Phnom Penh, Cambodia

Migrant and mobile populations are a difficult to reach population and often coined a 'hot population' in malaria control programs in the Greater Mekong Sub-region. Engaging in construction and agricultural production in remote areas where malaria is common, these workers often lack malaria knowledge or immunity, and information on and access to preventive measures and curative services.They could serve as a source of infection introducing malaria to historically low- or non-transmission areas. The PMI Control and Prevention of Malaria (CAP-Malaria) Project has implemented innovative approaches to reach migrant and mobile populations in remote border areas of Thailand, Cambodia, and Myanmar where malaria is concentrated. The project utilizes a multi-pronged approach that provides malaria information and services at multiple points that migrants typically visit or pass through while in the region and returning home. For instance, malaria information is provided by transportation services taking migrants to their employment destinations. Migrants may also obtain information and services during arrival or departure at malaria posts located at border crossings or border malaria clinics. Mobile malaria workers (community volunteers) and mobile clinics with medical staff frequent locations with high concentrations of migrants. Large multinational companies as well as smaller agricultural employers in both Cambodia, Thailand and Myanmar are participating in a lending scheme through which migrants can borrow a long-lasting insecticidal net (LLIN) for the duration of their employment. In Cambodia, a radio show reaches residents and mobile populations in remote areas, providing malaria information and promoting a malaria hotline for one-on-one malaria discussions. Engagement of taxi and bus drivers in providing information on malaria prevention and health seeking behavior are vital for mobile and migrant populations prior to reaching highly malaria endemic zones. During the past 2 years, the project has reached over 50,000 migrant workers in Cambodia, 439,345 in Myanmar, and 3,600 in Thailand.

924

USING INCIDENT MALARIA CASES TO FIND ASYMPTOMATIC MALARIA INFECTIONS IN SOUTHERN PROVINCE, ZAMBIA: WHICH CASES INCREASE THE PROBABILITY OF FINDING INFECTIONS?

David Larsen[1], Zunda Chisha[2], Tokozile Ngwenya-Kangombe[2], Sydney Kaweme[2], Sanford Cheelo[2], Busiku Hamainza[3], Mulakwa Kamuliwo[3], John Miller[4], Anna Winters[2], **Daniel J. Bridges**[2]

[1]Syracuse University, Syracuse, NY, United States, [2]Akros, Lusaka, Zambia, [3]Zambia National Malaria Control Center, Lusaka, Zambia, [4]PATH-MACEPA, Lusaka, Zambia

As malaria transmission declines it becomes heterogeneous, characterized by pockets of sustained transmission. Finding and removing the pockets of sustained malaria transmission is of utmost importance to malaria elimination programs. Incident malaria cases may be indicative of malaria transmission, and returning to the household of incident malaria cases may be one way in which to target pockets of residual malaria transmission. In Southern Province, Zambia reactive case detection (RCD) has expanded to included >1500 community health workers (CHWs) operating out of >260 health centers. CHWs return to the household of a rapid-diagnostic test (RDT) confirmed incident malaria case and screens individuals within 140 meters for a malaria infection using RDTs, treating those positive. Detailed records of each reaction have been kept by CHWs in Southern Province, and are currently being digitized for further data analysis. These records contain information on the incident malaria case demographics as well as the demographics of individuals screened during reactions. Preliminary analyses of aggregated RCD data suggest that RDT positivity during reactions exceeds RDT positivity during passive case surveillance more often in the dry season, when mosquito habitat as measured through remotely sensed enhanced vegetation index is limited, and when the ambient temperature as measured through remotely sensed land surface temperature is lower. These results suggest that incident malaria cases during the dry season may be more indicative of pockets of asymptomatic malaria infections than incident malaria cases during the wet season. Further analyses with the digitized CHW records will investigate what effect different demographics of incident malaria case affect the ability to find pockets of asymptomatic malaria infections using each individual reaction as the unit of analysis. A more robust analysis of the environmental factors affecting the probability to find pockets of asymptomatic malaria infections will also be conducted. Full analyses will be completed by June 2014.

925

SPATIO-TEMPORAL CHARACTERISTICS OF MALARIA INCIDENCE IN LUSAKA, ZAMBIA: TOWARDS MALARIA ELIMINATION IN AN URBAN ENVIRONMENT

Zunda Chisha[1], David Larsen[2], Clara Mbwili[3], Aves Hakalima[3], Busiku Hamainza[4], Mulakwa Kamuliwo[4], Daniel J. Bridges[1], **Anna M. Winters**[1]

[1]Akros, Lusaka, Zambia, [2]Syracuse University, Syracuse, NY, United States, [3]Lusaka District Health Office, Lusaka, Zambia, [4]Zambia National Malaria Control Center, Lusaka, Zambia

Malaria elimination may be more feasible from within urban areas than rural areas, due to differences in *Anopheles* habitat. Lusaka, Zambia has documented zero malaria infections in children under the age of five in Malaria Indicator Surveys conducted in 2010 and 2012 suggesting elimination of malaria transmission from within Lusaka is near. However, health facilities in Lusaka during this time period continue to record confirmed malaria incidence. Enhanced surveillance of incident malaria cases in Lusaka, Zambia was initiated in 2011. All confirmed incident malaria cases are questioned about history of travel outside Lusaka and previous malaria episodes. Case investigations are undertaken for incident malaria cases without a history of travel, wherein all members of the 9 adjacent households are screened with a rapid diagnostic test

and treated with artemether-lumefantrine if positive. We describe the temporal characteristics of health facility malaria incidence with and without a history of travel in relation to remotely sensed estimates of mosquito habitat. We use spatial scan statistics to estimate the location of spatial clusters of health facility malaria incidence without a history of travel within the catchment areas of 5 health centers in Lusaka using randomly generated population-weighted controls. Eighty percent of confirmed incident malaria cases report a history of travel outside Lusaka in the previous month, with travel being most common in December and January. Incidence rates of malaria without a history of travel follow the suspected seasonal pattern and are highly correlated with those of malaria reporting a history of travel (rho = 0.82). Significant clusters of malaria incident cases were found within each health center catchment area assessed. In all but 1 of the catchment areas, greater than 75% of incident cases without travel were located within clusters. The heterogeneous spatial pattern of malaria incidence in Lusaka is clear - identifying and targeting clusters of malaria transmission will be critical in the pursuit of elimination.

926

IMPLEMENTATION AND APPLICATION OF A MULTIPLEX ASSAY TO DETECT MALARIA ANTIBODIES: A PROMISING TOOL FOR ASSESSING MALARIA TRANSMISSION IN PRE-ELIMINATION AREAS

Karen Kerkhof[1], Lydie Canier[2], Somony Heng[2], Tho Sochantha[3], Niel Hens[4], Didier Menard[2], Marc Coosemans[1], Vincent Sluydts[1], Lies Durnez[1]

[1]Institute of Tropical Medicine Antwerp, Antwerp, Belgium, [2]Institut Pasteur du Cambodge, Phnom Penh, Cambodia, [3]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [4]University of Antwerp, Antwerp, Belgium

In countries that move towards pre-elimination, malaria is concentrated in hotspots. To identify these hotspots for targeted and effective interventions it is crucial to improve the understanding of the effectiveness of malaria control tools. However, in areas with a low prevalence and incidence of malaria, such as Cambodia, detection of parasitological indicators is very insensitive. Seroprevalence is based on the detection of antibodies against antigens of malaria parasites, which offers an advantage as anti-*Plasmodium* antibodies can persist for months after infection. Therefore, a multiplex assay has been recently developed for the simultaneous detection of multiple antibodies. An assay based on 14 *Plasmodium* specific peptides, 1 peptide specific for *Anopheles gambiae* saliva protein and 5 *Plasmodium* specific recombinant proteins was developed for the MAGPIX system and applied on blood spots from 2000 individuals collected in the Ratanakiri province, Cambodia. This project fits within the framework of a research project that aims to evaluate the effect of mass use of safe and effective mosquito repellents on the malaria transmission, in addition to the use of impregnated mosquito nets (MalaResT). For all antigens the assay performed equally well in monoplex and in multiplex formats ($p<0.001$). High specific antibody levels and a high seropositivity were detected for antibodies against Pf-LSA3-RE, Pf-CSP, Pf MSP1-19, Pf-GLURP, Pf-SALSA2 and Pf-GLURP-R2 with the comparison between the different serological markers. Blood samples positive for malaria by PCR showed a higher response to some of the antigens as compared to PCR negative samples. An increase in seropositivity was observed with increasing age. Differences in seropositivity were observed between different districts, indicating the heterogeneity of malaria transmission within the most endemic province of Cambodia.

927

MALARIA ELIMINATION - NOT YET ACCOMPLISHED IN ZANZIBAR

Deler Shakely[1], Abdullah S. Ali[2], Ali K. Abbas[2], Mwinyi Msellem[2], Achuyt Bhattarai[1], Netta Beer[1], Abdul-wahiyd Al-mafazy[2], Kristina Elfving[3], Jackie Cook[1], Weiping Xu[1], Ulrika Morris[1], Alison Rand[4], Max Petzold[5], Rahila Omar[2], Peter McElroy[6], Chris Drakely[4], Andreas Mårtensson[1], Anders Björkman[1]

[1]Karolinska Institutet, Stockholm, Sweden, [2]Zanzibar Malaria Elimination Programme, Ministry of Health, Zanzibar, United Republic of Tanzania, [3]Department of Infectious Diseases, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom, [5]Centre for Applied Biostatistics, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [6]Centers for Disease Control and Prevention, Dar es Salaam, Tanzania and Atlanta, GA, United States

Zanzibar represents a unique case study for potential malaria elimination in a previously high endemic area in sub-Saharan Africa. The Zanzibar Malaria Elimination Programme (ZAMEP) has implemented modern malaria control interventions since 2003. These interventions included combined vector control with long lasting insecticidal nets (LLIN) and indoor residual spraying (IRS) as well as improved malaria case management with rapid diagnostic tests (RDT) and artemisinin-based combination therapies (ACT) in all public health facilities. We have studied temporal trends of different malaria indices such as incidence of parasitologically confirmed malaria infections in public health facilities, crude child mortality and community parasite prevalence in two districts of Zanzibar, North A and Micheweni, between 1999 and 2013 in a population of about 200,000 people. Malaria transmission declined already after introduction of ACT and dropped further after addition of intensified vector control. Health facility data showed a 96% reduction in positivity rates (from 38.2% to 1.6%) of parasitologically confirmed malaria cases in the two districts between 2002 and 2012 with a relative shift towards older age groups and an increasing seasonability of the malaria incidence. In North A district, all cause <5 years mortality rate decreased by 70%. A population survey in 2013 revealed a malaria prevalence of 0.3% determined by RDT representing a 97% reduction compared to 2003 (10.3%). However, the parasite prevalence as determined by PCR was 10-fold higher, indicating a remaining reservoir of asymptomatic low-density parasitemias. Moreover, signs of minor increase of reported malaria infections from public health facilities occurred in Micheweni district in 2012 compared with 2010-2011. Malaria control in Zanzibar has reached a level equivalent to malaria pre-elimination. However, a new malaria epidemiological context has emerged necessitating new tools and strategies as well as re-orientations of ongoing control activities in order to further reduce malaria transmission.

928

STOCKS AND SHOPS: THE IMPORTANCE OF HEALTH SYSTEMS STRENGTHENING WITHIN PRIVATE SECTOR OUTLETS FOR THE DELIVERY OF ARTEMISININ COMBINATION THERAPIES (ACTS) TOWARDS MALARIA CONTROL AND ELIMINATION

V. Bhargavi Rao[1], Jamie Griffin[1], IMPACT 2 Study Team[2], Lucy Okell[1], David Schellenberg[3], Azra Ghani[1]

[1]Imperial College London, London, United Kingdom, [2]Ifakara Health Institute (Tanzania)/Centers for Disease Control and Prevention (USA)/ London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom

There remains debate regarding the inclusion of Informal sources of antimalarials, ranging from drug shops and pharmacies to general village stores, within national malaria control planning. The Affordable Medicines facility for malaria (AMFm) has been effective in increasing stocks of subsidised quality-assured Artemisinin Combination Therapies

(ACTs) within drug shops but the impact of this initiative remains unclear. We extended an existing mathematical model of malaria transmission to include health systems factors: access to sources of ACTs in the private, public and tertiary sectors, and quality of care for malaria and non-malarial febrile illness (NMFI). Data from the IMPACT 2 study in Tanzania was used to parameterise the model. Our aim was to estimate the impact of interventions in the private sector on malaria mortality and parasite prevalence (i.e. transmission risk). We compared the effect in three settings, which differed by epidemiology and prevailing health systems. Model outcomes suggest in areas where there is high private sector preference, strengthening of public facilities has less impact than anticipated. Improving stocks of ACTs in the private outlets, as per AMFm, was predicted to be more effective than in public clinics in all three regions considered, with a greater relative impact on parasite prevalence than mortality; likely due to high levels of NMFI overtreatment thereby opportunistically treating asymptomatic and sub-patent infections. Modelling the rollout of diagnostic led therapy in drug shops reduced missed cases and thus mortality without increasing prevalence, but only if adequate stocks of ACTs were present. At low and medium transmission settings, pharmacy accreditation schemes to limit the diversity of informal private sources, and improve ACT stock and dispensing practices may have the potential to interrupt transmission, reducing parasite prevalence by up to 86% in scenarios with private sector preference. However investment would be required across the spectrum of case management to improve the quality of care delivered. Innovative methods need to be found to harness the private sector cost-effectively in the push for control and elimination.

929

IDENTIFYING AND CHARACTERIZING ASYMPTOMATIC MALARIA INFECTIONS IN ZANZIBAR: THE RESIDUAL PARASITE RESERVOIR

Ulrika Morris[1], Weiping Xu[1], Delér Shakely[1], Jackie Cook[1], Mwinyi I. Msellem[2], Abdullah S. Ali[2], Achuyt Bhattarai[3], Gabrielle Fröberg[1], Anders Björkman[1], Andreas Mårtensson[1]

[1]Karolinska Institutet, Stockholm, Sweden, [2]Zanzibar Malaria Elimination Programme, Ministry of Health, Zanzibar, United Republic of Tanzania, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

Asymptomatic *Plasmodium* infections are an important reservoir for continued malaria transmission and must be targeted to achieve malaria elimination. Sensitive molecular methods have shown that asymptomatic infections are often of very low-density in low endemic settings, frequently falling below the detection limit of both rapid diagnostic tests (RDT) and microscopy. This study aimed to identify and characterise asymptomatic low-density malaria infections in Zanzibar by active case detection employing community-based screening for *Plasmodium* infections by real-time PCR. Dried blood spots on filter paper (Whatman 3mm) were collected from asymptomatic individuals participating in cross-sectional surveys conducted in 2005, 2009, 2011 and 2013. For each respective year 548, 2324, 2978 and 3038 samples were screened for parasite DNA using a highly sensitive Cytochrome B SYBR green real-time PCR for Pan-*Plasmodium* detection and restriction fragment length polymorphism assay for species determination. The PCR determined asymptomatic parasite prevalence was 25.7% (CI95% 21.7-29.0), 3.3% (2.6-4.0), 2.2% (1.6-2.8) and 2.3% (1.7-2.8) in 2005, 2009, 2011 and 2013, respectively. The corresponding yearly microscopy/RDT positivity rates were 10.9% (CI95% 9.2-12.7), 0.0% (0-0.3), 0.7% (0.4-1.3) and 0.3% (0.13-0.54). Children 5-15 years and young adults 15-25 years had higher prevalence of asymptomatic malaria (range 2.7-52% and 3.7-13.7%, respectively) as compared to children <5 years and adults (range 0.5-24.5% and 1.7-8.6%). *P. falciparum* remained the predominant species (2.0-25.4%) followed by P. malariae (0.3-3.1%); no cases of P. ovale or *P. vivax* were identified. Although the asymptomatic malaria prevalence has declined since 2005, this study revealed a substantial remaining reservoir of low-density, sub-microscopy/RDT but PCR detectable parasitaemias among asymptomatic individuals in Zanzibar. The results highlight the need for sensitive molecular methods for identifying and targeting the residual parasite reservoir in malaria elimination programmes.

930

CLONAL OUTBREAK OF *PLASMODIUM FALCIPARUM* IN EASTERN PANAMA

Nicanor Obaldia, III[1], **Nicholas K. Baro**[1], Jose E. Calzada[2], Ana M. Santamaria[2], Rachel Daniels[1], Wesley Wong[1], Hsiao-Han Chang[1], Elizabeth J. Hamilton[1], Myriam Arevalo-Herrera[3], Socrates Herrera[3], Dyann F. Wirth[1], Daniel L. Hartl[4], Matthias Marti[1], Sarah K. Volkman[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Instituto Conmemorativo Gorgas de Estudios de la Salud, Panamá City, Panama, [3]Centro de Investigación Científica Caucaseco, Cali, Colombia, [4]Harvard University, Cambridge, MA, United States

Reemerging parasite populations threaten to undermine efforts of global malaria eradication. Identifying the source of resurgent parasites is therefore paramount to strategic and successful intervention for malaria elimination. Genetic tools such as the malaria barcode to "fingerprint" parasite linages are anticipated to help identify sources of parasite outbreaks and track their spread. Panama has been notably successful in reduction of *Plasmodium falciparum* with only one reported case in 2011. Although malaria incidence in Panama has remained low over the past two decades, a major outbreak from 2001 to 2005 resulted in more than 60 percent of all cases reported over the past 35 years. We hypothesized that parasites from this epidemic might be highly related genetically and exhibit a largely clonal population structure. We thus tested the relatedness of *Plasmodium falciparum* parasites from this outbreak using informative single nucleotide polymorphisms and also examined drug resistance loci from among these parasites. We found the parasites to be clustered into three clonal subpopulations (AMOVA, 96%, $p = 0.001$) among eastern Panamanian isolates and shared genetic relatedness with parasites sampled from Colombia. Structure analysis revealed there was most likely two populations ($\Delta K = 108$), however given the clone-corrected clusters and AMOVA results, we found the second most likely number clusters ($\Delta K = 61$) revealed additional sub-population structure. Two clusters of Panamanian parasites from the epidemic shared identical drug resistance haplotypes, and all clusters shared a chloroquine resistance genotype matching the pfcrt haplotype found among parasites of Colombian origin. Our findings suggest these resurgent parasite populations are highly clonal and likely resulted from epidemic expansion of imported or vestigial cases. Outbreak investigation using genetic tools can illuminate the relatedness and potential sources of epidemic malaria cases and guide strategies to prevent further resurgence in areas of malaria elimination.

931

THE AGRICULTURE - MALARIA RELATIONSHIP AND THE CONTRIBUTIONS OF DELOS LEWIS VAN DINE TO THE MALARIA PROBLEM IN THE SOUTHERN U.S.

Edward D. Walker

Michigan State University, East Lansing, MI, United States

Human malaria has a strong association with agricultural activity. Increased food production via cultivation and irrigation often increases malaria risk in malaria-endemic zones, creating a conflict between agricultural enterprises and health. Conversely, malaria stultifies agricultural productivity by causing illness in laborers at critical times. This presentation highlights the efforts of USDA entomologist Delos Lewis Van Dine to quantify the impact of malaria on cotton production in Louisiana in 1914 and argues that it is was the first effort to connect malaria and agriculture fiscally. The study, conducted on the Hecla Plantation in the Mississippi delta, showed high malaria prevalence in black tenant farmers beholden to white plantation owners and that lost profits of $3.98 per acre could be attributed to malaria. It further demonstrated that impoundment of bayou waters and

water level manipulation eliminated *Anopheles* breeding near tenant farmers' shacks. Overall, this poorly known but seminal project provides an early, effective demonstration of intersectoral malaria control well before drugs and insecticides were available and that investment from the agricultural sector was key to malaria reduction.

932

SUBMICROSCOPIC PARASITEMIA IN RURAL AND URBAN SOUTHWESTERN NIGERIA

Adeola Y. Olukosi, Olusola Ajibaye, Bassey Orok, Bamidele Iwalokun, Chimere Agomo, Oluwagbemiga Aina, Oluwagbemiga Aina, Hilary Okoh, Davis Nwakanma

Nigerian Institute of Medical Research, Lagos, Nigeria

Epidemiology of submicroscopic parasitemia will become more relevant in determining malaria control measures in the era of elimination. This study evaluates the performance of microscopy and qPCR in detecting and quantitating malaria parasites in an urban and a rural sites in South West Nigeria.Cross-sectional surveys were conducted in hospital laboratories in rural Ijede and urban Lekki, spanning the period 2008-2010. Malaria parasite detection and quantitation using microscopy and qPCR were compared for these two sites. Prevalence at the urban site was 29% (330/1158) by PCR and 11%(111/997) by microscopy while at rural Ijede corresponding values were 30.2% (197/652) and 25%(168/658) respectively. The differences between the prevalence values at both sites were not significant by PCR (χ2=0.52, P=0.47) but very significant by microscopy (χ2=57.6, P<0.0001). Overall composite sensitivity for microscopy(41%) was lower compared to PCR (95%) while their respective specificities were 98% and 95%. At individual study sites, sensitivity of microscopy was better at the rural site with 98% than at the urban site 30% and specificities at both sites were 99% and 98% respectively. Sub-microscopic parasitaemia at the rural site was less, 74/197(37.5%), compared with the urban 211/336(62.2%), (χ2=30.34, P<0.0001). This was reflected in variance in their geometric mean parasite density(GMPD) observed-6999p/µL at the rural, and 2701p/µL at the urban sites by microscopy and corresponding values of 4693 p/µL and 1100 p/µL by qPCR (Cohen's Kappa agreement 0.48, Spearmans rank's correlation coefficient was 0.5, CI 0.37-0.59, mean difference of log units in the Bland Altman plot of 0.87; and 95% limits of agreement(mean ± 2SDs) between the two methods, 1.5 to 3.1.In spite of apparent lower prevalence of malaria parasites by microscopy in rural Ijede compared to urban Lekki, the actual prevalence when PCR technique is employed shows that parasite prevalence are similar in both study sites. Result from this study highlights a possible role for more sensitive diagnostic method in urban and other apparent regions of diminished parasitemia load and prevalence, over rural settings.

933

ON THE ESTIMATION OF MALARIA TRANSMISSION INTENSITY USING SEROLOGICAL DATA: POWER CALCULATIONS AND ESTIMATION BIAS

Nuno Sepulveda, Chris Drakeley

London School of Hygiene & Tropical Medicine, London, United Kingdom

Parasite prevalence and entomological infection rate are two popular measures of malaria burden in a given population. An alternative measure is the so-called sero-conversion rate that can be estimated from sero-prevalence data of specific anti-parasite antibodies in the serum using a series of reverse catalytic models. In theory, sero-conversion rate is the frequency by which individuals convert from a sero-negative state into a sero-positive one upon parasite challenge. In practice, sero-conversion rate has proven to be strongly correlated with the underlying force of infection, suggesting its routine use in malaria epidemiology. Statistically speaking, little is known about the expected behavior of the corresponding estimates under different estimation methods, sampling schemes, and disease dynamics. This knowledge is particularly instrumental in helping the design of future field studies. In this work we perform a comprehensive theoretical study using simulated data from typical cross-sectional, health-facility and school surveys. Specifically, we generate sero-prevalence data assuming either a constant sero-conversion rate over time or assuming a change in it after a field intervention. We assess the underlying estimation bias and statistical power in order to calculate the minimum sample size required to (1) estimate sero-conversion rate with a given precision, (2) or to detect any change in disease transmission after an intervention with a given probability. We also suggest different statistical strategies to reduce estimation bias introduced by using convenience sampling or the maximum likelihood estimation method. Finally, we present a new R package for sero-prevalence data analysis that can be easily used by malaria epidemiologists.

934

THE POTENTIAL TO TEST AND TREAT MALARIA IN NIGERIA: RESULTS FROM NATIONAL OUTLET SURVEYS (2009-2013)

Raymond K. Sudoi[1], Julius N. Ngigi[1], Chinazo N. Ujuju[2], Jennifer Anyanti[2], Vamsi Vasireddy[3], Megan Littrell[1]

[1]Population Services International, Nairobi, Kenya, [2]Society for Family Health, Abuja, Nigeria, [3]Independent Consultant, Chicago, IL, United States

The WHO T3 initiative: Test, Treat and Track promotes effective management of suspected malaria cases through diagnosis, effective treatment and accurate surveillance. In Nigeria, children with fever are taken to private (46%) or public sector providers (18%) for treatment. We assessed readiness of these outlets to provide malaria testing and treatment and the cost to the consumer. Nationally-representative antimalarial medicine outlet surveys were conducted in 2009, 2011 and 2013 as part of the ACTwatch project. A complete census of all outlets with the potential to stock antimalarials within selected clusters was conducted. Outlet types were classified as either public/not-for-profit or private for profit. The primary monitoring indicators were the weighted proportion of outlets offering/stocking any malaria diagnostics or Quality Assured Artemisinin-based Combination Therapy (QAACT). Data on the cost QAACTs to the consumer were also collected. Among outlets that had any antimalarial medicines in the public sector, 22% (2009) and 31% (2011) reported having malaria blood testing available on the day of interview. This compares with 2% (2009) and 4% (2011) of outlets in the private sector. Availability of QAACTs in the public sector remained stable at 44% (2009) and 48% (2011). In the private sector availability remained low at 7% (2009) and 10 % (2011). In the private sector, QAACT prices decreased from $3.94 (2009) to $1.40 (2011). 2013 survey findings will be reported at the conference. Across survey rounds, availability of malaria blood testing and QAACTs is lower in the private sector compared to the public sector. While readiness for malaria case management is improving in the public sector, nearly half of all children with suspected malaria in Nigeria are taken to the private sector where most of these providers are not equipped to appropriately test and treat cases. Improving readiness of the private sector for malaria case management in Nigeria may be an important strategy to improve effective coverage.

935

FORECASTING THE BURDEN OF MALARIA IN UGANDA USING CLINICAL AND ENVIRONMENTAL PREDICTORS

Kate Zinszer[1], Ruth Kigozi[2], Katia Charland[1], Grant Dorsey[3], Timothy Brewer[4], John Brownstein[5], Moses Kamya[6], David Buckeridge[1]

[1]McGill University, Montreal, QC, Canada, [2]Uganda Malaria Surveillance Project, Kampala, Uganda, [3]University of California San Francisco, San Francisco, CA, United States, [4]University of California Los Angeles, Los Angeles, CA, United States, [5]Harvard Medical School, Boston, MA, United States, [6]Makerere College of Health Sciences, Kampala, Uganda

Malaria thrives in poor tropical and subtropical countries where local resources are limited. Accurate disease forecasts can provide public health and clinical organizations with the information needed to implement targeted approaches for malaria control that make effective use of limited resources. Previous malaria forecasting work has not considered clinical predictors, such as antimalarial treatment, which is an important predictor of malaria burden in a population. The objective of the research was to identify the environmental and clinical predictors that produce the most accurate forecasts of malaria at six different health facilities in Uganda. Malaria forecasting models were developed using health facility data collected by the Uganda Malaria Surveillance Project and satellite-derived rainfall, temperature, and vegetation estimates. Short-term (4-week) and long-term (52-week) weekly forecasts of confirmed malaria from June 1, 2012 to May 31, 2013 were developed for each health facility using multivariate transfer function models. The model with the most accurate forecasts varied by site. Clinical predictors were retained in the most accurate models across all facility sites with the exception of one model. The average short-term error ranged from 20% to 96% over the forecasting period. The long-term models performed best for predicting the cumulative cases with error ranging from 2% to 22%. Study limitations included not knowing if the confirmed malaria cases were incident or recrudescent as well as measurement errors associated with the remote sensing and clinical data sources. Incorporating clinical predictors such as type of antimalarial treatment, improved the forecasting accuracy of several of the models. These results demonstrate the utility of using clinical predictors in conjunction with environmental predicators to forecast malaria. With the mounting cost of the global fight against malaria and the drive towards elimination in many countries, accurate forecasts of malaria remain essential.

936

FETAL GROWTH RESTRICTION IS A MAJOR FACTOR INVOLVED IN GESTATIONAL MALARIA IN BENIN

Valerie Briand[1], Jessica Saal[1], Caline Ghafari[1], Bich-Tram Huynh[2], Achille Massougbodji[3], Jennifer Zeitlin[4], Michel Cot[1]

[1]UMR216, Research Institute for Development, Université Paris Descartes, Sorbonne Paris cité, Paris, France, [2]Institut Pasteur Paris, Pharmacoepidemiology and Infectious Diseases Unit, Paris, France, [3]Faculté des Sciences de la Santé, Centre d'Étude et de Recherche sur le Paludisme Associé à la Grossesse et l'Enfance, Cotonou, Benin, [4]Institut national de la santé et de la recherche médicale, Obstetrical, Perinatal and Pediatric Epidemiology Research Team, Center for Epidemiology and Biostatistics (U1153), Paris-Descartes University, Paris, France

Malaria in pregnancy (MiP) leads to low birth weight, which is mainly due to intra-uterine growth restriction in Africa. However, when and how malaria impacts on fetal growth is still unknown. We investigated this issue using data on 774 Beninese pregnant women from the STOPPAM cohort. They were screened for malaria (using blood smears) every month during pregnancy until delivery and had 4 ultrasound scans at 20, 26, 30 and 36 weeks of gestation, allowing repeated measurements of growth within the same pregnancy. For the analysis, birth weights (BW) and estimated fetal weights for gestational age were converted into Z-scores using Tanzanian sex-specific charts as reference. First, BW Z-scores were compared between women infected and uninfected with malaria during pregnancy. Then, the effect of malaria on fetal growth, measured as a change in Z-scores between two consecutive measurements, was assessed. Both analyses were adjusted for potential confounding factors such as maternal undernutrition, maternal anemia and gravidity. More than 40% of women had at least one malarial infection during pregnancy. We showed that women infected in the first and at least one subsequent trimester of pregnancy had significantly lower BW Z-scores (-0.40 [-0.78; -0.01]) than uninfected women during pregnancy. We did not find that malaria limited to either the 1st trimester or the 2nd/3rd trimester was associated with BW Z-scores, suggesting a cumulative effect of malaria on fetal growth. A decrease in Z-scores during pregnancy was significantly associated with malaria infections that occurred several weeks before the decrease (Z-scores decrease in women infected with malaria compared to non infected women: -0.23 [-0.40; -0.05]), but not with recent malaria infections. In conclusion, we confirmed the effect of malaria on fetal growth in Africa. Our results suggest both a long-term and cumulative effect, starting from the 1st trimester onwards.

937

USING GENOME-WIDE SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) TO TRACK MALARIA SPREAD AMONG LOCAL COMMUNITIES IN THE MYANMAR-CHINA BORDER AREAS

Eugenia Lo[1], Winny Oo[1], Guofa Zhou[1], Elizabeth Baum[1], Liwang Cui[2], Guiyun Yan[1]

[1]University of California Irvine, Irvine, CA, United States, [2]Pennsylvania State University, Pennsylvania, PA, United States

Border areas are reservoirs of malaria because of frequent and recurrent parasite introductions via human movements. Areas along the international border of Myanmar and China have reported high malaria incidence in recent years. In Myanmar, civil unrest and establishment of internally displaced persons (IDP) camps along the Myanmar-China border have impacted much of the malaria transmission in the region. The growing IDP populations raise deep concerns about health impact on local communities. Moreover, in remote countryside of poor accessibility and general negligence, and where the ethnic minorities reside, over 60% of the populations are potentially at high risk of malaria. This study used genome-wide Single Nucleotide Polymorphisms (SNPs) and microsatellite markers to examine the source and spreading patterns of malaria parasites (*Plasmodium falciparum* and *P. vivax*) between IDP camps and surrounding villages in Myanmar, as well as villages/towns in the border areas of Myanmar-China. We compared genotypic composition of *Plasmodium* samples collected in 2011, 2012, and 2013 from the same area and examined demographic history with the goal to determine whether recent infections were caused by the same or different parasite genotypes. In addition, we tested if IDP camps are the source of malaria infections particularly concerning border-malaria cases based on genetic diversity differences among localities. Broadly, we tested the hypothesis of whether *P. vivax* is genetically more diverse than *P. falciparum* under the same environmental and temporal settings at a local scale. In-depth knowledge and information on the extent of malaria spread are keys to target disease control efforts in high-risk areas such as those near international borders and remote countryside. This is of particular relevance when most other parts of Southeast Asia are entering the malaria elimination phase.

938

USE OF GPS DATA LOGGERS TO DESCRIBE SPATIO-TEMPORAL HUMAN MOVEMENT PATTERNS IN AN AREA OF EFFECTIVE MALARIA CONTROL IN SOUTHERN ZAMBIA

Kelly M. Searle[1], Jailos Lubinda[2], Timothy Shields[1], Harry Hamapumbu[2], Frank Curriero[1], Phil Thuma[2], William J. Moss[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Macha Research Trust, Macha, Zambia

The role of human movement in malaria transmission dynamics, particularly in pre-elimination settings, has not been fully elucidated. GPS data loggers allow for micro-scale estimates of human movement in rural areas of sub-Saharan Africa, which can aid in explaining the micro-epidemiology of malaria importation and transmission in these areas. Participants currently enrolled in a longitudinal cohort study of the impact of malaria control measures in a region of declining malaria transmission in southern, Zambia (one of three Southern Africa International Centers of Excellence for Malaria Research sites) were invited to participate in a population movement study using GPS data loggers. Approximately 10 participants at a time were asked to carry the loggers for a one-month period. Data will be collected over 12 months to account for seasonality in movement patterns. Enrollment began in October 2013 and is ongoing. Serial numbers of GPS data loggers were matched to participant IDs and geographic position logged every two minutes. Movement data from the GPS loggers were imported into ArcGIS for pre-processing and analysis. The movement tracts were used to determine the cumulative amount of time spent in different areas, derived from the frequency of visits and amount of time spent during each visit to different areas. An intensity map was created to display the cumulative amount of movement in different areas. An analysis of time spent in and around the residence was conducted. The distribution of time spent in and around the household and time spent at varying distances from the household was analyzed, and the cumulative amount of time spent in areas of high and low malaria risk was calculated. An analysis to determine whether time spent inside an area of high malaria risk is due to residence in a hotspot or routine travel to a location of high malaria risk was conducted.

939

PLASMODIUM VIVAX MALARIA IS NOT A MAJOR THREAT IN MADAGASCAR

Milijaona Randrianarivelojosia[1], Jemima Mahatovo[1], Elisabeth Ravaoarisoa[1], Leonora Ravolanjarasoa[1], Dominique Rasoloson[2], Christophe Rogier[1]

[1]Institut Pasteur de Madagascar, Antananarivo, Madagascar, [2]Johns Hopkins University, Baltimore, MD, United States

Published reports on malaria in Madagascar in the last seven decades showed that *Plasmodium vivax*, *P. ovale* and *P. malariae* are present; however, *P. falciparum* remains the predominant species. Effort has been achieved with the introduction of artemisinin-based combination therapy (ACT) and the scaling-up of the vector control since 2006. The last two malaria index surveys among children in Madagascar from 6 to 59 months of age (n = 6154 in 2011 and n = 5504 in 2013) showed that malaria transmission is highly variable in different areas. Global malaria prevalence ranged from 1% in the highlands to 15% on the rainy eastern coast. *P. falciparum* infection was responsible for >99% of the cases. In those two surveys, only one *P. vivax* infection (1/863) and two *P. malariae* infections (2/863) were detected. It was shown recently that *P. vivax* is capable of breaking through the Duffy-negative barrier in Madagascar, leading some policy makers to consider introducing *P. vivax* specific treatments. Available data, however, indicate that at this time, *P. vivax* is not a real threat in Madagascar. With its local population of both Asian and African origin, Madagascar is an ideal place to investigate the fundamental aspects of the *P. vivax* biology including the molecular mechanisms underlying red blood cell invasion. The key issues in malaria elimination in Madagascar are the recurrent shortage of stocks of ACT; the interruption of insecticide indoor spraying and the irregularity in insecticide treated bed net distribution. Nonetheless, the impact of the intervention against malaria in Madagascar on *P. vivax* between 1940 and 2000 will be also discussed in our presentation.

940

G6PD GENOTYPE AND THE IMPACT ON PREGNANCY OUTCOME IN WOMEN INFECTED WITH *PLASMODIUM FALCIPARUM* AND *P. VIVAX* MALARIA: DESCRIPTIVE STUDY OF 1749 WOMEN

Germana Bancone[1], Nongnud Chowwiwat[1], Mary Ellen Gilders[1], Aye Min[1], Nay Win Tun[1], Rose McGready[1], Nicholas J. White[2], Francois Nosten[1]

[1]Shoklo Malaria Research Unit, Mae Sot, Thailand, [2]Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand

G6PD deficiency is the most common enzymatic disorder in human, and its distribution closely matches that of malaria. Nonetheless, a protection from malaria infection and parasitemia in G6PD deficient subjects does not seem to explain completely the rapid spread of this genetic trait in several populations. The associations between G6PD status, malaria and pregnancy outcome have not been well characterized although a protective effect might be hypothesized. A large cohort of pregnant women from malaria treatment and prevention studies carried out along the Thai-Myanmar border was characterized for G6PD genotype, and the impact of falciparum and vivax malaria infections on pregnancy outcomes was compared. The major local mutation, Mahidol, was detected through PCR-RFLP in DNA extracted from filter papers collected in the past 20 years in 1749 pregnant women. Malaria was detected by microscopy during active screening at ANC consultations. Demographic data, obstetric history, gestational age and clinical symptoms were recorded as well as pregnancy and neonatal outcomes. Significant differences in the proportion of women with symptomatic infections, onset of anaemia, miscarriage, stillbirth, birth weight and sex of the newborn were observed according to their G6PD*Mahidol genotypes. Protection from G6PD was not always observed for both falciparum and vivax infections.

941

MALARIA IN BORDERLANDS: A CASE STUDY FROM THE THAILAND-MYANMAR BORDER

Daniel Parker[1], Stephen A. Matthews[1], Jetsumon Sattabongkot[2], Jeeraphat Sirichaisinthop[3], Kirakorn Kiattibutr[2], Guiyun Yan[4], Guofa Zhou[4], Mieng-Chieh Lee[4], Qi Fan[5], Peipei Li[5], Liwang Cui[1]

[1]Pennsylvania State University, University Park, PA, United States, [2]Mahidol University, Bangkok, Thailand, [3]Bureau of Vector Borne Diseases, Pra Phuttabhat, Thailand, [4]University of California Irvine, CA, United States, [5]Dalian Institute of Biotechnology, Dalian, China

The central plains of Thailand have been mostly malaria free now for several decades. However, malaria continues to persist in the borderlands connecting the Thai Kingdom with Laos, Cambodia, Malaysia, and Myanmar. The reasons for this persistence are complex. These are areas with high ecological, ethnic, and linguistic diversity. These borderlands have been exposed to political instability that has existed across the borders in Cambodia, Laos, and Myanmar. The common logic on the ground is that the malaria situation on the Thai side is a spill-over effect of the malaria situation on foreign soil. In this research, we take a micro, spatial-epidemiological approach to understanding border malaria, using a study site along the Thai-Myanmar border. The underlying goals of this work were to empirically test for demographic factors (including migration) as risks for malaria infection; in the potential for asymptomatic carriers to exist in a region that is considered to be a "low transmission" area; and in mapping space-time patterns in malaria cases in households. We find that a large proportion of cases are missed by microscopic diagnosis, that migration does not appear to be a significant risk factor for individuals or household members who live with migrants, and that individuals without

citizenship (either in Myanmar or Thailand) exhibit a significantly higher risk of malaria infection when compared to any other demographic group. Finally, the spatial distribution of malaria cases clusters tightly around year-round water sources within the village during the dry season, but expands throughout much of the village during the wet season. Taken together, these factors indicate that the border malaria situation is much more complex than generally considered. While this research does not rule out the potential of malaria importation from Myanmar, it does show that such importation isn't necessary for continued persistence of malaria on Thai soil.

942

USING GEOSTATISTICAL METHODS TO ANALYZE SPATIO-TEMPORAL CHANGES IN CHILDHOOD MALARIA FOLLOWING SCALE-UP OF CONTROL EFFORTS IN CHIKWAWA DISTRICT, MALAWI

Emanuele Giorgi[1], Dianne Terlouw[2], Peter John Diggle[1]

[1]Lancaster University, Lancaster, United Kingdom, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Geostatistical methods are increasingly used to support disease control efforts and analyse spatial variation in disease prevalence. These methods are especially useful in settings where disease registries are non-existent or geographically incomplete, and data on disease prevalence must be obtained via cross-sectional surveys of the population of interest. In order to obtain timely, accurate and (sub-)district estimates of prevalence, however, continuous prevalence surveys could provide a more powerful approach for identifying local hotspots and guiding more targeted control efforts. Applying novel model-based geostatistical methods for malaria mapping, we estimated changes in the spatial distribution of malaria prevalence over time using data from a continuous Malaria Indicator Survey conducted within a 20 x 20 km area of Chikwawa District in Malawi, from May 2010 to June 2013, a period of district-wide scale-up of malaria control. We developed a statistical model that accounts for temporal and spatial variation in prevalence, and over-dispersion within households. This approach allows us to quantify the uncertainty in prevalence estimates more accurately than standard statistical methods that ignore the temporal and spatial correlation induced by unmeasured risk factors for malaria and, as a result, give an exaggerated impression of regression relationships. To model seasonality accurately we used a linear combination of sinusoidal curves at different frequencies. The resulting time-series of malaria prevalence maps show how the model can provide an evaluation of control progress. The method can also be used to predict changes in prevalence that would result from different control progress scenarios, by running the model with different values for the input variables. Finally, visualizations using animations of spatio-temporal prevalence allow for a more intuitive interpretation by end-users that can guide more targeted control efforts towards hotspots.

943

DETERMINING THE CHANGE IN MALARIA ACROSS AFRICA FROM 2000 TO 2012

Samir Bhatt, Daniel J. Weiss, Ewan Cameron, Bonnie Mappin, Ursula Dalrymple, Peter Gething

University of Oxford, Oxford, United Kingdom

Decreases in malarial prevalence over the past decade have been mainly associated with the expansion of vector control measures such as insecticide-treated nets (ITNs) and changes in environmental and socio-economic factors. Here we seek to formally evaluate the determinants and patterns of the change in malaria prevalence from 2000 to 2012 across all of Africa. We first build Bayesian spatial models of malarial prevalence using a large assembly of geopositioned parasite rate surveys, along with new dynamic environmental and socioeconomic covariates varying through time. Then, using a combination of Bayesian compartment and spatial modelling we build models of ITN usage through time. Using this rich data set of prevalence, environmental factors, socioeconomic factors and ITN usage, all modelled continuously at a 5km by 5km resolution across all of Africa, we characterise the change that has occurred in the last decade.

944

AIR TEMPERATURE SUITABILITY FOR *PLASMODIUM FALCIPARUM* MALARIA TRANSMISSION IN AFRICA 2000-2012

Daniel J. Weiss, Samir Bhatt, Ewan Cameron, Bonnie Mappin, Ursula Dalrymple, Peter W. Gething

University of Oxford, Oxford, United Kingdom

Air temperature affects multiple aspects of the malaria transmission cycle, including vector survival and sporozoite development. These factors have been combined within a biological model to produce a single metric (temperature suitability) that is useful as a predictor variable in modeling and mapping malaria endemicity. In this study we improve on previous research endeavors by creating a dynamic temperature suitability product for *Plasmodium falciparum* in Africa, from 2000-2012, with a 1km spatial resolution, and a monthly temporal resolution. The temperature suitability product is generated using land surface temperature data derived from satellite imagery, which is converted to air temperature using an approach we develop, and then ingested into an improved version of an established temperature suitability model. Results indicate that the dynamic temperature suitability product is an improvement over earlier synoptic products, particularly with respect to its ability to characterize spatio-temporal patterns in areas with seasonally variable infection rates.

945

CONSENSUS FORECASTS OF CLINICAL DISEASE BURDEN FROM *PLASMODIUM FALCIPARUM*: A STOCHASTIC MODELING APPROACH

Ewan Cameron, Samir Bhatt, Daniel Weiss, Bonnie Mappin, Ursula Dalrymple, Peter Gething

University of Oxford, Oxford, United Kingdom

Mechanistic transmission models for "micro-simulation"---the stochastic, computational realization of disease dynamics at the individual level within a mock human population---represent a key tool for forecasting the burden of clinical disease from community prevalence estimates. These models reveal a complex, non-linear relationship and age-dependence to the parasite prevalence--clinical incidence relationship, owing to the effects of exposure-driven immunity, which in turn depends on a combination of the historical mean entomological innoculation rate and its seasonality profile. In this study we examine the predictions of multiple model variants and forge consensus forecasts of *Plasmodium falciparum* disease burden on national and continental scales.

946

MALARIA TRANSMISSION, INFECTION AND DISEASE AT THREE SITES WITH VARIED MALARIA TRANSMISSION INTENSITY IN UGANDA: IMPLICATIONS FOR MALARIA CONTROL

Emmanuel Arinaitwe[1], Humphrey Wanzira[1], Agaba Katureebe[1], Chris Barusya[1], Simon Peter Kigozi[1], Maxwell Kilama[1], Andrew J. Tatem[2], Philip J. Rosenthal[3], Chris Drakeley[4], Steve W. Lindsay[5], Sarah G. Staedke[4], David L. Smith[6], Bryan Greenhouse[3], Grant Dorsey[3], Moses R. Kamya[7]

[1]Infectious Diseases Research Collaboration, Kampala, Uganda, [2]University of Southampton, Southampton, United Kingdom, [3]University of California San Francisco, CA, United States, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom, [5]Durham University, Durham, United Kingdom, [6]John Hopkins University, Baltimore, MD, United States, [7]Makerere University College of Health Sciences, Kampala, Uganda

Intensification of control interventions has led to reductions in malaria burden in some, but not all settings. To describe the malaria epidemiology in Uganda, we conducted entomologic surveys and cohort studies at 3 sites with varying transmission intensity. All households were enumerated in 3 sub-counties: Walukuba (peri-urban), Kihihi (rural) and Nagongera (rural). In each sub-county, 100 houses were randomly selected for measures of malaria transmission, infection, and disease over 24 months. Annual entomological inoculation rate (AEIR) was estimated from monthly collections using CDC light traps. All children aged 0.5-11 years were provided a long lasting insecticide treated bed net (LLITN) and followed using active surveillance for measures of parasite prevalence and anemia every 3 months and passive surveillance to measure the incidence of malaria. Episodes of uncomplicated malaria were treated with artemether-lumefantrine and complicated malaria treated with quinine. Transmission was highly seasonal at all 3 sites, with 2 annual peaks. Estimates from Walukuba, Kihihi, and Nagongera, respectively were as follows: AEIRs of 3.3, 31.5, and 315 infectious bites per person year (PPY); parasite prevalence of 8.8%, 15.0%, and 41.5%; and malaria incidence of 0.48, 1.67 and 3.52 episodes PPY. Comparing the 1st and 2nd years, there was a significant decrease in the incidence of malaria in Walukuba (0.55 vs. 0.36 PPY, p=0.01) and significant increases in Kihihi (1.24 vs. 2.13 PPY, p<0.001) and Nagongera (3.06 vs. 3.98 PPY, p<0.001). Of 3140 episodes of malaria diagnosed, only 9 (0.3%) met criteria for severe disease with no cases of cerebral malaria or deaths. The prevalence of moderate (Hb<10) and severe (Hb<7) anemia were 8.5% and < 0.4%, respectively, and did not vary by transmission intensity. In the setting of LLITNs and prompt effective treatment, the risk of complicated malaria and anemia was very low. However, the burden of malaria remains high and increased at two rural sites, suggesting that additional malaria control interventions are needed in Uganda.

947

STATISTICAL INFERENCE OF *PLASMODIUM FALCIPARUM* TRANSMISSION NETWORKS BASED JOINTLY ON EPIDEMIOLOGICAL AND GENETIC DATA

Alex Perkins[1], Rasmus Nielsen[2], David L. Smith[3], Bryan Greenhouse[4]

[1]University of California Davis, CA, United States, [2]University of California Berkeley, CA, United States, [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [4]University of California San Francisco, CA, United States

Decisions about how to best allocate finite resources for malaria surveillance and control depend on a range of factors, including epidemiological quantities such as the proportion of local versus imported cases, the origin of imported cases, and variation in local transmission potential. Measurement of these quantities is difficult, however. For a variety of viral and bacterial pathogens, recent advances in genetic sequencing and statistical inference methodology have enabled the estimation of transmission linkages between small geographic areas or groups of infected hosts, and in some cases between individual hosts. Methods for making such estimates currently depend on the assumption that ample genetic variation is generated by neutral mutations on a timescale that is fast relative to that of transmission. Because this assumption is likely violated for *Plasmodium* parasites, which undergo a sexual reproductive phase and have a lower mutation rate than viruses, existing methods to build transmission networks from genetic and epidemiological data cannot be applied to malaria. To overcome these obstacles, we have developed bespoke statistical methodology for making inferences about transmission linkages between human malaria cases. This methodology makes use of the multi-locus genetic composition of an individual's parasites, the individual's home location, and the date when the infection was detected. We make explicit assumptions about the processes that generate and erode genetic variation, including superinfection, the importation of parasites from source populations, and the stochastic loss of alleles over the course of multiple transmission events. Using this mathematical framework for epidemiological dynamics and parasite evolution, together with Bayesian statistical techniques, we made inferences about transmission networks using simulated data and data sets from Zanzibar and Swaziland. These networks can be used to determine both population and individual-level parameters, such as R0 and the probability of a given infection being imported, respectively. We furthermore used simulated data to explore the robustness of this method to assumptions about the proportion of cases detected, mechanisms of inheritance, and epidemiological heterogeneities. Our results demonstrate the potential for this method to estimate key epidemiological parameters for malaria surveillance and elimination.

948

THE RELATIONSHIP BETWEEN THE PREVALENCE OF MALARIA IN PREGNANT WOMEN AND THE PREVALENCE OF MALARIA IN CHILDREN AND NON-PREGNANT WOMEN IN SUB-SAHARAN AFRICA

Anna M. van Eijk[1], Jenny Hill[1], Robert W. Snow[2], Feiko O. Ter Kuile[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Malaria Public Health Department, Kenya Medical Research Institute-Wellcome Trust Research Programme, Nairobi, Kenya

In malarious areas, malaria is more frequent in pregnant women than in age-matched non-pregnant women and the magnitude of the excess risk varies with age and exposure to malaria in previous pregnancies. Pregnant women attending for their first antenatal clinic visit are a potential pragmatic sentinel group to track malaria transmission intensity, however the relationship between malaria infection prevalence in asymptomatic pregnant women and other transmission indices, such as the prevalence of malaria infection in children obtained from population based cross-sectional surveys, a commonly used indicator for malaria transmission intensity, is not yet known. To determine if pregnant women can provide an alternative source of transmission intensity information, we evaluated the relationship between the prevalence of malaria among asymptomatic pregnant women and a) asymptomatic non-pregnant women and b) asymptomatic children (0-59 months) in the same area. Studies in sub-Saharan Africa were obtained using the malaria in pregnancy library (January 2014) and national surveys. We used random effects meta-analysis and meta-regression. The summary risk ratio (RR) of the malaria prevalence among pregnant vs non-pregnant women was 1.45 (95% CI 1.33-1.59), I-square 68% among all gravidae (51 studies in 32 records), and 2.10 (1.82-2.43), I-square 74% among primigravidae (19 locations). Information from 58 studies (18 records) was available for the comparison between pregnant women and children aged 0-5 years. The prevalence was highest in children: compared to all gravidae RR 1.45 (1.31-1.60), I-square 80%, and compared to primigravidae RR 1.13 (1.00-1.28), I-square 72% (5 studies). The malaria prevalence among primigravidae at the first antenatal booking visit may be a good approximation of the prevalence of malaria in children obtained from household surveys.

949

VARIABLE MALARIA PREVALENCE ON ISLANDS IN LAKE VICTORIA, WESTERN KENYA

Zulkarnain Md Idris[1], Chim W. Chan[1], Chang Sheng Deng[2], Masatsugu Kimura[3], Isao Teramoto[3], Ahmeddin Omar[4], Willis Akhwele[4], James Kongere[4], Akira Kaneko[1]

[1]Department of Microbiology, Tumor and Cell Biology, Stockholm, Sweden, [2]Research Center of Qinghao, Guangzhou University of Chinese Medicine, Guangzhou, China, [3]Department of Parasitology, Graduate School of Medicine, Osaka City University, Japan, [4]Division of Malaria Control, Ministry of Public Health and Sanitation, Nairobi, Kenya

Kenya launched its second National Malaria Strategy with a notably ambitious vision for a "*malaria free Kenya*", but malaria remains a major health problem among communities in Lake Victoria, We conducted two malariometric surveys during the dry (January) and wet (August) seasons in 2012 to determine the prevalence, geographical and seasonal variation of malaria among communities in the coast (Ungoye: population ~2,000) and on islands of Lake Victoria (Mfangano [large island]: population ~25,000; Takawiri, Kibuogi, and Ngodhe [small islands]: population ~700 each). Overall, parasite rates (PRs) as determined by microscopy (18.9% vs 14.5%), rapid diagnostic test (36.9% vs 31.9%) and PCR (31.1% vs 25.8%) were higher in the dry season than in the wet season ($P<0.01$) with characteristic age distribution. The highest prevalence by RDT was observed in Ungoye (dry season): 54.4% in age group 0-5 years old, 68.4% in 6-10, 55.3% in 11-15, 13.2% in 16-30 and 11.3% in >30, while the lowest was in Takawiri: 13.1%, 21.5%, 11.5%, 7%, and 1%, respectively. Species-specific prevalence by PCR was 29.3% for *Plasmodium falciparum*, 8.5% for *P. malariae*, and 2.1% for *P. ovale* in the dry season, and 24.5%, 6.0%, and 2.1%, respectively, in the wet season. No *P. vivax* was detected. Prevalence of mixed infections was 8.3% and 5.8% in the dry and wet seasons, respectively. In both seasons, PRs were highest in the coast, followed by large island and lowest in the small islands, with significant fluctuations in islands but not the coast. Fluctuations in PRs among island settings were significant only in children and young adolescents but not in adults. PRs were correlated with prevalence of fever in both seasons ($P<0.05$), however they were correlated with enlarged spleen in the dry season only ($P< 0.01$). Paradoxically, PRs were either not correlated (wet) or negatively correlated (dry) with rates of anaemia. Overall prevalence of G6PD deficiency was 12% in male and 2% in female. No significant correlation between the deficiency rates and PRs was observed among islands and the coast. Variation in malaria prevalence reflected the different dynamic of malaria transmission between the islands and the coast of Lake Victoria. Our results provide baseline data for the planned feasibility study of malaria elimination on islands in Lake Victoria.

950

MALARIA AND ANEMIA PREVALENCE TWO YEARS FOLLOWING MASS NET DISTRIBUTION IN PLATEAU STATE, NIGERIA

Gregory S. Noland[1], Amy E. Patterson[1], Adamu K. Sallau[2], Abel Eigege[2], Christopher Bewa[3], Joseph Ajiji[3], James Damen[4], Bulus Mancha[2], Kal Alphonsus[2], Masayo Ozaki[1], Patricia M. Graves[1], Emmanuel Miri[2], Frank O. Richards[1]

[1]The Carter Center, Atlanta, GA, United States, [2]The Carter Center, Jos, Nigeria, [3]Plateau State Ministry of Health, Jos, Nigeria, [4]University of Jos, Jos, Nigeria

Nigeria suffers the world's largest malaria burden and in 2009 it launched a plan to provide two long-lasting insecticidal nets (LLINs) to every household via state-level mass distribution campaigns. In Plateau State (pop. 3.6 million) in central Nigeria, a baseline survey was conducted in September 2010 prior to mass net distribution of 1.4 million LLINs in December 2010. Two years later (October 2012), a modified malaria indicator survey was conducted to compare changes in net ownership, utilization, IRS coverage, *Plasmodium* prevalence and childhood anemia. In each survey >1,300 households in at least 58 clusters were sampled. Household ownership of at least one net increased from 35.1% in 2010 to 74.0% in 2012 ($P<0.001$), while ownership of two or more nets increased from 14.5% to 50.1% ($P<0.001$). In 2012, households had a mean of 1.6 nets per household, 0.62 nets per sleeping space and 0.31 nets per person. Overall reported net use the night before the survey among all individuals, children <5 years, and pregnant women was 49.0%, 59.0% and 60.5%, respectively in 2012 among all households (all $P<0.001$ versus 2010) and 64.5%, 77.6% and 79.1%, respectively, among households owning at least one net (all $P<0.001$ versus 2010). IRS coverage remained low (<1% in both surveys). Between 2010 and 2012, crude *Plasmodium* prevalence by microscopy decreased by 58% from 47.7% (n=4,209) to 19.9% (n=3,911; $P<0.001$). However, parasite prevalence by rapid diagnostic test (RDT) in 2012 (36.7%) was not significantly lower compared to 2010 RDT prevalence (40.5%, $P=0.16$) using the same test (CareStart Pf/PAN). Prevalence was highest among children 5-9 years old. *Plasmodium malariae* accounted for 3.7% of infections diagnosed by microscopy. Anemia in children ≤10 years was equally prevalent in 2012 (57.8%) and 2010 (57.1%). These results, believed to be the first state-level report of the impact of mass net distribution in Nigeria, document significant increases in net coverage and usage that correspond with a decrease in parasite prevalence as diagnosed by microscopy, but not RDT.

951

DETERMINANTS OF SOCIO-ECONOMIC STATUS AND RISK OF MALARIA INFECTION IN PANAMA (2009-2012)

Nicanor III Obaldia

Harvard School of Public Health, Boston, MA, United States

In the Americas malaria remains a major problem in areas with low socioeconomic-status (SES). In order to achieve elimination, current strategies must have a multi-sectorial approach that addresses social and environmental determinants of malaria infection. The current focal low incidence of malaria in Panama presents a unique opportunity to start an elimination phase. In this study we examine the epidemiology of malaria in Panama with special reference to social and environmental determinants of malaria infection at the corregimiento level (smallest political division), analyzing census demographic and epidemiological data for 2,295 malaria cases between 2009-2012 in 621 corregimientos. Our analysis indicated that the burden of *P. vivax* infections by health region was higher among Amerindian reservations (where the incidence of *Plasmodium falciparum* and vivax infections was estimated between < 1 % to 8.4 % respectively), and female individuals less than 15 years old. In order to test the hypothesis that those corregimientos with the highest proportion of type 2 households (build with deciduous construction materials) were more likely to be infected, we performed a multivariate logistic regression analysis to evaluate the association between risk of malaria infection and type 2 houses, controlling for other predictors of low SES such as dirt floor, lack of potable water, lack of electricity, lack of sanitary facilities, unemployment and illiteracy. Results indicated that those corregimientos with the highest proportion of type 2 households were more likely to be infected with malaria (OR = 43.24 (2009), 821.20 (2010), 1359.23 (2011) 729.52 (2012) ($p < 0.05$), all other predictors held constant. Pairwise correlations indicated a protective effect of type 1 households ($p < 0.05$), while risk of malaria infection was positively correlated with determinants of low SES such as type 2 houses, dirt floors, illiteracy and lack of electricity but was not with lack of potable water, sanitary facilities or unemployment. In conclusion, risk of malaria infection was associated with corregimientos having the highest proportion of type 2 households. We expect that this data will help implement a multi-sectorial approach for the elimination of malaria based on determinants of SES in Panama.

952

DETERMINANTS OF EFFECTIVE DELIVERY OF INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY (IPTP) IN SUB-SAHARAN AFRICA: A MULTI-COUNTRY ANALYSIS

Lia S. Florey[1], Erin Eckert[2]

[1]ICF International - The DHS Program, Rockville, MD, United States, [2]United States Agency for International Development - President's Malaria Initiative, Washington, DC, United States

Malaria infection during pregnancy leads to adverse health outcomes for both mothers and infants. IPTp of at least two doses of sulphadoxine-pyrimethamine (SP), administered at antenatal care (ANC) visits, is an effective malaria prevention intervention. Despite increasing investment in IPTp programs over the past decade, and despite high rates of attendance at ANC visits, use of IPTp remains low. To identify bottlenecks in IPTp delivery, service effectiveness analyses were performed on data from 16 Demographic and Health Surveys (DHS) and Malaria Indictor Surveys (MIS) conducted between 2007 and 2011 in malaria-endemic countries in SSA. Multi-country, pooled, multivariate logistic regressions were used to identify determinants of IPTp delivery. Distributions of key determinants were compared for lower IPTp coverage countries (<20% IPTp use) and higher IPTp coverage countries (≥20% IPTp use). IPTp was effectively delivered for only 18% of targeted women. Access to ANC services was not identified as a major reason for this low rate. In fact, 83% attended ANC at least once and 97% of those receiving one dose of SP attended ANC twice. However, levels of SP delivery to those attending ANC was low: 42% of those attending one ANC visit received one SP dose, and 57% of those attending two ANC visits received two SP doses. Determinants of IPTp use included number of ANC visits, receipt of other maternal health interventions, and malaria transmission level. Effectiveness of IPTp delivery systems varied substantially between higher and lower IPTp coverage countries. Women in higher coverage countries made fewer ANC visits, attended ANC for the first time earlier in gestation, and were more likely to use ANC services at public or religious facilities than were women in lower coverage countries. Results show that most pregnant women are obtaining ANC services at sufficient frequency and appropriate timing to permit IPTp delivery, but the intervention is not being effectively delivered in these settings.

953

ACCESS TO MALARIA CONTROL INTERVENTIONS AMONG SCHOOL-AGE CHILDREN IN MALAWI

Lauren M. Cohee[1], Jenny A. Walldorf[1], Jenna E. Coalson[2], Nelson Chimbiya[3], Kondwani Nkanauena[3], Andy Bauleni[3], Jacqueline Fiore[4], Atupele Kapito-Tembo[3], Don Mathanga[3], Terrie E. Taylor[5], Miriam K. Laufer[1]

[1]University of Maryland School of Medicine, Center for Vaccine Development, Baltimore, MD, United States, [2]University of Michigan School of Public Health, Ann Arbor, MI, United States, [3]University of Malawi College of Medicine, Malaria Alert Center, Blantyre, Malawi, [4]Michigan State University, College of Osteopathic Medicine, East Lansing, MI, United States, [5]University of Malawi College of Medicine, Blantyre Malaria Project, Blantyre, Malawi

In Malawi, school-age children have the highest rates of malaria illness at health facilities and asymptomatic infections in the community. Whether this is due to age-related biological susceptibility or difference in access to malaria control interventions is unclear. We used community-based surveillance data to assess interventions, including bednet use and prompt diagnosis and treatment, in school-age children (6-15years) compared to younger children (0-5years) and pregnant women, groups that are typically the target of anti-malaria interventions. During cross-sectional surveys in September 2012 and May 2013, we enrolled 7653 participants. School-age children were less likely to report using bednets (645/1130, 57%) than younger children (600/790, 76%, p<0.001) and pregnant women (57/74, 77%, p<0.001). Among school-age children, the likelihood of bednet use was not affected by gender or rural vs. urban setting, but they were more likely to sleep under a net in the rainy season and when the ratio of household members to nets was lower (2.6 vs 4.5 people per net, p<0.001). Effects of bednets in this age group will be determined by comparing rates of anemia, microscopic and submicroscopic parasitemia. Fever in the last two weeks was reported in fewer school-age children (163/1130, 14%) than younger children (200/790, 25%); p<0.001). Among those with fever, there was no difference between the groups in seeking treatment or duration of fever prior to seeking treatment. School-age children were most commonly taken to a shop for treatment compared to younger children, who were most commonly taken to a government health facility. By parent report, the two groups were equally likely to be tested for malaria and to receive antimalarial drugs. In Malawi, school-age children have less access to antimalarial interventions than younger children and pregnant women. This may partially explain their high rates of disease and infection. New interventions targeting this group or strategies to increase access to existing programs may decrease both disease burden and transmission.

954

MALARIA IN CHILDREN UNDER-TWO IN RURAL SINDH, PAKISTAN - RESULTS OF A COMMUNITY BASED ACTIVE SURVEILLANCE STUDY

Asad Ali[1], Tauseef Akhund[1], Gohar Warraich[1], Najeeb Rehman[1], Fayaz Ahmed[1], Molly Hughes[2]

[1]Aga Khan University, Karachi, Pakistan, [2]University of Virginia School of Medicine, Charlottesville, VA, United States

Burden of malaria is not clearly defined in Pakistan, and is believed to be sporadic and limited to certain high risk areas. These high risk zones have not been clearly defined. With the MDGs and Roll Back Malaria's targets approaching in 2015, it is important to characterize this malaria burden better. We aimed to determine the incidence of malaria in children less than two years old in a rural community typically considered at low risk of malaria. Prospective surveillance was carried out in children between 0 - 24 months over a period of two years from October 2011 - November 2013 in the district of Matiari, in Sindh, Pakistan. Children of parents and / or guardians able to give informed consent were recruited as newborns, and underwent routine fortnightly active surveillance for two years. Children meeting the World Health Organization's Integrated Management of Childhood Illness' criteria for febrile illness and severe pneumonia were tested for malaria using Malaria Pf/Pan rapid immunochromatographic tests. Positive malaria cases were confirmed by light microscopy. Febrile children had weekly follow-ups and were treated or referred. 817 children were followed and total child-years of follow-up were 1374 after adjusting for children lost to follow-up or missed on surveillance visits. 409 (50.1%) were male. Malaria incidence rate was 9.5 cases per 1000 child years (95% CI 5.0 - 16.1). No cases of neonatal malaria were detected. Clinical presentation was nonspecific and overlapped with pneumonia. There were no mortalities due to appropriate treatment and follow-up. Malaria in rural Sindh in Pakistan is a more common occurrence than previously recognized. This has implications for diagnostic and management algorithms used for young febrile children in the community.

955

THE TRIPLET METHOD: A QUICK AND RELIABLE METHOD FOR DELIVERING PARASITE INFECTION CLEARANCE AND DETECTION SENSTIVITY ESTIMATES

Mariabeth Silkey[1], Ingrid Felger[1], Natalie Hoffmann[1], Ivo Müller[2], Leanne Robinson[2], Rahel Wampfler[1], Thomas A. Smith[1]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Walter and Eliza Hall Institute, Melbourne, Australia

Malaria infection and clearance rates can be estimation by analysis of longitudinal molecular typing data. In general, infections appear and disappear over short time intervals, but these highly dynamic patterns

are mainly the result of imperfect detection owing to samples that fall below the detection limit of PCR. Several different statistical techniques, such as hidden Markov models, have been used to estimate infection and clearance rates allowing for imperfect detection, but these can be difficult to implement. We present improvements on a simple method for estimating force of infection for *Plasmodium falciparum* from longitudinal typing data. The longitudinal sequence of observations for any one genetic type of parasite is evaluated as overlapping triplets observations, at time steps zero, one and two in order to estimate the true clearance rate between times steps zero and one. Counts from the set of triplets beginning with a positive measurement at time step zero are evaluated as realizations of a multinomial distribution conditional on the true clearance and detection sensitivity. We amend the original method to estimate the force of infection, clearance, and detection rates, as part of the same Bayesian model. The triplet method is straightforward to calculate, does not require extensive statistical modelling, and produces estimates comparable to more involved approaches for longitudinal data. Missing data are naturally accommodated as a category of the multinomial. We provide a program that calculates the carriage prevalence for each genotype, estimates of infection incidence, and the clearance rate, all corrected for imperfect detection. The method applied to the Albinama cohort (ages five to nine) of the TransEpi study in Papua New Guinea produces overall *Plasmodium falciparum* parasite mean daily clearance rate of 0.142 [0.005, 0.743] and annual incidence of infection 4.011[3.837, 4.093] (median[95%CI]); both the recovery rate and the infection rate are higher than those found for younger children in Tanzania and Ghana using similar methodology.

956

INDIVIDUAL AND HOUSEHOLD LEVEL FACTORS ASSOCIATED WITH RAPID DIAGNOSTIC TEST (RDT)-POSITIVITY IN A HIGH MALARIA TRANSMISSION SETTING OF NORTHERN ZAMBIA, 2012- 2013

Jessie Pinchoff[1], Timothy M. Shields[1], Michael Chaponda[2], Mbanga Muleba[2], Modest Mulenga[2], William J. Moss[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Tropical Disease Research Centre, Ndola, Zambia

While malaria transmission has declined substantially throughout parts of Zambia, some areas continue to experience high transmission levels despite deployment of malaria control efforts. Understanding factors associated with continued malaria transmission in these areas may inform control efforts. Household malaria surveys were conducted in Nchelenge District, Luapula Province, Zambia. Households were enumerated based on satellite imagery, 5 x 5 km grid cells were overlaid, and households were randomly chosen within selected grid cells. Households were enrolled into cross-sectional (one visit) or longitudinal (visits every other month) cohorts; analyses were restricted to cross-sectional and the first visit to longitudinal households. During study visits, adults and caretakers of children were administered a questionnaire and a blood sample was collected for a malaria rapid diagnostic test (RDT). Individual and household level factors associated with RDT positivity were analyzed using logistic regression models. A total of 1,201 individuals from 339 households were enrolled. Over the study period, 43% of participants were RDT positive. Over half of RDT positive individuals were between the ages of 5 and 17 years, and half of RDT positive individuals had visited a health center or health post for malaria in the past 6 months. In the multi-variable logistic regression analysis, RDT positive individuals were over twice as likely to be between the ages of 5 and 17 years as compared to children younger than 5 years (OR=2.06; 95% CI: 1.23, 3.44), over half as likely to report a fever within the past two weeks (OR=1.57; 95% CI: 1.04, 2.37), and 73% more likely to live in a household using an open well as the main water source (OR=1.73; 95% CI: 1.15, 2.6). RDT positivity was highest among children and adolescents between the ages of 5 and 17 years. RDT positives were likely to experience symptoms and have sought care. Open wells may be a breeding site for mosquito vectors, potentially contributing to malaria transmission.

957

SPATIAL PREDICTION OF MALARIA RISK IN A HIGH TRANSMISSION AREA OF NCHELENGE DISTRICT IN NORTHERN ZAMBIA, 2012-2013

Jessie Pinchoff[1], Timothy M. Shields[1], Michael Chaponda[2], Mbanga Muleba[2], Modest Mulenga[2], William J. Moss[1], Frank C. Curriero[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Tropical Disease Research Centre, Ndola, Zambia

Despite gains in malaria control in much of Zambia, burden of malaria remains high in some areas. Transmission may be explained by proximity to environmental features associated with mosquito-breeding sites. Household malaria surveys were conducted in Nchelenge District, Luapula Province in northern Zambia from February 2012 through December 2013. Households were enumerated based on satellite imagery and randomly selected for study enrollment. At each visit, adults and caretakers of children were administered questionnaires and a malaria rapid diagnostic test (RDT). Data on the spatial distribution of malaria cases were used to generate a risk map based on environmental features, including proximity to category 1, 2, and 3 streams, slope (range 0 to 90 degrees), distance from lake Mweru, distance to health facilities, distance to roads, population density and vegetation. Streams were categorized using hydrological models based on a digital elevation model (DEM) derived from the Shuttle Radar Topography Mission version 3 and correspond to the size of the stream. Logistic regression modeling and spatial risk maps were built using programs from the R statistical software and ArcGIS. A total of 300 households were visited, comprising 1,171 participants, of whom 43% were RDT positive. Households within 500 meters of any stream, specifically located closer to a category 2 stream (per 50 m), and households located closer to the lake (per 50 m) were more likely to have RDT-positive residents. The odds of an RDT-positive resident also increased 14% per unit increase in the degree of slope where the household is located. These environmental features were used in the logistic regression model to predict and map malaria risk, along with a measure of risk uncertainty. Malaria transmission is heterogeneous in a high transmission area. Proximity to any streams within 500 meters, specifically distance to category 2 streams, higher degree of slope, and being closer to the lake increased the risk of transmission. Prediction maps may be useful in targeting control interventions.

958

PLASMODIUM VIVAX POPULATION STRUCTURE AND TRANSMISSION DYNAMICS IN CENTRAL CHINA

Yaobao Liu[1], Sarah Auburn[2], Jun Cao[1], Hidayat Trimarsanto[3], Huayun Zhou[1], Karen-Ann Gray[4], Taane G. Clark[5], Ric R. Price[2], Qin Cheng[4], Rui Huang[6], **Qi Gao**[1]

[1]Jiangsu Institute of Parasitic Diseases, Wuxi, China, [2]Menzies School of Health Research and Charles Darwin University, Darwin, Australia, [3]Eijkman Institute for Molecular Biology, Jakarta, Indonesia, [4]Australian Army Malaria Institute, Brisbane, Australia, [5]London School of Hygiene & Tropical Medicine, London, United Kingdom, [6]Medical College of Soochow University, Suzhou, China

In Central China the declining incidence of *P. vivax* has been interrupted by reemergence and outbreak since 2000. In this study the impact of these changes on the local parasite population, and concurrent risks of future resurgence, was assessed. *P. vivax* Isolates collected from Anhui (n=94) and Jiangsu (n=25) provinces, Central China by passive case detection between 2007 and 2010 were genotyped using capillary electrophoresis at 7 polymorphic short tandem repeat markers. Spatial and temporal analyses of within-host and population diversity, population structure, and relatedness were conducted on these isolates. Polyclonal infections were infrequent in the 94 isolates from Anhui (4%) and 25 from Jiangsu (12%), with a trend for increasing frequency from 2008 to 2010 (2 to 19%) when combined. Population diversity was high in both provinces and across the

years tested (HE = 0.8 - 0.85). Differentiation between Anhui and Jiangsu was modest (F'ST = 0.1). Several clusters of isolates with identical multi-locus haplotypes were observed across both Anhui and Jiangsu. Linkage disequilibrium was strong in both populations and in each year tested (IAS = 0.2 - 0.4) but declined two- to four-fold when identical haplotypes were accounted for, indicative of occasional epidemic transmission dynamics. None of 5 imported isolates shared identical haplotypes to any of the Central Chinese isolates. The population genetic structure of *P. vivax* in Central China highlights unstable transmission, with limited barriers to gene flow between the central provinces. The challenge of imported cases and risks of resurgence emphasise the need for continued surveillance to detect early warning signals. Although parasite genotyping has potential to inform the management of outbreaks, further studies are required to identify suitable marker panels for resolving local from imported *P. vivax* isolates.

959

PLASMODIUM VIVAX MORBIDITY AFTER RADICAL CURE: A TWO-YEAR COHORT STUDY IN THE PERUVIAN AMAZON

Annette Erhart[1], Veronica Sotto-Calle[2], Christopher Delgado-Ratto[3], Juan Jose Contreras-Mancilla[2], Eliana Torres[2], Luis Sanchez-Martinez[2], Freddy Alava[4], Hugo Rodriguez[4], Angel Rosas[2], Anna Rosanas-Urgell[1], Umberto D'Alessandro[5], Alejandro Llanos[1], Nico Speybroeck[6], Dionicia Gamboa[2]

[1]Institute of Tropical Medicine, Antwerp, Belgium, [2]Instituto de Medicina Tropical, Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Unit of International Health, ESOC Department, Faculty of Medicine, University of Antwerp, Antwerp, Belgium, [4]Region de Salud Loreto, Iquitos, Peru, [5]Disease Control and Elimination, Medical Research Council Unit, Fajara, Gambia, [6]Research Institute of Health and Society (IRSS), Université Catholique de Louvain, Brussels, Belgium

The current national treatment guidelines for vivax malaria in Peru include a 7-day primaquine (0.50mg/kg/day) course together with chloroquine (25mg/kg, 3days). In order to evaluate the efficacy of this protocol a cohort of *P. vivax* infected patients was treated and monitored for 2-years. The study was conducted between 2008 and 2011 in 29 communities around Iquitos city. *Plasmodium vivax* infected individuals were enrolled in the cohort after individual informed consent. Treatment was directly observed and participants were monitored actively to assess treatment efficacy at Day28, then visited monthly for clinical examination and blood sampling (microscopy and PCR). A total of 303 *P. vivax* infected individuals were recruited and treated, and 270 (89%) of them completed follow-up. At baseline, males and females were equally represented and the median age was 20 years [IQR: 11-38].Two late parasitological failures, both at day 28, were detected, though at the same time point 16 participants (5%) had a PCR detectable *P. vivax* infection. Almost half (144) of the participants had *P. vivax* recurrent infections most of them (70%) repeatedly (median=3, range [2-11]. The incidence of *P.vivax* recurrent infections by microscopy and by PCR was analyzed using negative binomial regression; and time to event was analysed by survival analysis and cox regression. Results will be compared to a sister cohort study carried out in Vietnam. In conclusion, after radical treatment, a high number of *P. vivax* recurrent infections were observed, most of them asymptomatic and sub-microscopic. Our results show that a substantial amount of the *P. vivax* transmission occurs silently in the Peruvian Amazon, a finding that calls for improved diagnosis and treatment if elimination is to be achieved.

960

SEIZURE OCCURRENCE DURING TWO-YEAR FOLLOW-UP OF PEDIATRIC MALARIA PATIENTS: PRELIMINARY OBSERVATIONS

Nicholas Sausen[1], Richard Idro[2], Robert Opoka[2], Chandy John[1]

[1]University of Minnesota, Minneapolis, MN, United States, [2]Makerere University, Kampala, Uganda

Seizures are common following cerebral malaria (CM). Previous studies have demonstrated that epilepsy develops in 5-10% of survivors of CM. Less is known regarding the incidence of provoked and unprovoked seizures in other forms of severe malaria. At Mulago Hospital, Kampala, Uganda we conducted a prospective cohort study of pediatric malaria between 2008 and 2013. Children with cerebral and severe malarial anemia (SMA) were enrolled, with community children (CC) without a history of seizures or neurodisability enrolled as controls. The children's caretakers were asked whether the child had seizures during follow-up at 6, 12 and 24-month visits. 218 children with CM, 180 children with SMA and 182 CC were enrolled. Seizures occurred before admission in 205 children with CM (94%) and 3 children with SMA (1.7%), and during admission in 125 children with CM (57%) and 2 children with SMA (1.1%). 182 children with CM, 164 children with SMA, and 171 CC completed 2-year follow-up. Among children who completed follow-up, 5 children with CM (2.7%), 11 children with SMA (6.7%) and 2 CC (1.2%) had febrile seizures during the follow-up period, while 4 children with CM (2.2%), 1 child with SMA (0.6%) and 0 CC (%) had unprovoked seizures during follow-up. 3 children with CM (1.6%), 1 child with SMA (0.6%) and no CC met the definition of epilepsy (two separate incidents of unprovoked seizures). In this cohort of children with CM or SMA, febrile seizures were reported, but epilepsy was infrequent, occurring in <2% of the cohort.

961

NEXT GENERATION DURABLE WALL LINERS, A NEW STRATEGY FOR MALARIA CONTROL: BASELINE RESULTS FROM A CLUSTER RANDOMIZED TRIAL

Joseph Mugasa[1], Robert Malima[1], George Mtove[1], Filemoni Tenu[1], Peter Mangesho[1], Said Magogo[1], Donald Shepard[2], Aaron Samuels[3], John Gimnig[3], Mark Rowland[4], Frank Mosha[5], William Kisinza[1]

[1]National Institute for Medical Research, Amani Medical Research Centre, Tanga, United Republic of Tanzania, [2]Brandeis University, Waltham, MA, United States, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Faculty of Infectious Tropical Diseases, London, United Kingdom, [5]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania

Despite widespread adoption of long lasting insecticide nets (LLINs) and indoor residual spraying for vector control, malaria remains a major source of morbidity and mortality. Pyrethroid insecticide treated wall liners (ITWL) have been efficacious in reducing malaria prevalence in small-scale studies, however the increasing prevalence and distribution of pyrethroid resistance poses a threat to many pyrethroid-based vector control strategies. A new generation ITWL made of polymer based fabric impregnated with a mixture of non–pyrethroid insecticides has been developed. ITWLs act as a physical barrier to vectors and they release insecticide gradually, killing vectors that come into contact with them. They are expected to last several years before needing to be replaced. Like IRS the efficacy of ITWLs does not depend as heavily on human behaviour as LLINs do. A three arm cluster randomized controlled trial is underway in Muheza district, north-eastern Tanzania to measure and compare the efficacy and cost effectiveness of (1) LLIN (reference group), (2) IRS + LLIN, and (3) ITWL + LLIN. We present baseline findings collected prior to the randomisation of clusters into study arms. The study area was mapped and a census was performed. Sixty clusters were created, and 15 to 20 houses were randomly selected from each. All consenting household members were

asked demographic and behaviour questionnaires. Blood samples were drawn for malaria diagnosis, anemia testing, and immunochromographic testing (ICT) for *Wuchereria bancrofti*. Entomologic evaluations by WHO cylinder tests were performed. A total of 92,538 individuals from 24,198 households in the study area were enumerated, and 3208 people from 954 houses were sampled in the baseline epidemiological survey. Malaria parasitemia by mRDT was 21.6 % (95% CI: 20.2 -23.1%) and 15.7% (95% CI: 14.4 -17.2%) were positive for *W. bancrofti* by ICTs. Anemia prevalence for under fives (haemoglobin <8g/dL) was 4.3% (95% CI: 2.7 – 6.0%). Malaria infection was more common in 5-12 years olds 37.2% (95% CI 33.5- 40.9%) compared with < 5 children 17.8% (95% CI: 14.6-21.0%) (p = 0.001). WHO cylinder tests showed reduced susceptibility to pyrethroids of *An. gambiae s.l* 24 h post exposure ranging from 51 to 90%. When completed, this study will provide important information to National Malaria Control Programes and international agencies to guide future malaria control strategy and allocation of resources.

962

FACTORS ASSOCIATED WITH DECREASED *PLASMODIUM FALCIPARUM* INFECTION RISK IN MALIAN CHILDREN

Anna A. Minta[1], Tuan M. Tran[2], Jill Coursen[2], Shanping Li[2], Aissata Ongoiba[3], Safiatou Doumbo[3], Didier Doumtabe[3], Younoussou Kone[3], Aboudramane Bathily[3], Seydou Dia[3], Cinzia Risso[4], Moussa Niangaly[3], Charles Dara[3], Jules Sangala[3], Ogobara K. Doumbo[3], Kassoum Kayentao[3], Bruno Arca[5], Philip L. Felgner[6], Boubacar Traore[3], Peter D. Crompton[2]

[1]Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2]Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [3]Mali International Center of Excellence in Research, University of Sciences, Technique and Technology of Bamako, Bamako, Mali, [4]Department of Public Health and Infectious Diseases, "Sapienza" University, Rome, Italy, [5]Department of Structural and Functional Biology, "Federico II" University, Naples, Italy, [6]Department of Medicine, University of California Irvine, Irvine, CA, United States

Malaria control efforts remain suboptimal for many reasons including knowledge gaps in malaria epidemiology and immunology. To better understand the epidemiologic and immunologic factors associated with risk of *P. falciparum* infection and clinical malaria, we conducted a longitudinal cohort study of 695 individuals aged 3 months to 25 years in the rural village of Kalifabougou, Mali. We did bi-weekly active surveillance for *P. falciparum* infection by PCR and weekly active clinical surveillance for clinical malaria. Nearly all adults and children over four years of age became infected during the malaria season at a rate that was independent of age (log-rank test, p =.37), indicating that sterile immunity to *P. falciparum* infection is not acquired through natural exposure; and as expected, the risk of clinical malaria decreased with increasing age (log-rank test, p =.0038). Surprisingly, we observed that children under 4 years of age were less likely to be infected with *P. falciparum* compared to older subjects (p <0.0001), and indeed, 24% of children under 4 years of age remained PCR negative throughout the intense 6-month malaria season. Exposure was measured by antibody response to gSG6, an *Anopheles gambiae* specific salivary protein, and results indicate that uninfected children were less likely to have serologic evidence of exposure to the mosquito vector over the course of the malaria season. Self-reported bed net use was not different between infected and uninfected children. Because evidence of decreased exposure did not fully explain decreased infection risk in young children, we are taking several approaches to test the hypothesis that uninfected children have enhanced pre-erythrocytic immunity and/or that developmental differences render young children less permissive to *P. falciparum* infection. Findings from this study may help inform strategies to prevent *P. falciparum* infection in malaria endemic areas.

963

INDIVIDUAL AND HOUSEHOLD LEVEL FACTORS ASSOCIATED MALARIA INFECTION AS DETERMINED BY PASSIVE AND REACTIVE CASE DETECTION OF MALARIA IN CHONGWE DISTRICT, ZAMBIA

Bryan Carter[1], Jessie Pinchoff[2], Sisa Hatwiinda[1], Busiku Hamainza[3], Benjamin Chi[4], Hawela B. Moonga[3], **German Henostroza**[5]

[1]Centre for Infectious Disease Research in Zambia, Lusaka, Zambia, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]National Malaria Control Center, Lusaka, Zambia, [4]University of North Carolina, Chapel Hill, NC, United States, [5]University of Alabama at Birmingham, Birmingham, AL, United States

In moderate and high malaria transmission areas, current surveillance systems rely solely on passive detection methods. As malaria transmission in Zambia declines, it will become important to identify continued foci of infection via active case detection to bolster malaria control efforts. Individuals seeking care at the Chinyunyu clinic within Chongwe District, Zambia with a chief complaint of fever were administered a questionnaire, a rapid diagnostic test (RDT) and a blood sample was obtained for microscopy. Field teams were dispatched to the homes of RDT positive individuals where an additional survey and RDTs were administered to members within the household. GPS coordinates were recorded for each household. Descriptive statistics and multi-level modeling methods were implemented to compare index cases to RDT positive household contacts, and compare RDT positive to RDT negative household contacts. A total of 472 index cases were identified between June 2012 and June 2013, with 1,621 household contacts investigated and 731 (45%) testing positive for malaria. Index cases were significantly more likely to report symptoms (fever, headache) and be of younger age than RDT positive household contacts (p<0.05 for all comparisons). The mean age of passively detected cases was 12.35 (SD=15.82), the mean age of RDT positive household contacts was 14.74 (13.86), and for RDT negative household contacts was 22.16 (19.88). The proportion of index cases with fever and headache was 97% and 85%, the proportion for RDT positive household contacts was 2.7% and 54.5%, and RDT negative household contacts was 1.1% and 19.3%, respectively. In conclusion, almost half of household contacts of index cases were identified as RDT positive for malaria during the 1-year study period, suggesting passive surveillance underestimates the malaria prevalence for the clinic catchment area. Age and symptoms were the most important factors in seeking care at the clinic. RDT positive household contacts were less likely to report symptoms and slightly older than those that sought care.

964

MODELING LONG-TERM MALARIA TRANSMISSION CHANGES IN A TANZANIAN VILLAGE USING CROSS-SECTIONAL DATA ON AGE SPECIFIC PREVALENCE AND LEVELS OF ANTIBODIES

Victor Yman[1], Michael White[2], Josea Rono[1], Bruno Arca[3], Faith H. Osier[4], Marita Troye-Blomberg[5], Stephanie Boström[5], Raffaele Ronca[6], Ingegerd Rooth[1], Anna Färnert[1]

[1]Karolinska Institute, Stockholm, Sweden, [2]Imperial College, London, United Kingdom, [3]Sapienza University of Rome, Rome, Italy, [4]KEMRI-Wellcome Trust Research Programme, Centre for Geographical Medicine Research-Coast, Kilifi, Kenya, [5]Wenner-Gren Institute, Stockholm University, Stockholm, Sweden, [6]Federico II University, Naples, Italy

Robust estimates of *Plasmodium falciparum* transmission intensity are imperative for planning, implementation and evaluation of malaria control interventions. Seroconversion rates (SCR) to asexual blood-stage antigens can provide estimates of transmission intensity that correlate with entomological inoculation rates. We study past transmission trends in a rural village and evaluate new models to improve SCR based methods by analysis of antibody levels from multiple cross-sectional serological surveys. The study was conducted in Nyamisati, Rufiji, Tanzania, where parasite

prevalence decreased from 65 to 18% from 1999 and 2010. A single intervention with ITNs was performed in 1999. IgG levels to recombinant *P. falciparum* antigens (MSP-1_{19}, MSP-2, MSP-3, AMA-1) and *An. gambiae* salivary protein gSG6 were measured in children (1-16y) sampled in cross-sectional surveys in 1999 and 2010. SCR and rates of antibody decay were estimated by fitting mathematical models to data from the two cross-sections, assuming three profiles of exposure: (i) stable; (ii) stepwise decrease; or (iii) continuous decrease. Results suggest an average 66% decrease in malaria transmission intensity and an 89% reduction in *Anopheles* exposure. Transmission trends were best described by a stepwise decrease model with a reduction predicted to occur shortly after distribution of ITNs. The new models provide estimates of the duration of antibody responses under this transmission decline. MSP-1_{19} seropositive individuals were estimated to convert to seronegative with a half-life of 12 (95% CI 7-20) years due to antibody decline with a half-life of 3 (95% CI 2-6) years. The reduction in transmission may in part be attributed to reduced anopheles exposure following the introduction of ITNs, but is not likely to be explained by ITNs alone. Despite reduced parasite prevalence many children remained seropositive to blood-stage antigens. The new sensitive models using antibody levels enabled detection of reduced exposure among seropositive children and provided estimates of both antibody and transmission dynamics.

965

MALARIA AT NATIONAL UNIVERSITY HOSPITAL, SINGAPORE

Revathi Sridhar, Hwang Ching Chan, Guat Kheng Goh, Indumathi Venkatachalam

National University Health System, Singapore, Singapore

Globally, malaria affects 300 to 500 million people each year. It is endemic to several countries in South East Asia. Singapore has maintained its malaria free status since 1982 despite occasional clusters due to local transmission. However, travel related malaria is seen in hospitals here. We describe the current epidemiology of malaria at our institution. The National University Hospital (NUH) is a 1000 bed multi-specialty, tertiary teaching hospital in Singapore. Laboratory surveillance for malaria is part of the infectious disease surveillance at NUH since 2004. A retrospective study, describing the epidemiology of malaria cases presenting between January 2009 and mid- April 2014, was conducted. A total of 44 cases were analyzed. 39 (88.6%) of them were male, 25 (6.8%) were Indian, 6 (13.6%) Chinese, 1 (2.27%) Malay and 1 (2.27%) Caucasian. 28 (63.6%) of the 44 patients had travelled to a malaria endemic area. All 9 Singaporeans with malaria had preceding travel history. The most common species of malaria was *Plasmodium vivax* (n=31, 70.5%). Of the other species, 7 (15.9%) were *P. falciparum*, 4 (9.09%) were *P. knowlesi* and 1 (2.27%) had a mixed *falciparum* and *vivax* infection. 17 of the 44 patients had traveled to India. 15 of the 17 patients who traveled to India were infected with *vivax*. Amongst the 4 patients with *P. knowlesi*, 3 had traveled to Malaysia. In particular, they had visited forested areas for recreation or training. Other travel destinations included Indonesia, Thailand, Hong Kong and Ghana. 39 of 44 patients were admitted and their mean length of stay was 4 days. 4 (9.09%) patients required ICU care and all 39 were discharged well. The global disease burden, modern travel dynamics and emergence of *P. knowlesi*, a zoonotic malarial species, contribute to the continued presence of cases of malaria in Singapore. The existence of *Anopheles* mosquito vectors on the island warrant ongoing vigilance to limit the risk of local transmission.

966

EARLY DETECTION OF MALARIA RESURGENCE IN THE PERUVIAN AMAZON REGION USING SEROLOGICAL MARKERS

Angel Rosas[1], Alejandro Llanos-Cuentas[1], Niko Speybroeck[2], Hugo Rodríguez[3], Juan Contreras-Mancilla[1], Dionicia Gamboa[1], Anna Rosanas-Urgell[4], Freddy Alava[3], Irene Soares[5], Edmond Remarque[6], Umberto D'Alessandro[7], Annette Erhart[4]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Université Catholique de Louvain, Brussels, Belgium, [3]Región de Salud Loreto, Iquitos, Peru, [4]Institute of Tropical Medicine, Antwerp, Belgium, [5]Universidade de São Paulo, Sao Paulo, Brazil, [6]Biomedical Primate Research Centre, Rijswijk, Netherlands, [7]Disease Control and Elimination, Medical Research Council Unit, Fajara, Gambia

In the past decade, increased support from international donors, e.g. the Global Fund-PAMAFRO Project (2005-2010), allowed for the scale-up of comprehensive malaria control strategies in the Amazon Region. During this period, malaria declined drastically in Peru from 87,805 reported clinical cases in 2005 to 29,355 and 23,075 cases in 2010 and 2011, respectively. Since 2011, malaria control activities are mainly supported by the MoH budget, prioritizing passive and reactive case detection and treatment of confirmed infections. Since 2012, several malaria outbreaks have been detected in diverse Amazonian areas, a phenomenon that had not been observed since 2007. In order to assess recent changes the malaria transmission intensity (MTI), a cross-sectional survey was conducted during November 2012 in eight peri-Iquitos villages using molecular and serological tools. After a full census of the study villages, each household was visited and all available children<7 years plus one randomly selected individual>7 years were enrolled. A total of 651 survey participants were interviewed, clinically examined and a blood sample taken for the detection of malaria parasites (microscopy and PCR) and antibodies to *Plasmodium vivax* (PvMSP119, PvAMA1) and *P. falciparum* (PfGLURP, PfAMA1) antigens by ELISA. Age-specific seroprevalence was analyzed using a previously published catalytic conversion model based on maximum likelihood for generating seroconversion rates (SCR). Overall parasite prevalence by microscopy and PCR were low, i.e. 1.8 and 3.9%, respectively for *P. vivax*, and 1.5 and 6.7%, respectively for *P. falciparum*, while seroprevalence was much higher, 23.3% for PvMSP119 and 18.0% for PfGLURP. Most of infections were asymptomatic (79.2%) and sub-patent (71.6%). Likelihood ratio tests supported age seroprevalence curves with two SCR for both *P. vivax* and *P. falciparum* indicating a significant increase in MTI since 2011. Additional data including antibody responses to two antigens for each species and a risk factor analysis for malaria infection and exposure will be presented. In conclusion, this sero-epidemiological analysis allowed for an in-depth characterization of the current malaria transmission pattern as well as for the identification of a recent increase in MTI in the peri-Iquitos area of the Peruvian Amazon following a reduction of control efforts since 2011.

967

ROLE OF AUTOPHAGY AND POLYMORPHIC VARIATION IN AUTOPHAGY GENES IN CONDITIONING CLINICAL OUTCOMES IN CHILDREN WITH MALARIAL ANEMIA

Prakasha Kempaiah, Karol Dokladny, Zachary Karim, Samuel B. Anyona, Kaitlin Ha, Evans Raballah, John M. Ong'echa, Pope L. Moseley, Douglas J. Perkins

University of New Mexico School of Medicine, Albuquerque, NM, United States

Plasmodium falciparum polymerizes free heme into hemozoin (*Pf*Hz) as a byproduct of hemoglobin (Hb) digestion. Phagocytosis of *Pf*Hz by leukocytes promotes dysregulation in the immune response and enhanced pathogenesis. Autophagy is a process that eliminates intracellular components to maintain homeostasis. Although no studies have investigated autophagy in malaria, inactivation of autophagy is associated

with enhanced susceptibility to infectious and inflammatory disorders. To investigate the role of autophagy in malaria, we genotyped autophagy genes [*ATG9* (i.e. -2896G/C, -4970C/T, -6561A/G) and *ATG10* (-2442G/C, -4322T/A, -7723G/T)] in parasitemic children (n=1220; 3-36 mos.) and determined the association between variation and severe malarial anemia (SMA, Hb < 5.0 g/dL). Regression analyses, controlling for covariates of anemia, revealed that carriage of CCA (-2896C/-4970C/-6561A) and GTA (-2896G/-4970T/-6561A) haplotypes (*ATG9*) were associated with reduced risk of developing SMA (Avg: OR, 0.26; 95% CI, 0.08-0.66; *P*<0.001). Similarly, carriers of CTG (-2442C/-4322T/-7723G) and GTT (-2442G/-4322T/-7723T) in *ATG10* had decreased susceptibility to SMA (Avg: OR, 0.52; 95% CI, 0.18-1.05; *P*=0.070). Analysis of intragenic haplotypes revealed that carriage of CCAGTT, CCGGTT, GCACTG, GTAGTT, and GTGGTT were correlated with a reduced risk of developing SMA (Avg: OR, 0.27; 95% CI, 0.05-0.94; *P*<0.001). Analysis of gene expression profiles showed increased levels of autophagy genes in children with SMA (*ATG9,* 1.57 fold, *P*=0.022; *ATG10,* 2.29 fold, *P*=0.001). In addition, treatment of cultured PBMCs with *Pf*Hz enhanced autophagy, as illustrated by elevated LC3-II (*P*<0.05). Consistent with previous studies showing the importance of inflammatory mediators in autophagy, analysis of malaria-associated cytokines/chemokines revealed that haplotypes associated with disease susceptibility had dysregulation of IL-1Ra, IL-1β, IL-8, and IFN-γ (*P*<0.05). Collectively, these studies suggest that autophagy plays an important role in conditioning clinical outcomes in children with malaria.

968

ASSOCIATION BETWEEN INDUCIBLE HEME OXYGENASE (*HMOX*)-1 GENE VARIANTS AND SEVERE MALARIAL ANEMIA AMONG CHILDREN RESIDENT IN A *PLASMODIUM FALCIPARUM* HOLOENDEMIC REGION OF WESTERN KENYA

Samuel B. Anyona[1], Kaitlin Ha[2], Prakasha Kempaiah[2], Zachary Karim[2], Evans Raballah[1], John M. Ong'echa[1], Douglas J. Perkins[2]

[1]University of New Mexico/KEMRI, Laboratories of Parasitic and Viral Diseases, Kisumu, Kenya, [2]University of New Mexico Health Sciences Center, Albuquerque, NM, United States

Inducible heme oxygenase (HO)-1, the rate-limiting enzyme in the catabolism of heme into biliverdin, releasing free ferrous iron and carbon monoxide, is a protective factor with potent anti-inflammatory, anti-oxidant, and anti-proliferative effects, and is upregulated by multiple stimuli. Polymorphisms in the *HMOX-1* gene have been associated with various disease entities, including pulmonary, cardiovascular, neurological and infectious diseases, renal impairment and transplantation, as well as hematological and serological disorders. The current study investigated the association between *HMOX-1* intronic 917A>G (rs5755720) and promoter -495A>T (rs2071746) variants and susceptibility to severe malarial anemia (SMA; hemoglobin, Hb<5.0g/dL) among parasitemic children (age: 3-36 months; n=1,224) with acute malaria presenting at Siaya County Hospital, western Kenya. Demographic, clinical and laboratory measures were determined and children were stratified based on Hb levels into non-SMA (Hb≥5.0g/dL; n=1,014] and SMA (Hb<5.0g/dL; n=210). Genotyping was performed using the TaqMan 5' allele discrimination assay. Proportions of *HMOX-1* 917 and -495 genotypes were comparable between the groups (*P*=0.479 and *P*=0.275, respectively). Similarly, frequencies of haplotype constructs failed to show differences between the groups [917A/-495A (AA; *P*=0.805), AT (*P*=0.133), GA (*P*=0.171) and GT (*P*=0.448), respectively]. Bivariate logistic regression analyses, controlling for covariates of anemia, revealed that carriers of *HMOX-1* 917 GG mutant genotype protected children against SMA (OR=0.569, 95% CI-0.328-0.988; *P*=0.045). Additionally, carriers of a mutant 917G/-495T (GT) haplotype had >41% reduced risk of developing SMA (OR=0.585, 95% CI-0.349-0.981; *P*=0.042). By contrast, carriage of the AT haplotype increased the risk of developing SMA (OR=1.867, 95% CI-1.063-3.279; *P*=0.030). Collectively, these results suggest that variation at 917 and -495 in the *HMOX-1* loci play an important role in conditioning the development of SMA in children resident in *Plasmodium falciparum* endemic areas.

969

INFERRING MOSQUITO POPULATION BIOLOGY FROM GENETIC DATA

Tin-Yu Hui, Samantha M. O'Loughlin, Austin Burt

Imperial College London, London, United Kingdom

Anopheline mosquitoes are malaria vectors, many questions concerning the vectors' demographics remain unanswered. Our study attempts to infer the seasonal dynamics of the effective population size, and estimate the average generation time of the vector species. We are also interested in what happens during the dry season - especially for the female mosquitoes. A dataset consisting of the full genome of 200 mosquitoes from Africa were collected and analysed. Such data contain information about demographic history of the species. Studying the genetic signals not only sheds light on the above problems, but also plays a critical role in designing and evaluating vector control technologies.

970

EVOLUTION OF THE MEROZOITE SURFACE PROTEIN 7 (MSP7) IN *PLASMODIUM* SPP., WITH EMPHASIS ON SPECIES CLOSELY RELATED TO *P. VIVAX*

Andreina Castillo[1], Maria Andreina Pacheco[2], Ananias Escalante[3]

[1]Arizona State University, School of Life Sciences, Tempe, AZ, United States, [2]Center for Evolutionary Medicine and Informatics, The Biodesign Institute, Arizona State University, Tempe, AZ, United States, [3]Arizona State University, School of Life Sciences, Tempe, AZ, United States

Multigene families are considered to be one of the main sources of genome innovation and diversification; particularly, in the case of *Plasmodium* spp. numerous multigene families are involved on processes such as immune evasion and host cell recognition. We have studied the evolution of the merozoite surface 7 (MSP7) multigene family, which it thought to participate on the initial steps of parasitic recognition and attachment to the host's erythrocyte surface. MSP7 paralog sequences for a total of twelve *Plasmodium* species were obtained by sequencing laboratory isolates and/or publicly available genomic data. An analysis of each paralog showed that similar amino acidic composition is shared among all members of the MSP7 multigene family. We observe numerous gene gain and loss events among *Plasmodium* species particularly within the clade that includes *Plasmodium vivax* (further referred as *P. vivax* clade) with a marked increment in the gene family size in this human parasite and its closely related macaque parasite *P. cynomolgi*. Specifically, there are several duplication events leading to MSP7 paralogs within the *P. vivax* clade and some of them increased the gene family size specifically in *P. vivax* and *P. cynomolgi*. Whether these events have been driven by natural selection is a matter that remains unclear. However, others have described five high activity binding peptides (HABP) to the host's erythrocyte in *P. falciparum* (gene PF3D7_1335100). We observe that three of them are frequently found in other PfMSP7 paralogs and other *Plasmodium* species. One, HABP_26114, shows 67 to 43% similarity (including conserved and same type amino acids) in all *P. falciparum* paralogs as well as in MSP7 paralogs found on species of the *P. vivax* clade (62-38%). This finding indicates that at least certain erythrocyte binding activity is to be expected in all members of the MSP7 multigene family as a mean to assure host infection. However, how the number of paralogs affects this putative binding activity across *Plasmodium* species is a matter that deserves further investigation.

971

ASCERTAINING THE DIVERSITY AND RATE OF EVOLUTION OF THE COMPLETE MITOCHONDRIAL GENOME IN HAEMOSPORIDIAN PARASITES

M. Andreina Pacheco[1], Nubia E. Matta[2], Ingrid A. Lotta[2], Angie D. Gonzalez[2], Juan S. Mantilla[2], Miguel Lentino[3], Ananias A. Escalante[1]

[1]Arizona State University, Tempe, AZ, United States, [2]Departamento de Biología, Universidad Nacional de Colombia, Bogota, Colombia, [3]Colección Ornitológica Phelps, Caracas, Bolivarian Republic of Venezuela

Our understanding about the origin of human malarias has been invigorating by the discovery of many new species in non-human primates. Many of those species, however, have been described solely using molecular data. Furthermore, zoonotic malarias have been misidentified due to the poor resolution of some morphological characteristics. Thus, the traditional view that relies solely on morphology to define malaria parasite species has been challenged. There are few systems that allow contrasting morphological and molecular data, one of those are the avian haemosporidians. In this study, we compare the rate of evolution of mitochondrial genes using standard molecular clock methods in both mammalian and avian parasites. We include newly described species belonging to three Haemesporidian genera: Leucocytozoon, Haemoproteus and *Plasmodium*. We contrast the results of single mitochondrial gene approaches with those from complete mitochondrial genomes and test both the potential and limitations of mitochondrial genes, including complete Cytb sequences, as ways to delimit species in malarial parasites that have been identified using morphology. Overall, we found that Cytb allows the correct differentiation of morphologically distinct species. However, the Cytb cannot, by itself, reliably uncover many recent phylogenetic relationships of species that radiated at a scale of 2-7 million years ago. Our conclusion is that single gene approaches do not provide enough information to properly differentiate or estimate molecular phylogenies on species that have recently diverged. Whether having morphological information is valuable in the description of species, molecular differences using multiple mitochondrial genes are sufficient to discover species.

972

ULTRASENSITIVE DETECTION OF *PLASMODIUM FALCIPARUM* BY AMPLIFICATION OF MULTI-COPY SUBTELOMERIC TARGETS USING QUANTITATIVE PCR

Natalie E. Hofmann, Felistas Mwingira, Ingrid Felger

Swiss Tropical and Public Health Institute, Basel, Switzerland

To inform up-scaled malaria control efforts, populations in endemic areas must be actively screened by molecular tools to identify the transmissive human reservoir including asymptomatic *Plasmodium* carriers. A major challenge is the large number of samples and the associated cost, which can be minimized by pre-screening of sample pools. Current molecular methods lack sensitivity and cannot reliably detect low-density infections, especially after dilution in sample pools. Novel and ultra-sensitive malaria detection assays are thus urgently needed. We therefore selected high-copy subtelomeric sequences to design two ultra-sensitive qPCR assays for detection of *P. falciparum* (Pf) infections. Amplification targeted the telomere-associated-repeat-element 2 (TARE-2, ca. 350 copies/genome) and the var-gene acidic terminal sequence (varATS, 60 copies/genome). In sensitivity tests using parasite culture, both assays reliably detected 0.034 parasites/µl blood, and could amplify from samples containing as little as 0.00034 parasites/µl blood. The sensitivity of TARE-2 and varATS assays relative to a 18S rDNA assay was assessed on 503 Tanzanian field samples covering all ages. The highest gain in Pf prevalence was observed in infants (0-1y) by TARE-2 qPCR (22.2%; varATS: 5.6%), and thus TARE-2 emerged as the most sensitive assay. From the age of four, TARE-2 and varATS qPCRs perfomed similarly with an average gain in Pf prevalence of 11.2% and 10.1%. To evaluate the applicability of our assays in pooling strategies, we produced 5-sample pools containing one low-density field sample (1-5 parasites/µl) plus four negatives and co-extracted DNA. Both TARE-2 and varATS assays reliably identified all pools containing a Pf sample. Our results demonstrate that a large proportion of asymptomatic Pf carriers is missed using 18S qPCR, leading to underestimation of the transmissive reservoir. Due to their enhanced sensitivity, TARE-2 and ATS qPCRs can be used to screen sample pools for high-throughput and cost-effective detection of Pf infections.

973

RHOPTRY PROTEINS ARE INVOLVED IN SPOROZOITE INVASION OF THE SALIVARY GLAND

Mamoru Nozaki[1], Tomoko Ishino[1], Naohito Tokunaga[1], Takafumi Tsuboi[2], **Motomi Torii**[1]

[1]Ehime University, Toon, Ehime, Japan, [2]Ehime University, Matsuyama, Ehime, Japan

It has been shown that rhoptry proteins are secreted and localized to the tight junction, during *Plasmodium* merozoite invasion of erythrocytes. One of rhoptry proteins, Rhoptry Neck Protein 2 (RON2) is known to form complex with RON4 and RON5. The interaction between RON complex and Apical Membrane Protein 1 (AMA1) at the tight junction confers the invasive motility on merozoites. Previously, we reported that RON2 is also localized to the rhoptries in sporozoites and that RON2 is involved in sporozoite invasion of the salivary glands by generation of sporozoite stage-specific ron2 silencing transgenic parasites. Here, we investigated whether RON complex is also formed in oocyst-derived sporozoites or not, by co-immunoprecipitation assay. Sporozoites were collected from infected *Anopheles* mosquito midguts at day 19th post-feeding and lysed in 1% CHAPS buffer. Solubilized proteins were then precipitated with anti-RON4 antibodies with protein G Sepharose beads. Western blotting showed that RON2 and RON5 were co-precipitated with RON4, indicating that RON complex is formed in oocyst-derived sporozoites. Next, the functions of RON4 and RON5 were examined by sporozoite-stage specific gene silencing system. The efficiency of salivary gland invasion was significantly decreased in ron4 or ron5 gene repressed sporozoites. These data suggested that sporozoite rhoptry proteins, RON2, RON4 and RON5, are involved in invasion of the salivary glands in a coordinated manner.

974

EFFECT OF DECREASING MALARIA TRANSMISSION ON THE GENETIC DIVERSITY OF THE *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN 2 IN A TANZANIAN VILLAGE

Manijeh Vafa Homann, Victor Yman, Josea Rono, Ingegerd Rooth, Anna Färnert

Karolinska Institute, Stockholm, Sweden

Plasmodium falciparum is a genetically highly diverse organism. The genes coding for blood stage antigens are particularly polymorphic being under immune selection. Understanding this diversity is important when considering potential vaccine candidate antigens. Merozoite surface protein-2 (MSP2) is one of the most polymorphic antigens and has been thoroughly studies as vaccine candidate. MSP2 is coded by the single copy gene, *msp2,* which is widely used as a marker to define *Plasmodium falciparum* diversity. This study assessed the diversity of *msp2* in relation to transmission intensity in a village in Tanzania, where malaria transmission has dramatically declined over the last decades. Asymptomatic individuals, aged 0-84 years, sampled in annual cross-sectional surveys 1994, 1999 and 2010 (n=577, 370 and 758, respectively), were included in the study. Parasite prevalence decreased significantly after 1999 (57.2%, 58.6% and 16.1%). Similarly, the mean number of concurrent clones declined significantly in 2010 (2.72, 2.15 and 1.66, respectively, $P<0.0001$). The proportion of infections that were multiclonal was 61.2% in 1994, 54.4% in 1999 and 36.1% in 2010 ($P<0.0001$). The number of different alleles (defined as 3 base pare size bins) of FC27 type were 21, 23 and 13; and of IC/3D7 was 82, 66 and 53 at the three respective surveys. These data

suggest that the genetic diversity of *P. falciparum* populations is affected by transmission. More in depth analyses including sequencing are in process to clarify more in detail the dynamics of the genetic diversity of the *msp2* gene in this setting.

975

FUNCTIONAL CHARACTERIZATION OF RNA REGULATORS IN MALARIA TRANSMISSION

Kevin J. Hart, Mark Kennedy, Scott E. Lindner

The Pennsylvania State University, University Park, PA, United States

The malaria parasite has a complex life cycle with two bottlenecks that occur during its transmission between mosquitoes and mammals. These transmission events require translational repression to ensure that only the proper proteins are being expressed, while allowing the parasite to prepare the mRNAs it will need for the next developmental stage. Puf2 is an RNA-binding protein and translational regulator, which when knocked out, causes the parasites in the salivary gland to become less infectious over time. In these *puf2*$^{-}$ parasites, the mRNA abundance of two putative deadenylases (CCR4, CCR4L) increases substantially.CCR4 is a deadenylase with the same functional domain as CCR4L. CCR4 is well characterized in yeast and mammals as a major translational regulator, and initiates the degradation of ribonucleic acids as part of the CCR4-Not complex. These proteins have yet to be well characterized in the malaria parasite, but the lack of a leucine-rich region in CCR4L may indicate that it does not interact with the CCR4-Not complexes suite of proteins and thus may have a specialized function. We have produced transgenic parasites that either knock-out the CCR4 or CCR4L genes, or express epitope-tagged versions of these proteins in order to compare their functions, localizations, binding partners and essentiality. Localization data in blood stage suggests that there is expression of CCR4L in schizonts but little to no expression in ring and trophozoite stages. Characterization of these proteins in the remainder of the life cycle may show that they are potential drug targets and by disrupting their function one might prevent parasite transmission, halt the infection, or reduce the infectivity of the parasite.

976

ENHANCING LONGEVITY OF *PLASMODIUM VIVAX* AND *P. FALCIPARUM* SPOROZOITES DISSECTED FROM MOSQUITO SALIVARY GLANDS

Emily J. Lupton[1], Rapatbhorn Patrapuvich[2], Alison Roth[1], Steven P. Maher[1], Naresh Singh[1], Jetsumon Sattabongkot[2], John H. Adams[1]

[1]University of South Florida, Tampa, FL, United States, [2]Mahidol University, Bangkok, Thailand

Many unknowns still exist regarding the liver stage forms of *Plasmodium* species that cause malaria. To investigate the liver stage invasion and development, malaria parasites need to be preserved in the infectious sporozoite form by preventing maturation during mosquito dissection. Mimicking salivary gland conditions with a dissection buffer comprised of insect media may aid in the preservation of sporozoites, by creating a more stable transition out of the mosquito. Our goal is to determine which buffer variation prolongs life and viability of sporozoites over time as measured by gliding motility. Variations of Hank's Balanced Salts and Grace's Insect Media were compared to RPMI 1640, the current standard dissection buffer. *Plasmodium vivax* and *P. falciparum* sporozoites were harvested from *Anopheles* mosquitoes into each test buffer, and gliding assays were performed at time 0, 4, 8, and 24 hours post dissection. Gliding percentages were compared for statistical difference between buffers and time points within three experimental groups. *P. vivax* experiments were split into two groups based on whether or not sporozoites were harvested from mosquitoes that were internationally shipped after blood feeding, and *P. falciparum* experiments composed the third group. At time 0 hours, RPMI and Grace's both showed strong gliding percentages in all groups (RPMI: 31-57%, Grace's: 33-57%), but by time 4 hours, RPMI consistently had the lowest gliding percentage (0-35%). Grace's had statistically higher ($p<0.001$) or equivalent gliding percentages compared to all other buffers at time 4, 8, and 24 hours (4 hours: 12-56%, 8 hours: 9-56%, 24 hours: 5-22%). Based on gliding percentages, our variation of Grace's preserved sporozoites over time better than both Hank's variations and the standard dissection buffer RPMI. Using a buffer variation such as Grace's that is more similar to the salivary gland environment, increases access to sporozoites for essential liver and pre-erythrocytic stage studies. *Rapatbhorn Patrapuvich and Alison Roth contributed equally

977

ANALYSIS OF THE GENETIC PROFILE OF *PLASMODIUM FALCIPARUM* ISOLATES FROM URBAN AND RURAL AREAS FROM GABON

Denise Patricia Mawili-Mboumba, Lelia Chadlene Bhongo Mavoungou, Noé Patrick Mbondoukwé, Prycil Nzamba, Blondel Madoungou, Marielle Karine Bouyou Akotet

Universite de Sciences de la Sante, Faculty of Medicine, Libreville, Gabon

Plasmodium falciparum malaria is a major public health problem in Africa. Indeed, in many African countries, new malaria control strategies recommended by the World Health Organization (WHO) have been adopted during the last decade. In Gabon, the deployment of these was followed by a decrease and a rebound in malaria prevalence, a change in the age of populations at risk, suggesting a changing epidemiology. It is therefore important to assess the impact of these strategies on the genetic diversity of circulating parasites. The aim of the study was to analyze and to compare the allelic diversity of *Plasmodium falciparum* isolates in Gabon. Febrile children and adults were recruited in 2011 at Oyem, Port-Gentil and Libreville. All patients had a malaria diagnosis based on microscopy. Peripheral blood samples were collected from and msp1 and msp2 were analysed by nested- PCR in malaria positive samples. The allelic family Ro33 was the most frequent (> 50%) in isolates from all sites. The highest diversity was found in the K1 allelic family with a total of 14 alleles while 10 and one Mad20 and Ro33 alleles was identified, respectively. The majority of 3D7 alleles was detected in Libreville and Oyem whereas most of the FC27 alleles was found at Oyem. Among isolates, 42 msp2-type alleles were detected, with nearly half belonging to each allelic family. The complexity of infections was the highest with msp1 gene: 1.95 in Port-Gentil, 1.91 and 1.66 in Oyem and Libreville. With the msp2 gene it was 1.33 in Port-Gentil, Libreville 1.24 and 2.15 Oyem. A significant allelic diversity was found in all isolates. The complexity of infections and the different genetic profile of the detected parasite strains varying according to the site suggest a heterogeneous transmission in the different sites. However, strategies for malaria control appear to have a limited impact on the diversity of *Plasmodium* strains.

978

GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* IN HAITI: INSIGHTS FROM MICROSATELLITE ANALYSIS

Tamar E. Carter[1], Halley Malloy[1], Alexander Existe[2], Jean R. Romain[3], Yves St. Victor[4], Bernard A. Okech[1], Connie J. Mulligan[1]

[1]University of Florida, Gainesville, FL, United States, [2]National Public Health Laboratory, Port au Prince, Haiti, [3]Hospital St. Croix, Leogane, Haiti, [4]Blanchard Clinic, Terre Noire, Haiti

Hispaniola is the only Caribbean island with endemic malaria, and the majority of malaria cases, all due to *Plasmodium falciparum*, are reported in Haiti. Recently, there have been renewed discussions on malaria elimination for Hispaniola, with the focus on interventions in Haiti. Effective antimalarial treatment policies are crucial to successful malaria elimination programs. Presently, Haiti employs chloroquine + primaquine regimen for patients diagnosed with malaria. Although antimalarial resistant *P. falciparum* is not present on a wide scale in Haiti, the emergence of chloroquine resistance remains a threat in Haiti.

Previous studies investigating *P. falciparum* evolution in Haiti in response to widespread antimalarial use have focused on specific genes associated with antimalarial resistance. The present study takes a broader approach to understanding *P. falciparum* evolution by measuring genetic diversity using microsatellite loci located on multiple chromosomes. Eighty-nine samples were collected on blood spot cards from Terre Noire, Leogane, Jacmel, Chabin, Nippes, North Cap Haitien, and Hinche between 2010 and 2013. DNA was extracted and amplified for 12 putatively neutral microsatellite loci. Based on analysis of five loci (*TA1*, *TA60*, *POLY*α, *ARA2*, and *Pfg377*), we identified multiple infections in 6.3% of our samples. Expected heterozygosities for the five loci ranged from 0.55 to 0.72, suggesting a highly diverse *P. falciparum* population in Haiti. Future analysis of all 12 loci will compare the level of diversity across multiple geographic sites and assess population structure within Haiti. Overall genetic diversity and geographic distribution of parasite diversity can aid in understanding present malaria transmission and impact of antimalarial drug use in Haiti.

978

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979

DISTINCT ESSENTIAL FUNCTIONS FOR POLYAMINES BIOSYNTHESIS ENZYMES IN MALARIA PARASITE BLOOD AND MOSQUITO STAGES DEVELOPMENT

Shaymaa Abdalal, Atif Ghaffar, Robert J. Hart, **Ahmed S. I. Aly**

Tulane University, New Orleans, LA, United States

Polyamines are positively charged organic molecules that play important roles in cell cycle regulation, cell proliferation, senescence and death of eukaryotic cells. Polyamine analogues have been considered and applied in cancer therapy. Moreover, DFMO (α-Difluoromethylornithine), one of the main drugs targeting African Trypanosomiasis, is an inhibitor of Ornithine Decarboxylase, an essential enzyme in the biosynthesis of polyamines. Despite of the importance of this pathway as possible target for multi-stage malaria intervention, little is known about the cellular functions of polyamine biosynthesis enzymes for *Plasmodium* development. We applied gene-targeting techniques for *Plasmodium yoelii* to target enzymes of this pathway for deletion. Our results indicate that the bifunctional Ornithine Decarboxylase/ S-Adenosylmethionine Decarboxylase (ODC/ SAMDC) enzyme is not essential for sexual and asexual blood stage (BS) development. However, BS growth was reduced in odc/samdc(-) and male gamete exflagellation was completely abolished with no development of mosquito stages. Spermidine Synthase, the downstream enzyme of ODC/ SAMDC, was shown to be essential for BS development by knock-out/ knock-in approach. These results indicate alternative essential roles for the polyamine biosynthesis enzymes during BS and early mosquito stages development. This validates the enzymes of this pathway as multi-stage drug targeting candidates.

980

START1 IS A MITOCHONDRIAL PROTEIN INVOLVED IN UBIQUINONE METABOLISM IN *PLASMODIUM FALCIPARUM*

Bethany J. Jenkins, Thomas M. Daly, Joanne M. Morrisey, Michael W. Mather, Akhil B. Vaidya, Lawrence W. Bergman

Drexel University College of Medicine, Philadelphia, PA, United States

The mitochondrial electron transport chain (mtETC), which utilizes ubiquinone (Coenzyme Q or CoQ) as an electron carrier, is a validated target for antimalarial drugs such as atovaquone. CoQ is also involved in the pyrimidine biosynthesis pathway, which is essential for parasite survival. Genes encoding several of the CoQ biosynthesis enzymes have been identified in *Plasmodium falciparum*; however, much remains unknown about CoQ synthesis and interactions in the mtETC. We have identified START1, a mitochondrial protein that complements *Saccharomyces cerevisiae* Coq10p, despite minimal homology. In *S. cerevisiae*, disruption of *COQ10* results in inhibition of mitochondrial respiration and impaired – but not inhibited – CoQ synthesis. It has been suggested that Coq10p binds to CoQ, and is likely involved in the transport of CoQ within the mitochondria. This is supported by overexpression of *COQ10*, which leads to deficient respiration, presumably through sequestering CoQ and preventing its use in the mtETC. Given *COQ10*'s essential role in yeast, and the pressing need for new antimalarial drug targets, our aim is to characterize the role of START1 in *P. falciparum*. To support the hypothesis that START1 is essential, we are attempting to disrupt the endogenous gene in wild type parasites and in parasites expressing an exogenous copy of the gene. We are also generating parasites in which endogenous *START1* is tagged with the GlmS ribozyme sequence for a regulatable mRNA knockdown. We are ascertaining whether overexpression of START1 in parasites results in a similar phenotype to that of *S. cerevisiae*. Finally, we are identifying whether START1 can be found in complex with other proteins in parasites by immunoprecipitation and blue native gels. This research will characterize a new and important element of the mtETC and ubiquinone pathways in *P. falciparum*.

981

EUPATHDB: AN ONLINE GENOMICS RESOURCE FOR EUKARYOTIC PATHOGENS

Susanne Warrenfeltz[1], Omar S. Harb[2], Jessica C. Kissinger[1]

[1]University of Georgia, Athens, GA, United States, [2]University of Pennsylvania, Philadelphia, PA, United States

The Eukaryotic Pathogen Database Resource (EuPathDB) is a family of free online databases that integrate genomic data with functional genomics and clinical/field isolate data for numerous eukaryotic pathogens within Amoebazoa, Apicomplexa, Diplomonadida, Microsporidia, Trichomonadida, Kinetoplastida. EuPathDB also integrates data informing upon host-parasite interactions from datasets that analyze mixed parasite-host samples such as human cells infected with parasites. An interactive data exploration platform, EuPathDB provides data mining and visualization tools for discovering meaningful relationships between

genomic features to support hypothesis-driven research. The databases are updated and expanded bimonthly with data ranging from genome sequence and annotation to expression data, to parasite field isolates, to host data in response to infection. Despite the breadth of data (140 genomes, 150 functional datasets), it is easy to mine, visualize, download and browse different data types. Data is mined using the Strategy System to search within and between datasets, developing in silico experiments that identify features with similar biological characteristics. Search strategies and results can be downloaded, saved and shared with a colleague. Data may be visualized in the context of the genome sequence and annotation using an interactive and configurable browser. Individual record pages that compile all available data for a feature (e.g. gene, isolate, genomic sequence) provide a comprehensive view of the feature. Our extensive user-support system includes video tutorials, a rapid-reply email question hotline, and hands-on workshops at locations worldwide. Attend our poster or exhibit booth for an overview of this NIH/NIAID-funded resource. Or visit one of our sites: AmoebaDB.org, CryptoDB.org, EuPathDB.org, GiardiaDB.org, MicrosporidiaDB.org, PiroplasmaDB.org, PlasmoDB.org, ToxoDB.org, TriTrypDB.org, TrichDB.org, OrthoMCL.org, and HostDB.org.

982

DETECTION OF *PLASMODIUM FALCIPARUM* IN THE BLOODMEAL OF *ANOPHELES GAMBIAE* USING QUANTITATIVE NUCLEIC ACID SEQUENCE BASED AMPLIFICATION (QT-NASBA)

Benjamin J. Krajacich[1], Nathan D. Grubaugh[1], Doug E. Brackney[1], Haoues Alout[1], Jacob I. Meyers[1], Lawrence S. Fakoli[2], Fatorma K. Bolay[2], Joseph W. DiClaro II[3], Roch K. Dabiré[4], Brian D. Foy[1]

[1]Colorado State University, Fort Collins, CO, United States, [2]Liberian Institute of Biomedical Research, Monrovia, Liberia, [3]United States Naval Medical Research Unit - 3, Cairo, Egypt, [4]Institut de Recherche en Sciences de la Santé, Bobo Dioulasso, Burkina Faso

Control of the human-to-mosquito transmission of *Plasmodium* is being increasingly explored through drugs against sexual parasite forms in the human and mosquito, transmission blocking vaccines, and mass drug administrations. Successful implementation of these measures will require a greater understanding and quantitation of stage-specific infections of mosquitoes from humans. Past work on the human to mosquito portion of the *Plasmodium* life cycle has primarily focused on dissection and staining of wild blood fed mosquito midguts for early sexual forms and later stage oocysts, or examination of human infectiousness by direct skin feeds and standardized membrane feeding assays. These approaches, while important, tend to be laborious and may lack sensitivity for early infection events. As an alternative, we present a method of detecting the acquisition of *P. falciparum* in the bloodmeals of field caught *Anopheles gambiae* utilizing the modern molecular technique of Quantitative Nucleic Acid Sequence Based Amplification. QT-NASBA was performed on individual and pooled RNA extracted from fresh bloodmeals preserved on FTA cards for detection of Pfs25 transcript. We were able to detect *P. falciparum* in 5 of 6 RNA pools and 5 of 6 individual mosquitoes. Negative controls of RNA extracted from bloodmeals taken from uninfected individuals were all negative. Future experiments will focus on discrimination of this technique and parasite quantification from laboratory infected and naturally-infected mosquitoes. We see a variety of uses for this approach, including the correlation of human gametocytemia detected by QT-NASBA on human blood spots to the presence of early sexual forms in mosquitoes that bite upon these same individuals, performing sensitive spatial and temporal microepidemiology, and investigating biting tendencies of wild mosquitoes as they relate to human gametocytemia in a natural setting. These measures should lead to a more complete understanding of *Plasmodium* transmission, and may become an important measure to validate transmission-blocking interventions.

983

DEVELOPMENT OF A SINGLE-CELL GENOMICS PLATFORM FOR MALARIA INFECTIONS

Shalini Nair[1], Standwell Nkhoma[2], David Serre[3], Peter A. Zimmerman[4], Karla Gorena[5], Benjamin J. Daniel[5], Francois Nosten[6], Tim J. Anderson[1], Ian H. Cheeseman[1]

[1]Texas Biomedical Research Institute, San Antonio, TX, United States, [2]Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi, [3]Cleveland Clinic Lerner Research Institute, Cleveland, OH, United States, [4]Case Western Reserve University, Cleveland, OH, United States, [5]University of Texas Health Science Center San Antonio, San Antonio, TX, United States, [6]Shoklo Malaria Research Unit, Mae Sot, Thailand

Mixed- or multiple-clone malaria infections are commonplace in areas of high endemicity, are a major outcome in drug and vaccine efficacy trials, and can confound most parasite genetic studies. Though, surprisingly, their composition is still poorly understood. A major roadblock in exploring multiple-clone infections is that current genome sequencing tools only allow us to examine infections in bulk, providing little information on individual parasite genomes within an infection. To address this we have developed a single-cell genomics approach to dissect multiple-clone infections. By combining cell sorting and whole genome amplification we are able to capture single malaria parasite-infected red blood cells and generate sufficient high quality material for genomic analysis. We optimized our approach by assaying >260 single cells across fourteen experimental conditions. To quantify accuracy, we created artificial mixtures of *Plasmodium falciparum* laboratory lines (Hb3/Dd2/3D7) and obtained highly accurate (>99.9%) single cell genotypes. We genome sequenced 4 single cell amplifications obtained from these mixtures (Hb3 (n=2), 3D7 (n=2)), confirming 99.29% of the 196,332 SNP calls made across these 4 sequencing reactions. We saw no contamination from other genomes present in the mixtures in single cell genome sequences. This single cell genomics platform can be extended to malaria parasite species where long-term culture is not possible, precluding the direct dissection of infections through dilution cloning. We performed single cell genotyping and single cell sequencing of *P. vivax* infections obtained directly from patients, and obtained comparable accuracy and coverage to *P. falciparum* assays. These methods open the door for large scale analysis of within-host variation of malaria infections at single cell resolution.

984

POPULATION GENETICS AND NATURAL SELECTION OF CANDIDATE MALARIA VACCINE ANTIGENS IN MALIAN *PLASMODIUM FALCIPARUM* ISOLATES

Amed Ouattara[1], Mahamadou A. Thera[2], Shannon Takala-Harrison[1], Drissa Coulibaly[2], Laurens B. Laurens[1], Amadou Niangaly[2], Youssouf Tolo[2], Travassos A. Travassos[1], Matthew Adams[1], Abdoulaye Djimdé[2], Ogobara K. Doumbo[2], Christopher V. Plowe[1]

[1]University of Maryland, Baltimore, MD, United States, [2]USTB, Bamako, Mali

Vaccination, in combination with other control measures, is an essential strategy for malaria eradication. However, the highly polymorphic nature of many candidate vaccine antigens can result in allele-specific immune responses that limit vaccine efficacy against diverse strains found in nature, as has been observed with vaccines based on apical membrane 1 antigen and merozoite surface protein 1. Little is known about the genetic diversity in field isolates of the next generation of vaccine antigens even as vaccines based on these antigens are entering Phase 1 clinical testing. Some of these candidates include the liver and blood stage antigen merozoite surface antigen 5 (MSP5), the blood stage antigens glycosylphosphatidylinositol-anchored micronemal antigen (GAMA), and the RH5-interacting protein (PfRipr). We estimated the haplotype prevalences and genetic diversity within vaccine antigen-encoding genes in 91 specimens collected from asymptomatic infections and clinical malaria

episodes experienced by children in Bandiagara, Mali. We hypothesized that field isolates of at least some of these candidate antigens would exhibit low genetic diversity that would predict cross-protective efficacy against heterologous strains found in endemic areas. We estimated heterozygosity of the full-length malaria vaccine antigen sequence by the parameter π, estimate D* and F* to detect significant departures from the neutral model with *Plasmodium* reichenowi, the chimpanzee malaria parasite as the out-group, and test for balancing selection using Tajima's D test. Preliminary results indicate that MSP5 and PfRipr are well-conserved while GAMA is highly polymorphic. The results from this study will be used to down select conserved candidate antigens and antigenic variants for possible inclusion in a broadly cross-protective, multivalent malaria vaccine.

985

A NEW MALARIA VACCINE CANDIDATE, GPI PROTEIN TRANSAMIDASE RELATED PROTEIN SHOWED GOOD PROTECTIVE IMMUNE RESPONSE IN MOUSE MODEL

Mahamoud S. Cherif[1], Mohammed Nasir Shuaibu[1], Yukinobu Kodama[2], Tomoaki Kurosaki[3], Gideon Kofi Helegbe[4], Evaritus Chibunna Mbanefo[1], Mihoko Kikuchi[1], Tetsuo Yanagi[1], Hitoshi Sasaki[1], Katsuyuki Yui[1], Nguyen Tien Huy[1], Juntra Karbwang[1], Kenji Hirayama[1]

[1]Nagasaki University, Institute of Tropical Medicine (NEKKEN), Nagasaki, Japan, [2]Nagasaki University, Hospital Pharmacy, Nagasaki, Japan, [3]Nagasaki University, Hospital Pharmacy, Nagasaki, Japan, [4]University for Development Studies, Tamale, Ghana

Following a genome-wide search for a blood stage malaria candidate with GPI anchor motif using bioinformatics, and DNA-based vaccine screening and mouse malaria model, our study identifies PyGPI8p-transamidase related protein (PyTAM) as hypothetical protein with a protective immune response (Shuaibu et al., 2010). The homology search of PYTAM molecule using Conserved Domain Architecture Retrieval Tool (CDART) reveled that it's belonging to Cysteine proteases C13 family. Cysteine peptidases of parasitic protozoa have been implicated in a variety of biological events including invasion of host cells, immune evasion, pathogenesis and virulence and proteolytic degradation of hemoglobin and therefore some have also been validated as drug targets. Interestingly, we have found by immunofluorescence staining and immune-electron microscopy that the molecule is cytosolic at ring stage, and then located finally onto the parasitophorous vacuole at the late schizonte. Also, we successfully demonstrated the vaccination efficacy of PYTAM candidate as DNA vaccine formulated with Nanoparticle delivery system in controlling malaria in mouse model, as previously reported. Although the protection enhancing mechanism is not clearly understood. It is clear that antibody levels, was not significantly increased but Th1-mediated antigen-specific immune responses (INF-γ producing CD4 and CD8 T cell) was significant. Currently, we are working on molecular characterization of PYTAM and its mechanism of protective immune responses. These data indicate that PyTAM could a promising malaria vaccine candidate for further development.

986

IMMUNOGENICITY AND EFFICACY OF CHAD63-MVA ME-TRAP PRIME-BOOST VACCINATION AGAINST *PLASMODIUM FALCIPARUM* INFECTION IN HEALTHY ADULTS IN SENEGAL

Athanasia V. Mensah[1], Babacar Faye[1], Tandakha Dieye[1], Magatte Ndiaye[1], Omar Gaye[1], Nick Anagnostou[2], Nick Edwards[2], Ines Petersen[3], Babatunde imoukhuede[2], Nicola Viebig[3], Rachel Roberts[2], Odile Leroy[3], Katie Ewer[2], Adrian Hill[2], Badara Cisse[1]

[1]Universite Cheikh Anta Diop, Dakar, Senegal, [2]Jenner Institute, Oxford University, Oxford, United Kingdom, [3]European Vaccine Initiative, Heidelberg, Germany

Malaria transmission is in decline in some parts of Africa, which is partly due to the scaling up of control measures. Previous attempts at malaria elimination ended with mixed success. It is currently agreed that additional control measures including vaccination will be required. Recent studies using viral vectors with prime-boost approach to deliver ME-TRAP (multiple epitopes thrombospodin related adhesion proteins) showed promising safety, immunogenicity and significant efficacy in sporozoite challenge studies. Our study reports on the efficacy and immunogenicity of the prime-boost vaccines in peri-urban area of Dakar West Africa. We conducted a single-blind, randomised controlled phase IIb efficacy trial of 120 healthy men aged 18-50 years, living in a malaria endemic area of Senegal.Eligible study participants were randomised to receive either the active vaccine ChAd63 encoding the pre-erythrocytic antigen ME-TRAP as prime vaccination, followed eight weeks later by Modified Vaccine Ankara also encoding ME-TRAP as booster or two doses of anti-rabies vaccine as comparator . They were all followed up for eight months. The immunogenicity was determined by ELISPOT to quantify T cells responses to TRAP, and ELISA to identify the specific antibodies. We used finger-prick thick blood film to evaluate parasitemia throughout the study follow-ups. We determined time to first *P. falciparum* infection and re-infection by Real time PCR. Prior to intensive PCR survey, all study participants received a 3-day malaria treatment with atovaquone-proguanil and artesunate at the end of the vaccinations to clear all traces of parasitaemia. We also assessed the reactogenicity by recording all adverse and serious adverse events which occurred during follow-ups. This pre-erythrocytic malaria vaccine is safe and induces high immunogenicity with a mean T-cell response at 1266 SFU/106 PBMCs compared to 84SFU/106 PBMCS for the control group. qPCR Data analysis is ongoing and efficacy results will be presented at the meeting Vaccine efficacy against infection in adults may be rapidly assessed in peri-urban areas using this efficient trial design

987

HIGH PARASITE DENSITY IS ASSOCIATED WITH DIMORPHISM VARIATION IN THE ID1 DOMAIN OF VAR2CSA DURING PREGNANCY-ASSOCIATED MALARIA

Justin Doritchamou[1], Audrey Sabbagh[2], Morten A. Nielsen[3], Emmanuelle Renard[2], Jakobs S. Jespersen[3], Philippe Deloron[2], Ali Salanti[3], Nicaise Tuikue Ndam[2]

[1]University of Abomey Calavi, Cotonou, Benin, [2]UMR216 - Paris Descartes University, Paris, France, [3]Centre for Medical Parasitology - University of Copenhagen, Copenhagen, Denmark

Sequestration of *Plasmodium falciparum* infected erythrocytes (EI) in the placenta is the key phenomenon that characterizes the placental malaria. This feature is conferred to EI by their ability to bind Chondroitin Sulphate A (CSA) molecules expressed on the syncytiotrophoblastes. Immunity acquired by multigravidae allows controlling parasite density which limits the impact on the pregnancy outcomes. VAR2CSA has been identified as the main *P. falciparum* erythrocytes membrane protein involved in this interaction and is therefore considered as the major target of the acquired protective antibodies. But, the sequences polymorphism observed in VAR2CSA is one of the biggest challenges to be overcome in order to achieve development of an effective VAR2CSA-based vaccine. The N-terminal part of VAR2CSA has been indicated to 1°) contains the minimal CSA-binding site and; 2°) induce adhesion blocking antibodies with cross-reactive properties. In this study, we analyzed the sequence polymorphism in the N-terminal part of VAR2CSA expressed by field isolates and investigated the relationship between a particular genotype and the ability to bind CSA, the parasite density and other mother-related factors and pregnancy outcomes. A total of 398 NTS-ID2a sequences were generated from transcripts of 45 isolates collected from Beninese pregnant women resulting in 92 distinct sequences at protein level. The analysis demonstrated the existence of a dimorphic region within the structurally critical ID1 domain that revealed a very interesting association with the occurrence of infections with very high parasite density. Primers specific for this polymorphism were designed and this association was further validated on a second study population. Sequence analyses have helped define a distinct cluster of parasites, without any geographical bias and representing 20% of all analyzed clones. These observations are of

relevance to understand the molecular mechanisms mediating the severity of malaria infection in pregnant women and indicate interesting ways for potential optimization in the ongoing effort to develop a VAR2CSA based vaccine.

988

INTRODUCTION OF MALARIA VACCINE IN NIGERIA: STATUS AND PROGRESS UPDATE

Victoria Ibeh, Olufemi Ajumobi, Godwin Ntadom

National Malaria Control Programme, Federal Ministry of Health, Abuja, Nigeria

Malaria is endemic in Nigeria. About 97% of the population is at risk and it accounts for 25% under-5 mortality. Artemisinin-based Combination Therapy and Intermittent Preventive Treatment are current curative and preventive interventions. Clinical trials using malaria vaccine were conducted in Enugu and Jos, Nigeria. The RTS,S/AS01 coadministered with Expanded Programme on Immunization (EPI) vaccines provided modest protection against both clinical and severe malaria in young infants. Meetings were held in-country to discuss the pros and cons, in preparation for the adoption of malaria vaccine in Nigeria. Nigeria officials held Stakeholder and advocacy meetings with facilitators from MVI PATH in 2011 and 2012. Key informants were interviewed about national policy decision making processes on adopting new malaria control interventions and new vaccines in Nigeria and the readiness of the health system in adopting the candidate vaccine. The results were analyzed by content analysis. Progress is being made with adoption of malaria vaccine in Nigeria. There was no National Immunization Technical Advisory Group/Committee for all vaccine preventable diseases in Nigeria, and no standard documented guideline for decision making process for the adoption of new vaccines. The existing public-private partnership for the adoption of Human Papilloma Virus vaccine and Expanded Programme on Immunization in Nigeria was proposed for Malaria vaccine. Challenges foreseen with the decision-making process include large population size, weak health system, resistance to change by health staff, inadequate trained staff, high cost of implementation, budgetary deficits, inadequate cold chain and storage supply. A Ministerial memo has been sent to the National Council of Health and the Federal Executive Council. A proposal presentation would be made to the Technical Working Group on Malaria and relevant stakeholders. Lessons will be learnt from neighbouring countries with functional health system. Funding will be harnessed from World Health Organization and Global Alliance for Vaccine and Immunization. A proper cost benefit analysis would be done to ascertain cost-effectiveness. High level advocacy will be carried out at all levels.

989

EVIDENCE OF SELECTION IN POLYMORPHIC *PLASMODIUM FALCIPARUM* MEROZOITE ANTIGENS DURING THE RECOVERY OF CHILDREN FROM MALARIA

Lynette I. Oyier[1], John Okombo[1], Kevin K. Tetteh[2], Kevin Marsh[1]

[1]KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

Several *Plasmodium falciparum* merozoite invasion genes are highly polymorphic, potentially allowing the parasite to evade the host's immune responses and utilise alternative invasion pathways. The extent to which immune evasion shapes the parasite's population dynamics in natural infections is unknown. The Reticulocyte binding homologue (Rh), erythrocyte binding antigen (EBA), merozoite surface protein (MSP) 3-like gene families, apical membrane antigen (AMA) 1 and MSP1 have been shown to be involved in invasion as antibodies to these proteins inhibit invasion *in vitro*. Fifteen of these merozoite genes were sequenced from parasite DNA isolated from children, under 5, with uncomplicated malaria recruited into a drug trial between 2007and 2008, which consisted of pre (samples at recruitment)- and post (malaria slide positive sample during 84 days of weekly follow up) -treatment samples. The AMA1, MSP142, EBA175, MSPDBL1, MSPDBL2 and Rh2b loci were 100% heterologous genotypes in pre- and post-treatment parasite pairs. In contrast, 70% of the pre- and post-treatment parasites were homologous genotypes at the Rh5 locus. An analysis of the proportion of individual alleles revealed that the proportion of certain alleles in MSP142, EBA175, EBA181, Rh5 and AMA1 loci were significantly different ($p<0.05$) pre- and post-treatment. These changes in allele frequencies reflect the highly polymorphic nature of these antigens and suggest there may be a selective mechanism potentially allowing the parasite to evade immune responses, by allele-specific immunity. The allele proportions of MSP3, MSP6, MSPDBL2 and Rh1 remained similar pre- and post-treatment, suggesting that they may generate cross-reactive immune responses and thus there is no distinction between the multiple allelic types. In summary, the antigens which showed allele frequency changes may require a multi-allelic approach for vaccine development. Also, the antigens that showed 100% heterologous parasite pairs are good candidates for discriminating between recrudescent and new infections in antimalarial drug trials.

990

IDENTIFICATION OF THE NOVEL TRANSMISSION-BLOCKING VACCINE TARGET EXPRESSING ON THE SURFACE OF MALE GAMETES

Mayumi Tachibana[1], Motomi Torii[1], Moe Sudo[1], Takafumi Tsuboi[2], **Tomoko Ishino**[1]

[1]Ehime University, Toon, Japan, [2]Ehime University, Matsuyama, Japan

Plasmodium transmission from mosquitoes to mammalians requires sexual stage parasite development and fertilization in mosquito midguts. After ingestion of gametocytes by mosquitoes, fertilization occurs to form zygotes, which develop into invasive forms, ookinetes. These stages could be promising targets for transmission blocking strategy, which intends to break malaria life cycle inside the mosquito vector. Despite large research effort on screening for parasite molecules expressing on the surface of gamete, zygote or ookinete, the number of the candidate antigens of transmission-blocking vaccine is still limited. Previously, we reported that a novel male specific protein, designated PyGM75, is localized to the osmiophilic bodies of male gametocytes then transported to the surface of microgametes in *Plasmodium yoelii*. Furthermore, we demonstrated that pygm75 disrupted parasites impaired the exflagellation ability. In this study, we investigated whether PyGM75 can be a novel transmission-blocking vaccine target. First, to examine if anti-PyGM75 antibodies can prevent parasite transmission to mosquitoes, we mixed the specific antibodies and parasitized RBCs, then let them feed on *Anopheles* mosquitoes using membrane-feeding system. As the result, the numbers of oocysts formed on the mosquito midgut were greatly reduced by adding anti-PyGM75 antibodies, as a dose dependent manner. In addition, the antibodies strikingly impaired the motility of microgametes *in vitro*. Taken together, our data demonstrates that anti-PyGM75 antibodies have potential to prevent the malaria transmission, presumably by interfering fertilization occurred in midguts. Since PyGM75 is conserved among *Plasmodium* species, including *P. falciparum* and *P. vivax*, this study strongly suggests that GM75 is a novel candidate target of transmission-blocking vaccine.

991

INVESTIGATING THE ROLE OF GILT ON IMMUNE RESPONSES TO A MALARIA VACCINE ANTIGEN

Kristen M. Merino, Vinay Kumar, Geetha Kumar, Nirbhay Kumar

Tulane University, New Orleans, LA, United States

Malaria ranks fifth as leading cause of death from infectious diseases. Our overall research goal is focused on the development of a malaria transmission-blocking vaccine (TBV). One such vaccine target (Pfs48/45 protein) is based on antigens expressed on male and female gametocytes, which establish infection in the mosquito vector. The targets of blocking antibodies are disulfide bond constrained conformational epitopes. As Pfs48/45 has not been crystallized, precise location of the disulfide bonds

and the topology of epitopes are unknown. The disulfide bonds have been shown to greatly influence the ability of an APC to process and present epitopes, and elicitation of an appropriate immune response. GILT, a thiol reductase constitutively expressed in APCs, mediates endocytic reduction of antigens and display of peptides on MHC class I and II. Using recombinant Pfs48/45 as the model antigen, this project seeks to identify mechanisms involved in presentation of relevant epitopes to T and B cells leading to effective antibody responses and generation of the memory B cell pool. We hypothesize that reduction of disulfide bonds in Pfs48/45 will dramatically impact the generation of T cell epitopes, and thus influence downstream B cell stimulation and protective antibody responses. We have conducted immunogenicity studies in wildtype and GILT-/- mice using both non-reduced and reduced forms of Pfs48/45 and analyzed the responses using full length and five overlapping (~100aa long, spanning full-length Pfs48/45) sub-fragments and 39 peptides by ELISA, western blotting, ELISpot and T cell proliferation assays. Results from these studies have indeed revealed significantly different patterns of recognition of putative B and T cell epitopes. These ongoing studies have revealed the presence of immunodominant B cell and T cell epitopes in the sub-fragment 2 (aa 108-200) and sub-fragment 5 (aa 341-420), respectively. We are now initiating studies to investigate the influence of immune epitopes in functional responses and transmission-blocking protection. Results of the study will impact vaccine considerations.

992

IDENTIFICATION OF CORRELATES OF DISEASE AND PROTECTION IN A CONTROLLED HUMAN MALARIA INFECTION MODEL

Remko Schats[1], Edwin Quinten[1], Else M. Bijker[2], Erik van Zwet[1], Krista E. Meijgaarden[1], Fionn Mag Uidhir[1], Tom HM Ottenhoff[1], Robert W. Sauerwein[2], Leo G. Visser[1], Marielle Haks[1]

[1]Leiden University Medical Center, Leiden, The Netherlands, [2]Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Long-lasting protection against homologous *Plasmodium falciparum* infection can be induced in malaria naive subjects taking chloroquine prophylaxis during immunizations with bites of infected mosquitoes followed by a Controlled Human Malaria infection (CHMI). However, identification of correlates of disease and protection, which could be prospectively explored as biomarkers for host protective immunity to malaria, is still critically limited. Here, we monitored the kinetics of the human immune response at the transcriptomic and cellular level during immunizations and controlled infection. Direct ex-vivo whole blood gene expression profiling and cell subset analysis was performed using dual-color Reverse-Transcriptase Multiplex Ligation-dependent Probe Amplification (dcRT-MLPA) and polychromatic (16-color) flow cytometry, respectively. Volunteers from three CHMI trials were included in this study: (1) a mosquito/sporozoite dose titration CHMI using chloroquine prophylaxis during immunizations, (2) a non-inferiority CHMI comparing the efficacy of chloroquine to mefloquine prophylaxis during immunizations, and (3) a heterologous challenge of previously (under chloroquine prophylaxis) immunized and homologously protected volunteers. Genes differentially expressed between protected and unprotected volunteers were identified by the Global test at each time point during vaccination and challenge. Biomarker signatures for disease and protection were generated using Lasso and Ridge regression analysis. Critical signalling networks that discriminate between protective and non-protective immune responses will now be generated by pathway analysis. In addition, key cellular changes, both quantitative and qualitative, have been analyzed by multi-parameter flow cytometry. This will be followed by integration of the molecular and cellular datasets to correlate data to protective immunity and/or disease.

993

PROFILING THE HUMORAL IMMUNE RESPONSES TO *PLASMODIUM VIVAX* INFECTION AND IDENTIFICATION OF CANDIDATE IMMUNOGENIC RHOPTRY-ASSOCIATED MEMBRANE ANTIGEN (RAMA)

Feng Lu[1], Jian Li[1], Bo Wang[1], Yang Cheng[1], Deok-Hoon Kong[2], Liwang Cui[3], Kwon-Soo Ha[2], Jetsumon Sattabongkot[4], Takafumi Tsuboi[5], Eun-Taek Han[1]

[1]Department of Medical Environmental Biology and Tropical Medicine, Kangwon National University, Chuncheon, Republic of Korea, [2]Department of Molecular and Cellular Biochemistry, Chuncheon, Republic of Korea, [3]Department of Entomology, Pennsylvania, PA, United States, [4]Mahidol Vivax Research Unit, Bangkok, Thailand, [5]Division of Malaria Research, Matsuyama, Japan

Completion of sequencing of the *Plasmodium vivax* genome and transcriptome offers the chance to identify antigens among >5000 candidate proteins. To identify those *P. vivax* proteins that are immunogenic, a total of 152 candidate proteins (160 fragments) were expressed using a wheat germ cell-free system. The results of Western blot analysis showed that 92.5% (148/160) of the targets were expressed, and 96.6% (143/148) were in a soluble form with 67.7% of solubility rate. The proteins were screened by protein arrays with sera from 22 vivax malaria patients and 10 healthy individuals to confirm their immune profile, and 44 (27.5%, 44/160) highly reactive *P. vivax* antigens were identified. Overall, 5 candidates (rhoptry-associated membrane antigen [RAMA], Pv-fam-a and -b, EXP-1 and hypothetical protein PVX_084775) showed a positive reaction with >80% of patient sera, and 21 candidates with 50% to 80%. More than 23% of the highly immunoreactive proteins were hypothetical proteins, described for the first time in this study. One of the top immunogenic proteins, RAMA, was characterized and confirmed to be a serological marker of recent exposure to *P. vivax* infection. These novel immunoproteomes should greatly facilitate the identification of promising novel malaria antigens and may warrant further study.

994

IMMUNOGENICITY OF A SYNTHETIC VACCINE BASED ON THE *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN REGION II

Francis B. Ntumngia[1], Samantha J. Barnes[1], Amy M. McHenry[2], Miriam T. George[1], Jesse Schloegel[3], John H. Adams[1]

[1]University of South Florida, Tampa, FL, United States, [2]Southwestern Adventist University, Keene, TX, United States, [3]University of Sydney, Sydney, Australia

Molecules that play a role in *Plasmodium* merozoite invasion of host red blood cells represent attractive targets for blood-stage vaccine development against malaria. In *Plasmodium vivax*, merozoite invasion of reticulocytes is mediated by the Duffy binding protein (DBP), which interacts with its cognate receptor, the Duffy antigen receptor for chemokines (DARC) on the surface of reticulocytes. The DBP ligand domain, known as region II (DBPII), contains the critical residues for receptor recognition making it a prime target for vaccine development against blood-stage vivax malaria. In natural infections DBP is weakly immunogenic and DBPII allelic variation is associated with strain-specific immunity, which may compromise vaccine efficacy. In a previous study, a synthetic vaccine termed DEKnull that lacked an immunodominant variant epitope in DBPII induced functional antibodies to shared neutralizing epitopes on the native Sal1 allele. Anti-DEKnull antibody titers were lower than anti-Sal1 titers but produced more consistent, strain-transcending anti-DBPII inhibitory responses. In this study, we further characterized the immunogenicity of DEKnull, finding immunization with rDEKnull produced an immune response comparable to native recombinant DBP alleles. Further investigation of DEKnull is necessary to enhance its immunogenicity and broaden its specificity.

995

TARGETING MALARIA PARASITE INVASION OF RED BLOOD CELLS: HOST-CELL ENGAGEMENT AND ANTIBODY NEUTRALIZATION

Niraj H. Tolia[1], Joseph D. Batchelor[1], Brian M. Malpede[1], Edwin Chen[1], Daniel H. Lin[1], May M. Paing[1], Nichole D. Salinas[1], Jacob A. Zahm[1], Natalie S. Omattage[1], Gregory T. DeKoster[1], Katherine A. Henzler-Wildman[1], B. Kim Lee Sim[2]

[1]Washington University School of Medicine, St. Louis, MO, United States, [2]Protein Potential, Rockville, MD, United States

Host-cell invasion is a critical step in the pathogenesis of malaria. Erythrocyte Binding Like (EBL) ligands mediate critical interactions during RBC invasion by *Plasmodium falciparum* and *P. vivax*, and are targets of antibodies that play a role in naturally acquired immunity. However, the structural basis and mechanisms of host-cell engagement, as well as the neutralization mechanisms of antibodies that prevent parasite growth have not been defined. Lack of this knowledge has hampered the effective rational design of agents to disrupt invasion. We will present structural, mechanistic and functional studies of EBL ligands from both *P. falciparum* and *P. vivax* in association with receptors and antibodies. These studies demonstrate that multimeric assembly of receptor-ligand interactions is crucial for host-cell invasion, and highlight critical functional regions that can be exploited for targeted disruption, including receptor-binding pockets and multimeric assembly interfaces. We found that potently neutralizing antibodies target the assembly interfaces and receptor-binding residues, while non-neutralizing antibodies target decoy epitopes far removed from functional regions of ligands. These results explain why only a subset of antibodies that recognize EBL ligands are neutralizing. The results reveal the complex nature of EBL-RBC interactions and highlight new approaches to target the molecular mechanism of invasion. Vaccine efficacy may be improved by targeting critical functional regions and protective epitopes of EBL ligands, while avoiding decoy-epitopes identified by these studies.

996

A NEXT GENERATION GENETICALLY ATTENUATED *PLASMODIUM FALCIPARUM* PARASITE CREATED BY TRIPLE GENE DELETION

Viswanathan Lakshmanan

Seattle BioMed, Seattle, WA, United States

A malaria vaccine could dramatically reduce the tremendous morbidity and mortality due to *Plasmodium* parasites. Immunizations with live-attenuated sporozoites in animal models and humans have shown the feasibility of a pre-erythrocytic malaria vaccine that confers complete and protracted protection against infection. Genetic engineering offers a versatile platform for controlled and consistent design of pre-erythrocytic genetically attenuated parasites (GAPs) as vaccine candidates. We previously generated a GAP by deleting the *P. falciparum* P52 and P36 genes (Pf p52-/p36- GAP) preclinical assessment of which indicated an early liver stage growth defect. However, human exposure to >200 Pf p52-/p36- GAP-infected mosquito bites caused peripheral parasitemia in 1 of 6 safety trial volunteers, revealing that this GAP was severely but incompletely attenuated. We modeled this phenotype with rodent malaria *P. yoelii* p52-/p36- GAP in highly susceptible Balb/cByJ mice. Encouragingly, Pf p52-/p36- GAP induced substantial human immune responses including antibodies that blocked *in vitro* hepatocyte infection by sporozoites. We have now created a triple gene deleted GAP by additionally removing SAP1 (Pf p52-/p36-/sap1- GAP). SAP1 deletion alone was sufficient to completely attenuate *P. yoelii* in Balb/cByJ mice. Deletion of genes whose encoded proteins perform distinct biological functions (as do P52, P36 and SAP1) should improve the robustness of attenuation and greatly reduce the possibility of compensatory changes. Pf p52-/p36-/sap1- GAP and wildtype parasites were indistinguishable in blood and mosquito stages of development. Using an improved humanized mouse model transplanted with human hepatocytes and erythrocytes, we demonstrate that despite a high dose sporozoite challenge, Pf p52-/p36-/sap1- GAP did not transition to blood stage infection and appeared completely attenuated. We also used FLP/FRT recombination to remove all drug selectable markers from Pf p52-/p36-/sap1- GAP. Preparations are underway to test this next generation GAP in a human phase I clinical trial.

997

IMMUNOSCREENING OF THE TARGET PROTEINS CONTRIBUTING TO GROWTH INHIBITORY ACTIVITY OF HUMAN IGG AGAINST *PLASMODIUM FALCIPARUM*

Masayuki Morita[1], Eizo Takashima[1], Bernard Nganga Kanoi[1], Kazutoyo Miura[2], Takafumi Tsuboi[1]

[1]Division of Malaria Research, Proteo-Science Center, Ehime University, Matsuyama, Japan, [2]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Residents in malaria endemic area acquire the protective antibodies against the disease by continuous infection with *Plasmodium falciparum*. It has been shown that a part of the antibodies have growth inhibitory activity (GIA) against *in vitro* cultured *P. falciparum*. We then hypothesized that identification of responsible antigens to GIA may support the discovery of novel blood-stage vaccine candidates. The GIA of individual IgG purified from a Malian immune adult was measured (n=51). To identify the antibody responses correlating with GIA, we established a modified AlphaScreen system for high-throughput detection of antigen-antibody reaction. Genome-widely expressed 1,848 parasite proteins by a wheat germ cell-free system were screened with the Malian adult IgGs. Known malaria vaccine candidates were also included in the 1,848 proteins. First, we selected approximately 900 immunoreactive proteins with the Malian adult IgGs (giving the AlphaScreen count more than negative control). Of those, we selected antigens which showed significant positive correlations between their AlphaScreen counts and GIA. As a result, the ratio of the known malaria vaccine candidates among the finally selected antigens was significantly higher than that in the original 1,848 proteins. The result suggests that this screening system would be effective to discover novel malaria blood-stage vaccine candidates. In addition to the analysis of the individual antigens, we are now analyzing which combination of antigens will better explain the GIA of total IgG. The results of this analysis will be discussed in this presentation.

998

CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* MAS170 AS NOVEL MALARIA BLOOD-STAGE VACCINE CANDIDATE

Chisa Sasaoka[1], Hirokazu Sakamoto[1], Daisuke Ito[1], Satoru Takeo[1], Jetsumon Sattabongkot[2], **Eizo Takashima**[1], Takafumi Tsuboi[1]

[1]Division of Malaria Research, Proteo-Science Center, Ehime University, Matsuyama, Japan, [2]Mahidol Vivax Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

A novel 170-kDa merozoite apical surface protein of *Plasmodium falciparum* (PfMAS170) was characterized as a novel malaria blood-stage vaccine candidate in this study. The PfMAS170 is conserved among *Plasmodium spp.* and is predicted as 170-kDa protein with a signal peptide at the N-terminus. We expressed recombinant protein corresponding to the C-terminal region of PfMAS170 using a wheat germ cell-free system to obtain anti- PfMAS170 sera. Western blot analysis detected approximately 170-kDa signal corresponding to the full-length of PfMAS170 in late blood-stage parasites, and PfMAS170 fragments from approximately 120-kDa to 30-kDa were released in the culture medium. Immunofluorescence assay of free-merozoite without Triton X-100 permeabilization revealed that PfMAS170 localizes on the surface of apical end of merozoite. Erythrocyte binding assay of the conditioned culture medium showed that the secreted 30-kDa fragment of PfMAS170 binds to erythrocyte

surface. In order to test whether antibodies to PfMAS170 could block merozoite invasion, growth of *P. falciparum* 3D7 parasite in the presence of anti-PfMAS170 antibody was tested. The anti-PfMAS170 antibody significantly inhibits the merozoite invasion to erythrocytes. Since anti-PfMAS170 antibodies inhibited merozoite invasion *in vitro*, we decided to investigate whether PfMAS170 is exposed to the human immune system in *P. falciparum*-infected individuals. The sera from *P. falciparum* infected individuals in Thailand reacted with the recombinant PfMAS170, indicating PfMAS170 is immunogenic in humans. Taken together, these results suggested that PfMAS170 plays important role in the merozoite invasion process. The C-terminal erythrocyte-binding domain is of interest for the development of blood-stage malaria vaccine.

999

EVALUATING THE POTENTIAL IMPACT OF TRANSMISSION BLOCKING VACCINES AGAINST *PLASMODIUM FALCIPARUM* INFECTION ALONGSIDE EXISTING INTERVENTIONS

Robert Verity, Tom Churcher, Azra Ghani

MRC Centre for Outbreak Analysis and Modelling, Imperial College London, London, United Kingdom

Transmission-blocking vaccines are under development with the aim that they will reduce or interrupt transmission in malaria endemic settings when used alongside existing interventions. However, their utility will depend not only on their efficacy and durability, but also on characteristics of the transmission setting including the intensity and seasonality of transmission. We extended a published mathematical model to identify settings in Sub-Saharan Africa in which a TBV could interrupt transmission if implemented alongside existing interventions, and the characteristics required of the TBV and of the vaccination programme. In all settings repeated annual rounds of vaccination will be required, with more frequent rounds required if the duration of protection is shorter or if the initial transmission intensity is high. The protective efficacy of a typical TBV vaccine with a 1-year half-life is predicted to be substantially higher in seasonal settings compared to perennial settings with the same average transmission intensity, with greater efficacy achieved if the vaccination programme is aligned with the start of the transmission season. Overall the protective efficacy is predicted to be greatest in areas of low transmission but the number of cases averted greatest in areas of moderate transmission. Thus the optimal location to undertake Phase III trials for candidate vaccines would be in existing low to moderate transmission areas.

1000

IMMUNOGENICITY OF DNA VACCINES ENCODING PFS48/45, A *PLASMODIUM FALCIPARUM* TRANSMISSION-BLOCKING VACCINE ANTIGEN IN RHESUS MONKEYS BY *IN VIVO* ELECTROPORATION INCLUDING EVALUATION OF CODON OPTIMIZATION AND N-LINKED GLYCOSYLATION

Dibyadyuti Datta[1], Geetha Bansal[1], Brooke Grasperge[2], Mario Philipp[2], Rajesh Kumar[1], Dietlind Gerloff[3], Drew Hannaman[4], Nirbhay Kumar[1]

[1]Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Tulane National Primate Research Center, Covington, LA, United States, [3]Foundation for Applied Molecular Evolution, Gainesville, FL, United States, [4]ICHOR Medical Systems Inc., San Diego, CA, United States

Antigens expressed on various sexual stages of *Plasmodium falciparum* are being pursued as targets of transmission blocking vaccines (TBV). These include gamete surface proteins (Pfs230 and Pfs48/45) and zygote/ookinete surface protein (Pfs25) expressed after fertilization in the mosquito midgut. Ingestion of antibodies against these antigens effectively blocks parasite development in the midgut. Efforts are underway to develop vaccines based on either recombinant proteins-adjuvant formulations or as DNA vaccines. While our laboratory has previously expressed Pfs48/45 as a functionally effective recombinant molecule in E. coli, structural complexity has continued to be a challenge for further development of an effective vaccine. The goal of this study was to investigate Pfs48/45 using DNA vaccine platform. The rationale is to develop a TBV that is: (i) technically less challenging in comparison to recombinant protein production, (ii) cost-effective, (iii) stable, and (iv) easy to manufacture. In addition, DNA vaccine platform allows a multivalent approach by combining several antigens. Our vaccine design included codon optimization of DNA sequence for optimum expression, in-vivo electroporation for enhanced immunogenicity, mutations to block any N-linked glycosylation in the expressed protein, and a heterologous prime-boost approach. Additionally, we evaluated a combination of DNA vaccines encoding Pfs48/45 and Pfs25. All 7 putative N-linked glycosylation sites in Pfs48/45 were mutated (N to D or K) based on available sequences of P48/45 orthlogs in various *Plasmodium* species. Rhesus macaques (N=4) were assigned to each of three groups and immunized (IM) with 3 DNA vaccine doses (2.5mg), using *in vivo* electroporation at 4 week intervals followed by a recombinant protein boost (50ug protein in Alum). Antibody titers were determined by ELISA and functional activity in mosquito membrane feeding assays. Results on various test parameters as well as outcome of combining two different TBV target antigens will be discussed. (Funded by AI47089, AI101427 and AI 103466).

1001

DISTRIBUTIONAL IMPACT OF RTS,S VACCINATION IN SUB-SAHARAN AFRICA: IMPLICATIONS FOR POLICY IMPLEMENTATION

Katya Galactionova, Fabrizio Tediosi, Thomas A. Smith, Melissa A. Penny

Swiss Tropical and Public Health Institute, Basel, Switzerland

A growing body of evidence has demonstrated that many public health interventions developed to aid the poor are not reaching their intended target. These concerns are relevant for the introduction of a malaria vaccine, the most advanced of which is RTS,S, currently in Phase III clinical trials. Initial results indicate that RTS,S can provide modest protection against both clinical and severe malaria in young infants. We examine the potential distributional impact of the RTS,S vaccine in 6 African countries by evaluating differences in the relative risk of malaria against projected vaccine coverage and health benefits across beneficiaries grouped by socio-economic characteristics using an asset index. To accomplish this we first link country Demographic and Health Surveys with the distributions of entomological inoculation rate derived from the prevalence data assembled by the Malaria Atlas Program. We then combine information on transmission, access to health services and baseline vector control interventions coverage to assess the impact of vaccine deployment on disease burden and its distribution across different population groups using a stochastic simulator of malaria epidemiology and control. We estimate the extent of forgone health due to disparities in access to immunization services by simulating a scenario assuming immunization coverage of the group in the highest socio-economic quintiles for the whole vaccination cohort. We highlight the importance of malaria case management in sustaining the health gains achieved with the RTS,S by simulating vaccine impact at levels of highest wealth quintile for both immunization and case management coverage. Our findings suggest that substantial additional reductions in burden could be realized with RTS,S by targeting the underserved population with either extensive outreach or through innovative distribution channels. We further illustrate the gains in program effectiveness if vaccine deployment in combined with systems strengthening to improve access to malaria case management.

1002

DEVELOPMENT OF *PLASMODIUM FALCIPARUM* RETICULOCYTE BINDING-LIKE HOMOLOGOUS PROTEIN 2 (PFRH2) AS A BLOOD-STAGE MALARIA VACCINE CANDIDATE

Tajali Sahar, Alok Kumar Pandey, Archana Sharma, Amlabu Emmanuel, K. Sony Reddy, Virander S. Chauhan, Deepak Gaur

International Centre for Genetic Engineering and Biotechnology, New Delhi, India

The *Plasmodium falciparum* reticulocyte binding-like homologous (PfRH) family of proteins are key determinants of different erythrocyte invasion pathways. Out of five functional PfRH proteins, we report that native PfRH2 undergoes processing yielding fragments that exhibit differential erythrocyte binding specificities. Consistent with previous PfRH2 knockout studies, the RBC binding specificity of native PfRH2 was sialic acid-independent, trypsin resistant and chymotrypsin sensitive. However, a smaller processed fragment bound erythrocytes with a different phenotype. To further characterize the processing sequence and localization of the resulting fragments, we have raised specific antibodies against different regions of the PfRH2 ectodomain. We have mapped the erythrocyte binding domain of PfRH2 to a conserved 40kDa N-terminal region (rPfRH240). Recombinant rPfRH240 bound to erythrocytes in a sialic acid independent, trypsin resistant, chymotrypsin sensitive manner, consistent with the binding of the native protein. PfRH2 antibodies against only the 40 kDa receptor binding domain were able to efficiently block erythrocyte invasion and further produced synergistic inhibition of erythrocyte invasion in combination with antibodies against other parasite ligands such as EBA-175 and AARP. A recent study has demonstrated that PfRH2 is naturally immunogenic in humans residing in malaria endemic regions and that PfRH2 antibodies exhibited the highest association with protection against malaria among a pool of 91 recombinant blood-stage antigens. We have developed and optimized a process for the production of rPfRH240 with cGMP specifications for it's clinical development. Thus, rPfRH240 is a major candidate antigen under the ICGEB portfolio for the development of a new generation combination based blood-stage malaria vaccine.

1003

INDUCTION OF IMMUNITY FOLLOWING VACCINATION WITH A CHEMICALLY ATTENUATED MALARIA VACCINE CORRELATES WITH PERSISTENCE OF A SUB-PATENT INFECTION

Jennifer M. Reiman[1], Amber Raja[1], Bibiana Rodriguez[1], Sai Lata De[1], Stephanie Yannow[2], Terry Spithill[3], Moses Lee[4], Virginia McPhun[1], Michael F. Good[1]

[1]Griffith University, Southport, QLD, Australia, [2]University of Alberta, Edmonton, AB, Canada, [3]La Trobe University, Melbourne, Australia, [4]Georgia State University, Atlanta, GA, United States

Antigenic polymorphism presents a major hurdle for subunit malaria vaccine development. To overcome this obstacle we are developing whole parasite approaches using DNA alkylating agents to chemically attenuate blood stage parasites which will be used as vaccines. We have demonstrated that attenuated *Plasmodium chabaudi* or *P. yoelii* parasitized RBCs can induce immunity in mice. The RBCs in the vaccine had to remain intact for the induction of immunity and killed parasites did not induce immunity. To further understand the nature of immune induction as we move to a clinical trial, we have attempted to follow the fate of attenuated parasites post-inoculation. Mice were immunized once with 10^6 *P. chabaudi* pRBCs attenuated with 2μM tafuramycin A (TfA). qPCR and qRT-PCR were used to monitor parasite DNA and RNA in blood and various tissues. Adoptive transfer studies were used to investigate whether sub-microscopic levels of parasites could transfer immunity between animals. Surprisingly, parasite DNA was detectable for up to one week within the RBCs of vaccinated mice. In contrast, inoculation of mice with killed pRBCs did not result in persisting levels of parasite DNA in blood or tissues of recipient mice. Irraditated whole blood from vaccinated mice could adoptively transfer immunity to recipient mice. Furthermore, treatment of vaccinated mice with anti-malaria chemotherapy was able to reduce the level of immunity in vaccinated mice. Our results suggest that immunity in mice is dependent on a persisting sub-patent attenuated infection. These results have informed our strategy to develop a *P. falciparum* vaccine with a major goal of utilizing an attenuating dose of TfA that enables a persisting sub-patent infection.

1004

THE USE OF A TRANSGENIC RODENT MALARIA CHALLENGE MODEL FOR ASSESSMENT OF NOVEL LIVER-STAGE MALARIA VACCINES

Ahmed M. Salman[1], Rhea J. Longley[1], Alexandra J. Spencer[1], Shahid M. Khan[2], Chris J. Janse[2], Adrian V.S. Hill[1]

[1]The Jenner Institute, University of Oxford, Oxford, United Kingdom, [2]Leiden Malaria Research Group, Leiden University Medical Center, Leiden, Netherlands

Malaria remains one of the most important global infectious diseases. At present there is no completely effective/licensed malaria vaccine. Most of the severe pathologies and deaths due to malaria are associated with *Plasmodium falciparum* (Pf) strain, and developing effective vaccines remains a priority. Unfortunately Pf does not infect small animals and a number of the Pf candidate vaccine antigens either differ from or are absent in rodent parasites, limiting pre-clinical efficacy studies in murine models. In this work we sought to rank-order the protective immune responses to several novel Pf vaccine candidates using a rodent challenge model. We first immunized mice with different Pf pre-erythrocytic vaccine antigens delivered using a viral vectored vaccine (ChAd63/MVA prime-boost) approach and tested the responses to these antigens in mice. For each antigen, we created transgenic *P. berghei* (Pb) parasites expressing the Pf vaccine-candidate gene of interest thus enabling a vaccine efficacy/challenge assessment *in vivo*. Consequently, we were able to perform a screening of 11 Pf vaccine candidates - and several others are in progress. We rank ordered these antigens based on protection studies in different mice strains, selecting the most promising ones to be taken forward for further development. We created two sets of mutants: (i) Pf candidate-antigen genes were expressed in sporozoite and liver-stage using the Pbuis4 promoter; and (ii) when the Pf antigen had a homolog in Pb, the Pb gene was replaced by its Pf equivalent and expressed under the corresponding Pb promoter. Both sets of mutants were used in the immunization-challenge studies. Antigen screening using this challenge model identifies PfLSA1 and PfLSAP2 as more protective than PfCSP or PfTRAP in Balb/c mice with high efficacy: 81.25% and 75% respectively. Also, these two antigens show good efficacy in CD1 outbred mice: (87.5%) and (70%) respectively. Based on results from our initial candidate antigen immunogenicity and efficacy ranking experiments we have also created double transgenic parasites that express different combinations of the most promising candidates. These reagents, we believe, are powerful tools that can help in the rapid assessment of multiple-antigen vaccine approaches.

1005

OPTIMIZATION OF NON-IV ADMINISTRATION OF A RADIATION-ATTENUATED SPOROZOITE MALARIA VACCINE IN A MURINE MODEL

Richard Ruck[1], Maria Belmonte[2], Steve Abot[2], Sumana Chakravarty[3], Arnel Belmonte[2], Thomas L. Richie[2], Eileen Villasante[2], Judy Epstein[2], Steven L. Hoffman[3], Martha Sedegah[2]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Naval Medical Research Command, Silver Spring, MD, United States, [3]Sanaria Inc., Rockville, MD, United States

Radiation-attenuated sporozoites (SPZ) have been known to induce high-level protection against malarial infection for nearly 5 decades. This was

initially proven in mice with intravenous (IV) administration of rodent malaria SPZ, and was followed by successful immunization of humans by the bite of irradiated *Plasmodium falciparum* (Pf)-infected mosquitoes. It was recently reported that subcutaneous (SC) and intradermal (ID) administration of radiation-attenuated, aseptic, purified, cryopreserved PfSPZ Vaccine was poorly immunogenic and protective in a clinical trial. The same authors also reported that it required 7-10 times as many irradiated (irr) *P. yoelii* (Py) SPZ administered SC or ID as compared to IV to achieve similar protection in BALB/c mice. Subsequently, a clinical trial involving IV administered PfSPZ Vaccine demonstrated protection in 6/6 subjects against controlled Pf malaria infection. Many think it would be optimal if PfSPZ Vaccine was administered by a non-IV route. We utilized the PySPZ-BALB/c model system to systematically address how to optimize non-IV administration of irrPySPZ, using 3 doses of $2x10^3$ irrPySPZ administered IV as the gold standard. We assessed IM, SC, and ID routes of administration, and varied volume of administration (1-25 µL), number of sites of administration (2-30), number of doses (3-5), and number of irrPySPZ administered (2 - $7.5x10^4$ irrPySPZ). Protection > 80% was generally seen after IV administration. However, we were not able to achieve > 80% protection with the other routes. The SC route was superior to IM and ID routes, and injection in multiple sites with smaller volumes was superior to larger volumes in fewer sites, with the SC route achieving the highest protection at 56%. The complete results will be presented, as will plans for studies with higher doses and adjuvants. These results highlight the inefficiency of non-IV as compared to IV administration of SPZ. However, the data indicate that a systematic approach has the potential to significantly increase the efficiency of administration by non-IV routes.

1006

IMMUNE RESPONSES INDUCED BY AN ATTENUATED, WHOLE PARASITE, BLOOD-STAGE *PLASMODIUM YOELII 17X* MALARIA VACCINE

Amber I. Raja[1], Virginia McPhun[1], Jennifer Reiman[1], Moses Lee[2], Danielle I. Stanisic[1], Michael F. Good[1]

[1]Griffith University, Gold Coast, Australia, [2]Georgia State University, Atlanta, GA, United States

We are investigating the protective efficacy of a chemically attenuated, whole parasite, blood-stage *Plasmodium yoelii 17X* vaccine in a rodent malaria model. Optimization of an immunization regimen using a chemically attenuated, asynchronous *P. yoelii 17X* vaccine has demonstrated that 3 doses results in protection in BALB/c mice against death, severe anemia and weight loss following homologous challenge. Furthermore, parasite burden is limited in the blood of immunized mice compared to control mice. Immune responses induced by vaccination are being characterized, with data demonstrating that 3 immunizations induces both significantly higher levels of proliferative cellular responses and antibodies compared to control mice. The induction of antibodies following vaccination is novel to the *P. yoelii 17X* model as they were not detected following vaccination with a chemically attenuated *P. chabaudi* vaccine. However, the precise role of these antibodies in vaccine-induced protection is still unclear as no protection was observed when passively transferred to naive mice followed by homologous challenge. Lymphocyte depletion studies were undertaken to investigate the role of $CD4^+$ and $CD8^+$ T cells in vaccine-associated protection, demonstrating a crucial role for $CD4^+$ T cells only. Following *in vitro* stimulation with parasitized red blood cells, IFN-γ and IL-2 were detected. The cellular origin of these cytokines is being investigated. The induction and longevity of memory T cells in vaccinated mice is being investigated. Preliminary data show significantly higher effector memory T cells are observed up to 3 months post-vaccination in the blood of vaccinated mice compared to control mice, with the significance waning by 6 months. No difference in central memory T cells was observed. Additionally, we are investigating whether cross-stage protection against sporozoite/ liver-stage challenge is induced by this blood-stage vaccine. Our data show that vaccination is capable of inducing activated $CD8^+$ T cells ($CD8^{lo}CD11a^+$), which are known to play a role in liver-stage immunity.

1007

THE RELATIVE IMPORTANCE OF MALARIA VACCINE PROPERTIES

Melissa A. Penny, Peter Pemberton-Ross, Thomas Smith

Swiss Tropical and Public Health Institute, Basel, Switzerland

Currently many malaria vaccines directed at *Plasmodium falciparum* are under development and in clinical trials, with one candidate, RTS,S, in large scale Phase III trials across multiple sites in Africa. A policy recommendation from WHO is expected in 2015 on RTS,S and last year WHO announced their updated malaria vaccine roadmap with the strategic goals of developing both vaccines with protective efficacy of 75% against clinical malaria suitable for administration to appropriate at-risk groups in malaria-endemic areas, and vaccines that reduce transmission of the parasite and thereby substantially reduce the incidence of human malaria infection. In this new roadmap no length of protection is explicitly define and neither the period for which 75% protection must be achieved. Using simulations from an individual based stochastic model of malaria, we address the relative importance of coverage, vaccine efficacy and measures of duration of protection (such as half-life and shape of decay curve) for different outcomes, including reductions in transmission, parasitological and clinical effectiveness, and the overall public health impact at different time points. We investigate the drivers of optimal impact of pre-erythrocytic, blood-stage, and transmission blocking vaccines for these different outcomes, considering the coverage, target age-group and the initial transmission intensity. We consider the value of using alternative metrics that summarise the coverage, initial efficacy, decay shape and half-life as single parameters. These metrics could facilitate comparison across a portfolio of vaccine candidates, considering different endpoints measured in first-in-man challenge studies, Phase II, and Phase III clinical trials.

1008

REVERSIBLE CONFORMATIONAL CHANGE IN THE *PLASMODIUM FALCIPARUM*

Raul Herrera[1], Charles Anderson[1], Krishan Kumar[1], Alvaro Molina-Cruz[1], Vu Nguyen[1], Martin Burkhardt[1], Karine Reiter[1], Richard Shimp, Jr.[1], Randall F. Howard[2], Prakash Srinivasan[1], Michael J. Nold[3], Mark DeCotiis[1], Joan Aebig[1], Lynn Lambert[1], Kelly Rausch[1], Albert Jin[4], Steven G. Reed[2], Carolina Barillas-Mury[1], Patrick E. Duffy[1], Nicholas J. MacDonald[1], **David L. Narum**[1]

[1]National Institutes of Health, Rockville, MD, United States, [2]Infectious Disease Research Institute, Seattle, WA, United States, [3]Waters Corporation, Milford, MA, United States, [4]National Institutes of Health, Bethesda, MD, United States

The *Plasmodium falciparum* circumsporozoite protein (CSP) is the only malaria parasite antigen to advance to phase 3 clinical trials as part of a virus-like particle vaccine identified as RTS,S. The CSP is filamentous and comprised of three dominant domains: a charged N-terminus that binds heparan sulfate proteoglycans, a central NANP repeat domain and C-terminus comprised of a thrombospondin-like type I repeat (TSR) domain. RTS,S which contains numerous NANP repeats and the TSR domain protects about 50% of children against clinical disease. Hypothetically, a second generation CSP vaccine might improve vaccine efficacy by incorporating the N-terminal domain that is absent in RTS,S. Using a panel of CSP-specific monoclonal antibodies, well-characterized recombinant CSPs, and label-free quantitative proteomics, we show here that native CSP is N-terminally processed in the mosquito host. The processed CSP undergoes a conformational change from a filamentous, open form to a closed form in the salivary gland, which masks the N- and C-terminal domains until the sporozoite interacts with hepatocytes in the liver as determined by inhibition of sporozoite invasion, *in vitro*.

Interestingly, in mice the cleaved N-terminal domain contains a T-helper cell epitope(s) that enhances the immunogenicity of recombinant full-length CSP. Our findings show the importance of understanding the unique biophysical nature of the CSP and its impact on vaccine design and suggest that the conformational change is due to a mechanical or molecular signal.

1009

DOUBLE-BLIND DOSE-ESCALATING RANDOMIZED CONTROLLED PHASE 1 STUDY IN MALARIA EXPOSED ADULTS OF THE SAFETY AND IMMUNOGENICITY OF PFS25-EPA/ALHYDROGEL®, A TRANSMISSION BLOCKING VACCINE AGAINST *PLASMODIUM FALCIPARUM* IN BANCOUMANA, MALI

Issaka Sagara[1], Sara A. Healy[2], Mahamadoun Hamady Assadou[1], Merepen A. Guindo[1], Mamady Kone[1], Erin Gabriel[3], Kourane Sissoko[1], Intimbeye Tembine[1], Djibril Traore[1], Moussa L. Diakite[1], Sintry Sanogo[1], Sekouba Keita[1], Kelly Rausch[2], David L. Narum[2], David S. Jones[2], Nicholas MacDonald[2], Daming Zhu[2], Amagana Dolo[1], Yimin Wu[2], Patrick E. Duffy[2], Ogobara Doumbo[1]

[1]Malaria Research and Training Center, Mali-NIAID ICER, University of Science, Techniques and Technologies of Bamako, Bamako, Mali, [2]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [3]Biostatistical Research Branch, National Institute of Allergy and Infectious Diseases, Rockville, MD, United States

Transmission blocking vaccines (TBV) are a critical strategy for malaria elimination and eradication in Sub-Saharan Africa. A double blind, randomized, controlled Phase 1 clinical trial is being conducted to assess the safety and immunogenicity in malaria exposed Malian adults. The *Plasmodium falciparum* TBV vaccine, Pfs25-EPA/Alhydrogel®, contains a recombinant protein of Pf25 and a recombinant mutant non-toxic protein corresponding to sequence of ExoProtein A (EPA) of *Pseudomonas aeruginosa* with the adjuvant, Alhydrogel®. In May 2013, 120 healthy adult volunteers aged 18-45 years old living in the village of Bancoumana (or the surrounding area), Mali were enrolled into the study. Among the 120 participants, 20 (low dose of 16µg Pfs25-EPA/Alhydrogel® or control) have received 2 doses (Days 0 and 56) and 100 (high dose of 47µg Pfs25-EPA/Alhydrogel® or control) have received 3 doses (Days 0, 56, and 112.) Vaccinations started in May/June 2013 with the third vaccination occurring in September/October 2013 prior to the predicted peak of the transmission season. The fourth and final vaccine dose for the high dose group (47µg Pfs25-EPA/Alhydrogel® or control) is planned in September 2014. Vaccinations have been well tolerated. The related adverse events reported have been mostly mild or moderate injection site reactions and transient neutropenia cases which occurred both in Pfs25-EPA/Alhydrogel® and Euvax B/Hepatitis B vaccine (control) groups. In the group who received the high dose of 47µg of Pfs25-EPA/Alhydrogel®, antibody responses to Pfs25 increased with each subsequent dose of vaccine given, but dimished quickly following vaccination. Overall, Pfs25-EPA/Alhydrogel® TBV has been well tolerated and produced significant antibody responses in a malaria exposed adult population.

1010

TARGETING OUTDOOR MALARIA VECTORS USING ODOR-BAITED MOSQUITO LANDING BOX (MLB) EQUIPPED WITH LOW-COST ELECTROCUTING GRIDS

Nancy S. Matowo[1], Salum A. Mapua[2], Fredros O. Okumu[2]

[1]Ifakara Health Institute/University of the Witwatersrand, Dar-es Salaam/Johannesburg, United Republic of Tanzania, [2]Ifakara Health Institute, Dar-es Salaam, United Republic of Tanzania

Residual malaria transmission, especially the proportion that occurs outdoors, is now among the major obstacles in achieving malaria elimination goal. This outdoor transmission is attributed to outdoor mosquito bites by mosquitoes that are behaviorally resilient or resistant to existing indoor insecticidal interventions. Field experiments were conducted against free-flying wild mosquitoes, to evaluate an improved version of the recently developed odor-baited mosquito landing box (MLB), fitted with solar-powered low-cost electrocuting grids on its sides to rapidly kill even mosquitoes that only make very short contacts with the devices. Three MLBs were equipped with EC grids made from locally purchased mosquito racket zappers. One MLB was fitted with the EC grids on only one of its sides; another MLB had EC grids on two sides while the third MLB had EC grids on three of its sides. The three were comparatively evaluated using a 3 by 3 Latin square experiment, with outcome measure being average number of mosquitoes of different species. A total of 4986 dead mosquitoes were collected from the 3 odor-baited MLBs equipped with EC grids, 29% (1432) of which were from MLB with grid on one side, 35% (1738) from the MLB with EC grids on 2 sides while 36% (1816) were from the MLB with EC grids on 3 sides. More mosquitoes were caught from the MLB with more than one grid, which might be due to in the higher surface area of contact for mosquitoes with the EC grids. As targeting host-seeking mosquitoes with insecticide-based methods is increasingly challenging due to either behavioral or adaptive resistance of the mosquito vectors after prolonged use, MLBs equipped with low-cost EC grids regularly charged by solar energy, could have significant advantages, as it would also effectively control even those species that are behaviorally resilient or physiologically resistant to insecticidal interventions like long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS) after prolonged use.

1011

EFFECTIVENESS OF FREE DISTRIBUTION OF INSECTICIDE TREATED NETS (ITN) IN RURAL HEALTH DISTRICT: RESULTS FROM CROSS SECTIONAL SURVEY IN NOUNA HEALTH AND DEMOGRAPHIC SURVEILLANCE SITE, BURKINA FASO

Maurice Ye, Ali Sie, Eric Diboulo, Justin Tiendrebeogo

Nouna Health Research Center, Nouna, Burkina Faso

Malaria remains the global cause of morbidity and mortality with most of the burden being in sub-Saharan Africa. Insecticide Treated Nets (ITNs); one of the most effective strategies of Roll Back Malaria is currently rolled out on a large scale. However no much is known about the effectiveness of such a strategy in terms of coverage, use, and equity in protecting vulnerable groups.We used data from a cross-sectional household survey which was conducted in the Nouna Health and Demographic Site in 2012 after a large campaign of free ITNs distribution in 2010. The primary objective was to monitor household coverage in ITN and its use and acceptability. Data were collected from a total of 1050 households, selected using a three-stage cluster sampling procedure including 1202 pregnant women and 1114 children. Overall 97 % of households revealed a possession of at least one ITN in 2012 compared to 89% in 2009. In 2012, 69% of children have slept under ITN the last night compared to 25% in 2009. It was 69 % for pregnant women compared to 28% in 2009. The prevalence of presumptive malaria among under five years was 21% in 2011 compared to 20% in 2009. Meanwhile the malaria mortality decreased from 4% in 2009 to 2,7% in 2012 ($P<0.05$) in general population probably due to the high uptake of ITNs among vulnerable groups. However the household compliance to ITNS was high during rainy season (93%) than dry season (28.4%). 2.5% of general population reported a side effects dominated by pruritis (41.4%) and dyspnoea (32.9%). Although significant progress have been made to improve access of population to ITNs resulting in slight decrease of malaria morbidity due to high ITNs use in NHDSS, still much remains to do for achieving the MDGs goals. More attention should by pay to the side effects of ITNS which could play a role in adherence.

1012

INTEGRATED APPROACH TO MALARIA PREVENTION AT HOUSEHOLD LEVEL IN UGANDA: EXPERIENCES FROM A PILOT PROJECT

David Musoke[1], George Karani[2]

[1]Makerere University School of Public Health, Kampala, Uganda, [2]Cardiff Metropolitan University, Cardiff, United Kingdom

Background Malaria is a major public health challenge in sub-Saharan Africa. In Uganda, malaria is the leading cause of morbidity and mortality especially among children under five years of age. This pilot project promoted prevention of malaria at household level using an integrated approach in 2 rural communities in Wakiso district, Uganda. This involved advocating and implementing several strategies in a holistic manner geared towards reduction in the occurrence of malaria. The specific strategies included installing mosquito proofing in windows and ventilators, draining stagnating water, closing windows and doors early in the evenings, and sleeping under ITNs. Methods The objectives of the project were to: carry out a baseline survey on malaria prevention; train community health workers and increase awareness on the integrated approach of malaria prevention; and establish demonstration sites using the integrate approach. Results The project conducted a survey among 376 participants which generated information on the knowledge, attitudes and practices of the community on malaria prevention. 25 community health workers were trained and over 200 members among the general population sensitized on the integrated approach of malaria prevention. 40 demonstration households using the integrated approach were established. Conclusion The results from this pilot project showed that the integrated approach to malaria prevention was well received by the study communities, which could be scaled up to more areas in Uganda and other malaria endemic countries.

1013

EFFECTS OF A NEW ODOR-BAITED MOSQUITO CONTROL DEVICE, THE MOSQUITO LANDING BOX, ON MALARIA VECTOR DENSITIES AND SURVIVAL INSIDE A SEMI-FIELD SYSTEM IN TANZANIA

Arnold S. Mmbando, Joseph P. Mgando, Fredros O. Okumu, Dickson W. Lwetoijera

Ifakara Health Institute, Morogoro, United Republic of Tanzania

Malaria transmission is increasingly occurring outdoors due to changing vector behavior and effects of existing indoor insecticidal interventions. The commonly used Long Lasting Insecticide treated Nets (LLINs) and Indoor Residual Spraying mostly kill human-biting, indoor-feeding and indoor-resting mosquitoes. We assessed effects of a new odor-baited device that mimics humans, the mosquito landing box (MLB), which was recently developed for killing vectors outdoors to complement LLINs on vector densities and survival. Experiments were conducted in a semi-field system (SFS) in Tanzania for 40 nights. 400 unfed, laboratory-reared female *Anopheles* arabiensis mosquitoes were released in each chamber. Two MLBs, baited with nylon socks emanating human foot odor and CO_2 gas from yeast-molasses, were placed in one chamber, and another chamber left as control. The MLBs were dusted with either 10% larvicidal pyriproxyfen (PPF) powder, spores of entomopathogenic fungi (M. anisopliae IP46), or 5% pirimiphos methyl (PM). Human volunteers in each chamber collected and individually stored mosquitoes from their legs, or from walls of the SFS, using new collection tools for each specimen. The recaptured mosquitoes were assessed for contact with the MLBs by: 1) introducing them into beakers holding 10 3-4th instar An. arabiensis larvae and observing PPF effects, 2) observing the growth of fungus on mosquito carcasses, or 3) monitoring mortality rates (experiment with PM). In tests with PPF, 60% of mosquitoes recovered from human volunteer legs were contaminated compared to 6% in controls ($P<0.05$). 43% of mosquitoes found on walls in the treatment chamber were contaminated with fungus compared to 0% in controls ($P<0.05$). Lastly, in tests with 5% PM, daily survival of mosquitoes was significantly lower than in controls ($P<0.001$). The high contamination rates and reduced mosquito survival shows MLBs can significantly reduce densities and survival of malaria mosquitoes, thus being a potentially effective complementary intervention for use outdoors.

1014

IMPROVING MATERNAL AND NEONATAL HEALTH: COMPLEMENTARY ROLE OF THE PRIVATE SECTOR INCREASING UPTAKE OF INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA IN PREGNANCY IN KENYA

Augustine M. Ngindu[1], Muthoni M. Kariuki[1], Sanyu Kigondu[1], Johnstone Akatu[2], Isaac M. Malonza[1]

[1]United States Agency for International Development-MCHIP, Nairobi, Kenya, [2]Bungoma County Hospital, Bungoma, Kenya

Malaria in pregnancy (MIP) is associated with poor pregnancy outcomes including maternal anaemia, intrauterine growth retardation and low birth weight. Kenya changed its policy on intermittent preventive treatment using Sulfadoxine Pyrimethamine (IPTp-SP) in 1998 however, IPTp coverage rates have remained low 4% in 2003, 14% in 2007, 15% in 2008 and 25 % in 2010. To increase the coverage rate, MCHIP supported malaria control and reproductive health divisions of the ministry of health, first to harmonize knowledge among service providers on provision of IPTp-SP in 2011, and second to train community health workers (CHWs) on sensitization of pregnant women to start early antenatal care (ANC) attendance in 2012. A community survey conducted in 2013 showed a significant increase in the proportion of pregnant women receiving two or more IPTp doses from 25% to 63%, the highest increase in IPTp uptake since 1998. Following the successful scale up of IPTp, one sub-county conducted an assessment of its health facilities to determine quality of data on ANC clients accessing IPTp-SP. A total of 15 (58%) out all 26 health facilities in the sub-county (public - 6 out of 8, faith-based - 2 out 3 and private - 7 out of 15) were selected. Data on new ANC clients, revisits and IPTp doses given was collected from the ANC registers. Among the assessed health facilities 13 (87%) out of the 15 were registering new ANC cases, revisits and provided IPTp-SP (public 6, faith based 2, private 5. One private clinic provided ANC services to revisits and IPTp2 doses only after the clients had been registered in public facilities, the second did not offer ANC services. In 2013 the government declared provision of free maternity services in public facilities but ANC clients have continued to utilize services from the private sector. This is an indication of the untapped potential in the private sector in increasing access to high impact interventions and importance of supporting the sector by all partners to provide these interventions. Such complementary efforts if implemented will not only result in enabling the country to move towards achievement of set targets but also improve pregnancy outcomes through reduction in effects of malaria in pregnancy.

1015

FACTORS AFFECTING INSECTICIDE TREATED NET USE AMONG CHILDREN UNDER AGE OF FIVE YEARS IN MAINLAND TANZANIA

Ritha A. Willilo[1], Issa Hamisi Issa[2], Naomi Kaspar[3], Praveen Sravanam[4], Renata Mandike[5], Ramsan Mahdi[1], Abdallah Mkopi[6], Jeremiah Ngondi[1], George Greer[3]

[1]RTI International, Dar es salaam, United Republic of Tanzania, [2]Institute of Rural and Development Plan, Dodoma, United Republic of Tanzania, [3]USAID, Dar es salaam, United Republic of Tanzania, [4]Kilimanjaro Christian Medical University College, Kilimanjaro, United Republic of Tanzania, [5]National Malaria Control Program, Dar es salaam, United Republic of Tanzania, [6]Ifakara Health Institute, Dar es salaam, United Republic of Tanzania

Recently, there has been successful universal coverage of Insecticide Treated Nets (ITNs) in households through the national campaigns. Despite the free-distribution and high coverage of ITN, gap in ownership and

usage remains a challenge in achieving the National targets. Data from the 2011-12 Tanzania HIV/AIDS and Malaria Indicator survey (THMIS) was used to assess the combined effect of child level factors, maternal factors, household factors and community factors on ITN usage among children under five years of age. Using logistic regression, associations between usage of ITN in children under five years (<5) of age and potential risk factors were analyzed. A total of 8624 children under five who slept under an ITN the night before survey were included in the analysis. Education level of mothers, age of the child <5 and number of children in a household were significantly associated with net usage among Children <5 years in adjusted model. Households with children aged 36-47 months [Odds Ratio (OR) =0.74; 95% CI 0.58-0.95], children aged 48-59 months (OR=0.75; 95%CI 0.57-0.99) were associated with decrease usage of ITN. Household with three or four children (OR=1.68; 95% CI 1.35-2.09), one or two children <5 years (OR=3.25; 95% CI 1.14- 9.29) and mothers who are educated (OR =1.3; 95% CI 1.09 -1.61) were associated with higher usage of ITN among children <5 years of age. To improve use of ITN among children <5 years, policies promoting women's education, education on malaria prevention methods to society especially women (mothers and care-givers of children) as well as distribution of nets must be coupled with sensitization about use should be encouraged and strengthened. However, further research and efforts are needed to address barriers and determine the strategies to increase the ITN usage.

1016

LIGHT-REGULATED BLOOD-FEEDING AND FLIGHT BEHAVIOR AND A LIGHT PHASE RESPONSE CURVE FOR THE *ANOPHELES GAMBIAE* MALARIA MOSQUITO

Aaron D. Sheppard, Gary George, Erin Clark, Hannah Yu, Samual S. Rund, Giles E. Duffield

University of Notre Dame, Notre Dame, IN, United States

Biting behaviors in anopheline mosquitoes are time-of-day specific, with a greater abundance of biting occurring during the dark phase of their photoperiod. We investigated how a single light pulse administered at the beginning of the night effected biting behavior. Additionally, *Anopheles gambiae* locomotion has a distinct circadian rhythm, characterized by nocturnal activity bouts. We investigated how precisely timed light pulses delivered throughout the circadian cycle can shift the activity rhythm, leading to the synthesis of an *An. gambiae* Phase Response Curve (PRC). To investigate biting inhibition, two incipient *An. gambiae* species (S and M molecular forms) were treated with white light (10 min, 150-800 lux) at the onset of complete darkness and the percentage taking a blood meal was recorded every 2 hr up to 8 hr. To produce an anchored PRC, S-form mosquitoes received a single 30 min pulse of light at various times during the immediate 24 hr transitioning from a light-dark cycle to constant darkness. The pulse significantly reduced biting tendency in the S-form mosquito for 2 hr after administration (at 0.20 hr and 2 hr), with variable responses observed at 4 hr, and no differences detected at 6 and 8 hr. Conversely, M form mosquitoes, were unresponsive to the light treatment, *i.e.* their biting tendency did not change (*n.s.*). For the PRC analysis, as seen in most other examined species, *An. gambiae* mosquitoes demonstrated distinct delays and advances in circadian phase when light was presented during the early and late subjective night, respectively. These data reveal a strain-specific effect of acute light treatment on biting behavior that is both immediate and sustained. The *An. gambaie* PRC is qualitatively similar to several model insect and vertebrate organisms. At present, insecticidal treated bed-nets designed to prevent mosquito-human contact and kill mosquitoes, are relied upon to prevent malaria transmission; as mosquitoes and malaria parasites are becoming increasingly resistant to insecticidal and drug treatments, respectively, there is a necessity for the development of innovative control strategies. The inhibitory and phase shifting effects of light may prove to be an effective tool in assisting with these strategies.

1017

NEW LINES OF *ANOPHELES GAMBIAE* REFRACTORY TO *PLASMODIUM FALCIPARUM*: SELECTION, CHARACTERIZATION AND SPECIFIC MARKER GENE SELECTION

Lenka Richterova, Elizabeth Peat, Lisa Ranford-Cartwright

University of Glasgow, Glasgow, United Kingdom

Anopheles gambiae is a principal vector of *Plasmodium falciparum* malaria in Africa. Some individual mosquitoes within a population are naturally refractory to infection. The only existing refractory line of *An. gambiae* (G3) melanises *P. falciparum* parasites, a refractory behaviour uncommon under natural transmission. Understanding common, non-melanising mechanism of natural refractoriness could be used for development of transmission blocking vaccines or GMO vector strategies. We have selected a new, non-melanising, refractory line of *An. gambiae* named GU-REF. GU-REF was selected for refractoriness to *P. falciparum* clone 3D7 over 11 generations of selection. At the same time, the GU-CON line was selected at random as a control for inbreeding effects. The refractory line was then tested for genotype specificity, parasite stages affected, timing of blood meal digestion after feeding on infected and uninfected blood, expression of candidate genes previously linked with refractoriness, and for fitness costs of refractoriness. GU-REF mosquitoes exhibit a significantly lower infection prevalence with *P. falciparum* compared to GU-CON and the parent Keele line of *A. gambiae*. The refractory behaviour is not specific to the parasite clone (3D7) used for selection, in that refractoriness is seen to an unrelated parasite, HB3. The refractory mechanism affects the parasite stages before the oocyst. GU-REF mosquitoes do not appear to exhibit fitness costs associated with refractoriness, as measured by fecundity. Protein digestion of the blood meal is slightly faster in GU-REF after an infectious blood-meal, compared to GU-CON. There is no difference in speed of digestion after a non-infected blood-meal. Analysis of candidate marker genes for refractoriness identified one locus with significant allele frequency differences between GU-REF and GU-CON and the parent line. We are currently developing a further line, GU-REF2, homozygous at this locus, to confirm its involvement in the refractoriness.

1018

A SCALABLE ASSESSMENT OF MALARIA TRANSMISSION IN THE STANDARD MEMBRANE FEEDING ASSAY USING TRANSGENIC GFP-LUCIFERASE *PLASMODIUM FALCIPARUM* GAMETOCYTES

William J. Stone[1], Thomas S. Churcher[2], Wouter Graumans[1], Geert-Jan van Gemert[1], Martijn W. Vos[1], Kjerstin H. Lanke[1], Marga G. van de Vegte-Bolmer[1], Rianne Siebelink-Stoter[1], Koen J. Dechering[3], Ashley M. Vaughan[4], Nelly Carmargo[4], Stefan H. Kappe[5], Robert W. Sauerwein[1], Teun Bousema[6]

[1]Radboud University Medical Center, Nijmegen, Netherlands, [2]MRC Centre for Outbreak Analysis and Modelling, Department of Infectious Disease Epidemiology, Imperial College London, London, United Kingdom, [3]TropIQ Health Sciences, Nijmegen, Netherlands, [4]Seattle Biomedical Research Institute, Seattle, WA, United States, [5]Seattle Biomedical Research Institute & Department of Global Health, University of Washington, Seattle, WA, United States, [6]Radboud University Medical Center and Department of Immunology and Infection, London School of Hygiene & Tropical Medicine, Nijmegen, Netherlands

The development of drugs and vaccines to reduce malaria transmission is an important part of plans to eradicate the disease. The transmission reducing activity (TRA) of these agents is currently determined in the standard membrane feeding assay (SMFA), which is based on subjective microscopical read-outs which significantly limit the techniques throughput. Utilising a *Plasmodium falciparum* strain expressing the firefly luciferase protein, we present a luminescence based approach to SMFA evaluation that eliminates the requirement for mosquito dissections in favour of a simple approach where whole mosquitoes are homogenised

and examined directly for luciferase activity. Analysis of 6860 *Anopheles* stephensi mosquitoes across 68 experimental feeds shows that the luminescence assay was as sensitive as microscopy for infection detection. The mean luminescence intensity of individual and pooled mosquitoes accurately quantifies mean oocyst intensity and generates comparable TRA estimates. The luminescence assay presented here could increase SMFA throughput so that 10-30 experimental feeds could be evaluated in a single 96-well plate. This new method of assessing *Plasmodium* infection and transmission intensity could expedite the screening of novel drug compounds, vaccine candidates and sera from malaria exposed individuals for TRA.

1019

AN ASSOCIATION BETWEEN THE 1014F KDR ALLLELE AND *PLASMODIUM FALCIPARUM* INFECTION IN *ANOPHELES GAMBIAE* SENSU LATO

Traoré Alphonse[1], Athanas Badolo[2], Wandaogo Moussa Guelbeogo[1], Antoine Sanou[1], Hilary Ranson[3], N'falé Sagnon[1]

[1]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [2]University of Ouagadougou, Ouagadougou, Burkina Faso, [3]Vector Group, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Malaria vectors in Burkina Faso are highly resistant to pyrethroid insecticides but little is known about the impact of this resistance on malaria transmission. One approach to address this epidemiologically important relationship is to test for associations between known insecticide resistance mechanisms and infection with malaria parasites. Adult mosquitoes were collected over three years from four sites across Burkina Faso using pyrethrum spray catches, exit traps pit shelters. Genomic DNA of *Anopheles gambiae* s.l mosquitoes was identified to species and genotyped for the L1014F mutation. The circumsporozoite antigen was detected in mosquitoes using sandwich ELISA. *An. arabiensis* and *Anopheles gambiae* s.s were incriminated as vectors of *Plasmodium falciparum* with overall sporozoite rates of 3.8% for *An. arabiensis*, 4.4% for M-form, and 6.6% for S-form. The L1014F kdr allele was significantly associated with the sporozoite positive population of *An. arabiensis* s.l. (p=0.03).The current study shows a significant association between the presence of a kdr mutation and infection with the malaria parasite. By extending this work to include additional genes linked to pyrethroid resistance a clearer picture of the impact of resistance on malaria transmission will emerge.

1020

ELIMINATION OF MALARIA: THE CURRENT APPROACHES HAVE FAILED TO REDUCE MALARIA TRANSMISSION IN LOWLANDS IN WESTERN KENYA

Ednah N. Ototo[1], Patrick J. Mbugi[1], Christine L. Wanjala[1], Guofa Zhou[2], Andrew K. Githeko[3], Guiyun Yan[2]

[1]Kenyatta University, Nairobi, Kenya, [2]University of California Irvine, Irvine, CA, United States, [3]Kenya Medical Research Institute, Kisumu, Kenya

Insecticide treated bed nets (ITN) are a powerful tool in the control of malaria in Africa as most vectors there bite indoors at night. The World Health Organization recommended universal coverage of ITNs for all people at risk of malaria. The mass distribution of ITNs in Kenya in 2011 marked a milestone toward universal coverage. The study was conducted in six study sites in Western Kenya, three in the highlands and three in the lowlands from 2010 to 2013. ITN ownership and coverage were surveyed annually during the same study period. Mosquito vectors were collected monthly using the indoor PSC catch method, blood samples from school children aged between 6 and 15 years were collected in May and June each year using finger-prick method and malaria parasite prevalence was determined microscopically. Overall, 6,677 *Anopheles* were caught with 8,762 trap-nights; 4,018 blood samples were examined with 826 infections identified; 6,552 households with 20,267 individuals were surveyed for ITN ownership and coverage and 7,609 ITNs were surveyed. There was a marked increase in vector densities in four out of the six study sites but this depended on the vector species. Parasite prevalence increased in all the lowland sites while decreased in the highland sites. ITN ownership and coverage increased from an average of 64% in 2010 to 84% in 2013. This lead to significant decrease in indoor resting vector densities and in parasite prevalence in places where vector densities were low, however in the lowland regions an increase in the ITN coverage did not transform into a decrease in the parasite prevalence as the vector densities are high and there is a resurgence of An. funestus in the lowlands. The current malaria control strategies are not working in the Western Kenya lowlands as indicated by increased vector densities and malaria prevalence. Different or modified strategies are needed in order to achieve malaria elimination in the lowland areas in Kenya.

1021

FIELD EVALUATION OF YEAST-MOLASSES FERMENTATION AT A PRACTICAL SOURCE OF CARBON DIOXIDE FOR BAITING OUTDOOR DEVICES AGAINST HOST-SEEKING MOSQUITO VECTORS IN RURAL TANZANIA

Emanuel W. Kaindoa[1], Nancy S. Matowo[2], Salum A. Mapua[1], Arnold Mmbando[1], Fredros O. Okumu[1]

[1]Environmental Health and Ecological Sciences Thematic Group, Ifakara Health Institute, Morogoro, United Republic of Tanzania, [2]Wits Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Odour baited traps have been recently developed and increasingly used for sampling and controlling disease vectors. Carbon dioxide (CO_2) gas is recognized as an important stimuli mediating host attractiveness to mosquitoes. Currently, the main sources of CO_2 for mosquito sampling include dry ice, sugar-yeast fermentation and bottled industrial CO_2, all of which are expensive, labor-intensive and impractical for regular field use, beyond small-scale experimentation. We assessed molasses-yeast fermentation as an alternative organic and locally available source of CO_2 for sampling mosquito vectors in Tanzania. Mosquito trapping experiments were conducted in the field by using *Ifakara* OBS baited with 3 different sources of CO_2 and an unbaited *Ifakara* OBS as control. In each trap, the CO_2 was produced in two separate pots by adding 40g in one pot and 75g of yeast in another, adding 0.25 liters of molasses in each pot, then mixing these with 1.5 liters of water. Attractiveness of the molasses-yeast CO_2 to mosquitoes was tested and compared with conventional industrial CO_2, yeast-sugar fermentation generated CO_2 and control, i.e. unbaited traps.We also assessed whether CO_2 generated from molasses yeast-fermentation can enhance the attractiveness of synthetic human-derived cues to mosquitoes. Traps baited with CO_2 obtained from molasses-yeast fermentation caught significantly more *An. gambiae* mosquitoes than traps baited with yeast-sugar CO_2 ($P \leq 0.01$) and unbaited traps (control) ($P \leq 6.5E\text{-}05$). The trap baited with industrial CO_2 had higher mosquito catches than trap baited with molasses CO_2 but was not statistically significant ($P \leq 0.75$). Addition of molasses derived CO2 to traps baited with human odour significantly increased trap catches. There were no significance difference on the catches between the traps baited with molasses derived CO_2 and traps baited with industrial CO_2 ($P \leq 0.6$). Yeast-molasses fermentation is an effective, locally available and cheap source of CO_2 for mosquito vector studies. The CO_2 could potentially be used to replace yeast-sugar CO_2 in odor-baited devices for mosquito sampling, so as to sustainably monitor disease vector and control in low income communities.

1022

USING MOSQUITO PROOF HOUSING TO PROTECT ITINERANT RICE FARMERS IN SOUTHEASTERN TANZANIA AGAINST MOSQUITO-BORNE INFECTIONS

Johnson K. Swai, Fredros O. OKech, Silas Majambere

Ifakara Health Institute, Ifakara, United Republic of Tanzania

Poor housing increases exposure to insect bites and pathogens that these insects transmit. In rural south-eastern Tanzania, rice farming is a major economic activity, with most rice farmers relocating to their rice paddies in distant fields to tend to their crops for many days, weeks or months. While in the farms they live in semi-open improvised structures, which do not prevent mosquito entry, and indoor vector control tools such as insecticide treated bed nets (ITNs) and Indoor residual Spraying (IRS) cannot be effectively used, making these farming households disproportionately more vulnerable to nuisance biting and mosquito-borne diseases. We are exploring the use of portable mosquito-proof houses to protect these farmers while at their fields. Initial qualitative surveys through semi-structured interviews and participant observations assess community members' views and preferences regarding designs and use of the portable houses have been completed. The houses have been designed and initial prototypes ready for construction. The prototypes will be tested against farm house replicas that are currently used, to compare their protective efficacies in both semi-field and field settings, with human volunteers in each hut type. In the semi field, tests will be done in a 120m screened tunnel where the two hut types will be placed 50m apart, 500 3-5 days old female *Anopheles* arabiensis released in the middle and numbers of mosquitoes entering each hut recorded. In the field setting, the prototype will again be kept 50m away a farm house replica in an open field near mosquito breeding sites. Mosquito collections will be done using CDC light traps all night and indoor-resting catches in the morning to determine number of mosquitoes that enter the houses. Findings will be presented at the meeting. We expect that there will be significantly fewer mosquitoes entering the mosquito proof prototypes than the farm house replicas, and that by including community preferences into the design, these portable houses will be highly acceptable and scalable in these communities. The study will demonstrate potential of such low-cost interventions for effectively controlling mosquito-borne infections among itinerant communities, who are otherwise mostly disenfranchised from organized disease prevention campaigns

1023

COMPARATIVE EVALUATION OF A NEW EXPOSURE-FREE TOOL THE M-TRAP, AGAINST OTHER EXISTING SAMPLING TOOLS FOR OUTDOOR-BITING MOSQUITOES

Alex J. Limwagu, Stephen P. Mwangungulu, Fredros O. Okumu, Roberty D. Sumaye

Ifakara Health Institute, Morogoro, United Republic of Tanzania

Outdoor sampling of disease transmitting mosquitoes is a major challenge for experts when monitoring pathogen transmission risk and evaluating new interventions. Human landing catches (HLC), a potentially risky and labour-intensive method requiring use of human volunteers to collect mosquitoes attracted to their legs, unfortunately remains the standard for sampling host-seeking mosquitoes outdoors and indoors. In recent years alternatives to HLC have been tried including Ifakara tent trap (ITT-B), Ifakara odour baited stations (OBS), Mosquito Magnet (MMX), and BG sentinel traps. We designed a new exposure-free mosquito trap, known as M-Trap, as an outdoor surveillance tool for disease-transmitting mosquitoes, and comparatively evaluated it against ITT-B, MMX, Ifakara OBS, BG Sentinel and HLC. The study was conducted in rural southern-east Tanzania during dry and rainy seasons. The experiment was a 5 by 5 latin-square design in which the different traps were randomly allocated to five locations >30m apart, and rotated such that at the end of any five experimental days, each trap type had been to each of the five locations at least once. In the first study, all traps except HLC were baited with the same synthetic mosquito attractant, while in the second experiment, human volunteers slept in only ITT-B and M-trap, whereas synthetic odour baits were used in MMX, Ifakara OBS and BG Sentinel traps. tests were conducted from18:00hrs to 06:00hrs nightly for 75 nights. A total of 9302 mosquito were collected comprising 66.8% (6212) *Mansonia* species, 22.2% (2066) Culex species, 6% (558) *Anopheles coustani*, 4.5% (415) *Anopheles arabiensis* and 0.2% (15) *Anopheles funestus*. Trap perfomances were ranked as follows: MMX (43.4% (4038)), M-trap (24.1% (2244)), BG Sentinel (17.9% (1662)), ITT-B (17.9% (1662)) and Ifakara OBS (9.4% (875)) of total mosquitoes caught There was consistency in the sampling proportionality between the MMX and M-trap catches for both Anopheline and Culicine mosquitoes. The M-trap is less efficient than MMX while it is more effective than BG Sentinel, ITT-B and Ifakara OBS and suitable for use in large scale surveillance, Also M-trap is less cost effective in terms of development than this other traps, and it can be locally available.

1024

KNOWLEDGE AND USE OF MALARIA PREVENTIVE MEASURES BY CAREGIVERS OF UNDER-FIVES IN RURAL SOUTHWESTERN NIGERIA

Mobolaji M. Salawu[1], Oluwaseun Akinyemi[2], Akindele Adebiyi[2]

[1]University College Hospital, Ibadan, Nigeria, [2]University College Hospital/ University of Ibadan, Ibadan, Nigeria

Malaria is highly endemic in sub-Saharan Africa, and Nigeria is one of the worst hit countries causing about 30% of child mortality. Malaria is responsible for high infant and childhood mortality rates in this region. Several common and effective measures had been used to control malaria; and some African countries have reported up to 50% reduction in malarial cases. However, data from Nigeria have not shown significant reduction. This study therefore assessed the use of malaria preventive measures by caregivers of under-fives in rural South Western Nigeria. This was a cross sectional survey conducted in 2010 among 274 caregivers of under-fives who were selected using the cluster sampling technique. Logistic regression analysis was used to model for predictors of use of malaria preventive measures at 5% level of significance. About 86.1% of the caregivers were females and their mean age was 28.95±8.1 years. Almost all (92.7%) were married. They were largely unskilled workers 63.5%. About 95.6% of the care givers knew that malaria can be prevented and 78.1% had good knowledge of known malaria preventive measures. However, the use of malaria preventive measures was varied as only 19.7% used indoor residual spraying, 15.0% used anti malaria drug, 8.4% used personal and environmental hygiene, 8.0% used insecticide treated net and 6.0% used window net. Respondents who were professionals were more likely to have a good knowledge of malaria preventive measures $p<0.05$. The predictor of use malaria preventive measures was respondents who had a good knowledge of causes of malaria (OR 9.3, 95% C.I: 1.35-64.3). The knowledge of caregivers of under-fives about malaria infection and prevention were adequate, however, use of malaria preventive measures was poor. There is a need to provide more information and education on the causes of malaria and the need to use malaria preventive measures in order to prevent children from developing malaria.

1025

FACTORS RELATED TO THE USE OF LONG-LASTING INSECTICIDE-TREATED BEDNETS IN THE PERUVIAN AMAZON

Mitchel Guzmán[1], Angel Rosas[2], Crsitiam Carey[3], Hugo Rodriguez[3], Raul Chuquiyauri[2], Alejandro Llanos-Cuentas[2], Joseph M. Vinetz[4]

[1]Laboratorio ICEMR-Amazonia, Laboratorios de Investigacion y Desarrollo, Facultad de Ciencias y Filosofia, Universidad Peruana Cayetano Heredia, Iquitos, Peru, [2]Instituto de Medicina Tropical 'Alexander von Humboldt', Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Directorate of Health, Iquitos, Peru, [4]Division of Infectious Diseases, University of California San Diego, San Diego, CA, United States

With the support of the Global Fund's Multi-Country Malaria Project PAMAFRO, Peruvian Malaria Program has incorporated long-lasting insecticide net (LLIN) distribution as important strategy to prevent and control malaria. From October to December 2010, 21174 LLIN Olyset Net® were delivered in 70 communities of San Juan district in the Peruvian Amazon, covering 32337 people (ratio: 0.65 LLIN/person). Then, from January to March 2012 a household survey was conducted in a representative sample of 400 households located in 20 communities, in order to identify factors associated with the usage of LLIN after one year of the LLIN delivery. Data about usage of LLIN and potential associated factors (demographics, availability of LLIN, knowledge of malaria and preventive measures) were collected through interviews with the head of the household, using a semi-structured questionnaire. Households with low LLIN usage were those, in which less than 80% of their members slept under a LLIN the previous night. Household vulnerable rate was determined by the sum of children under 5 years and pregnant women divided by the total number of household members. Ownership ratio was defined as the number of LLIN divided by the number of household members. Ownership ratio decreased from 0.70 to 0.52 resulting in LLIN retention of 73% after 1 year of intervention. Almost 75% of children under 5 years old and 80% of pregnant women slept under a LLIN the previous night. While increasing ages of household heads were associated with households with low LLIN usage (OR=1.024, IC95%=1.01-1.04); decreasing vulnerable household rates (OR=0.972, IC95%=0.96-0.99) and ownership ratios (OR=0.966, IC95%=0.96-0.98) were associated with households with low LLIN usage. Despite a reduction of LLIN ownership seemed to be an important determinant of low usage LLIN usage, LLIN usage remained high after 1 year of intervention in the targeted communities.

1026

A CASE-CONTROL STUDY OF THE EFFECT OF HOLES ON MALARIA INFECTION AMONG USERS OF LONG-LASTING INSECTICIDE-TREATED BEDNETS - MALAWI, 2013

Keren Z. Landman[1], Laura Steinhardt[1], Don Mathanga[2], James Sutcliffe[1], Monica Shah[1], Dyson Mwandama[2], Christian Lengeler[3], Jodi Vanden Eng[1], Kimberly Lindblade[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]University of Malawi College of Medicine, Blantyre, Malawi, [3]University of Basel, Basel, Switzerland

Insecticide-treated nets (ITNs) are the cornerstone of malaria prevention, but as holes develop, ITNs may be less effective. We conducted a case-control study among children 6-59 months old who visited an outpatient clinic in Liwonde, Malawi for an acute febrile illness and who reported consistently using an ITN during the prior two weeks to explore whether the number, size or location of holes reduced protection from malaria. Cases were children who were positive for *Plasmodium falciparum* by slide microscopy, while controls were microscopy negative. The ITNs used by participants were collected and mounted on a frame where the number, size category and location of all holes were noted by visual inspection; the WHO-recommended proportionate hole index (pHI) was calculated. Log-binomial models were used to relate the pHI, hole size category and hole location to malaria while controlling for potential confounders. A total of 105 cases and 171 controls were enrolled, and 276 Olyset-brand nets were evaluated. Overall, 86% of nets had at least one hole; 69% had at least one hole between thumb and fist size; 36% had at least one hole between fist and head size ; and 17% had at least one hole larger than a head. The median (mean) pHI was 86 (578), and 77% of ITNs were categorized as in good or acceptable conditions (pHI<643). In univariate analysis, having at least one thumb-to-fist-sized hole in the net roof was associated with malaria (prevalence ratio [PR] 1.43, 95% confidence interval [CI] 1.01, 2.03), but no associations with other net variables, including pHI, were found. In a multivariate model adjusting for potential confounders, having at least one thumb-to-fist-sized hole in the net roof remained significantly associated with malaria (PR 1.48, 95% CI 1.06, 2.05). The association may be explained by mosquitos' preference for resting on net roofs rather than sides. This is the first study to find that hole size and location impact malaria prevalence. Digital image analysis of ITNs in this study will further explore these relationships.

1027

THE CRY TOXIN OPERON *CLOSTRIDIUM BIFERMANTANS MALAYSIA* IS HIGHLY TOXIC TO LARVAL MOSQUITOES

Swati Chawla

University of California Riverside, Riverside, CA, United States

The management and control of mosquito vectors of human disease currently relies primarily on chemical insecticides. However, larvicidal treatments can be effective, and if based on biological insecticides they can also ameliorate the risk to human health of chemical insecticides. The aerobic bacteria *Bacillus thuringiensis* and *Lysinibacillus sphaericus* have been used for vector control for a number of decades. But a more cost effective use would be an anaerobic bacteria because of the ease with which these can be cultured. More recently the anaerobic bacterium, *Clostridium bifermantans malaysia* (Cbm), reportedly has high mosquitocidal activity, and a series of proteins were identified as potentially mosquitocidal. However, the cloned proteins showed no mosquitocidal activity. We show here that the four toxins in the Cry operon, Cry16A, Cry17A, Cbm17.1 and Cbm17.2, are all required for toxicity, and these toxins collectively show remarkable selectivity for *Aedes* rather than *Anopheles* mosquitoes, even though Cbm is more toxic to *Anopheles*. Hence toxins that target *Anopheles* are different from those expressed in the Cry operon.

1028

WORSENING SOCIO-ECONOMIC DISPARITIES IN INSECTICIDE-TREATED NETS (ITN) OWNERSHIP, ACCESS AND USE FROM 2006 TO 2011 IN ANGOLA

Ana C. Franca-Koh, Rebecca Winter, Lia Florey, Yazoume Ye

ICF International, Rockville, MD, United States

Insecticide-treated nets (ITNs) are a key malaria control intervention as they significantly decrease malaria morbidity and mortality. However, in many malaria endemic countries, ITN coverage remains low and is inequitable among different socio-economic groups. In Angola, despite an increase in ITN use among children under five (from 18% 2006/7 to 26% in 2011) for example, it still remains far below the target of 85%. Using data from the two Malaria Indicator Surveys (MIS), this paper investigated change in equity in ITN ownership, access, and use among children under five and pregnant women between 2006/7 and 2011 in Angola. Lorenz Concentration Curve and Index (C-Index) was used to assess the magnitude of the disparities between wealth quintiles between both surveys. Disparities in ITN ownership, access and use worsened between socio-economic groups. ITN ownership was less equitable in 2011 (C-index: 0.17) compared to 2006/7 (C-index: 0.05). In 2011, 44% of households from the least poor wealth quintile owned at least one ITN compared to 15% in the poorest quintile. This disparity was less in 2006/7; 31% compared to 26% respectively. Similar results were found for ITN

access (C-index: 0.16 and 0.02), use among children under five (C-index: 0.17 and -0.004) and pregnant women (C-index: 0.14 and -0.06). Despite an increase in ITN ownership, access and use between 2006/7 and 2011 in Angola, inequity became greater, favoring the least poor quintiles. This could be due to different ITN distribution strategies implemented. Before the MIS 2006/7, there was a massive free net distribution campaign implemented in seven provinces. Afterwards, ITNs were mainly distributed through routine antenatal care. Universal coverage distribution began in 2010, but was limited to specific municipalities. ITNs therefore became more accessible to those living closer to facilities (most in urban areas) and who could afford them. A different strategy for ITN distribution that results in both higher and more equitable coverage is needed in Angola.

1029

THE IMPORTANCE OF PARASITE DENSITY IN ASSESSING THE EFFECTIVENESS OF TRANSMISSION-BLOCKING INTERVENTIONS FOR MALARIA

Patrick M. Brock, Andrew M. Blagborough, Leanna M. Upton, Robert E. Sinden, Azra C. Ghani, Thomas S. Churcher

Imperial College London, London, United Kingdom

Transmission-blocking interventions (TBIs) have the potential to reduce malaria transmission by targeting *Plasmodium* life-stages in the mosquito. However, the density-dependent effects of parasite numbers on malaria transmission remain poorly understood and may influence the effectiveness of TBIs. We fitted mathematical models to 2550 mosquito and 575 vertebrate host experimental infections, carried out as part of a laboratory population assay that simulates human-to-mosquito and mosquito-to-human infection over successive transmission cycles, to assess the influence of sporozoite and asexual parasite density on infection probability. Models categorised parasite density into groups, each with their own infection probability, and were compared with likelihood ratio tests (LRT). For mosquito-to-mouse transmission, the best model (LRT, $p = 0.023$) was the most complex, with the probability of infection increasing with the number of sporozoites present in the salivary glands following injection: 0 (1%); 1-10 (33%); 11-100 (54%); 101-1000 (64%); and >1000 sporozoites (88%). For mouse-to-mosquito transmission, the best model (LRT, $p = 0.0006$) also fitted infection probabilities that increased with asexual parasite density. To gauge the impact of this density-dependence on TBI assessment, we incorporated asexual parasite density into a chain binomial model of transmission, and fitted it to data from the laboratory population assay. Intervention effectiveness was assessed using effect size, the ability of a TBI to reduce the reproduction number of the parasite. The best model (LRT, $p = 0.00004$) showed that effect size was highest in mice with the lowest parasite density. This provides the first direct evidence that, in this laboratory model, parasite density influences the ability of TBIs to reduce transmission, and that TBIs are more likely to reduce transmission when parasite density is low. Current evaluations based on prevalence, and the relatively high parasite densities of laboratory assays, may, therefore, under-estimate the potential impact of TBIs.

1030

IMPACT OF A BEHAVIOR CHANGE INTERVENTION ON CARE AND REPAIR BEHAVIORS FOR LONG-LASTING INSECTICIDAL NETS IN NASARAWA STATE, NIGERIA

Hannah Koenker[1], Albert Kilian[2], Emmanuel Obi[3], Richmond A. Selby[4], Gabrielle Hunter[1], Angela Acosta[1], Matthew Lynch[1]

[1]Johns Hopkins Center for Communication Programs, Baltimore, MD, United States, [2]Tropical Health, Montagut, Spain, [3]Malaria Consortium, Abuja, Nigeria, [4]Malaria Consortium, Kampala, Uganda

Improvements in long-lasting insecticidal net (LLIN) durability have programming implications for national malaria programs. LLIN durability varies depending on user behaviors, household and environmental characteristics, and the strength and knitting pattern of the textile itself. A behavior change communication intervention was designed to promote household-level behaviors related to the care and repair of LLINs in Nasarawa State, Nigeria. The intervention was carried out in one local government area (LGA) in Nasarawa from October 2012 to April 2013, and again from October 2013 to April 2014. A second LGA served as a control area. The intervention included radio spots, local radio pronouncements, community events, and house-to-house visits. A baseline survey, nested within an ongoing ITN durability study using two-stage cluster sampling design, was conducted in April 2012 to measure LLIN ownership and existing patterns of net care and repair in both control and intervention LGAs, among 600 households. Control and intervention areas were similar at baseline in LLIN ownership and retention, net condition, observed repairs, and proportional hole index (PHI). Radio ownership was slightly higher in the intervention LGA. A midline survey conducted in April 2013 showed that in the intervention LGA, 28.2% (95%CI 21.4%-37.2%) of households had heard or seen at least one component of the BCC intervention, while in the control area, 12.3% (95%CI 8.1%-18.8%) reported exposure to care and repair messaging. The proportion of campaign nets with holes that had been repaired was not significantly different at midline, with 28.8% (95%CI 19.9%-39.6%) in the intervention LGA and 20.0% (95%CI 15.2%-25.9%) in the control LGA. An endline survey was conducted in April 2014 to determine whether exposure to the intervention contributed to any changes in care and repair behaviors, and whether changes in care and repair behaviors contributed to differences in net durability, measured by PHI. The results from this study will provide evidence as to whether BCC interventions have the potential to improve care and repair behaviors. These findings have implications for national malaria control programs' and donor agencies' programming decisions.

1031

CONTINUOUS DISTRIBUTION OF LONG-LASTING INSECTICIDAL NETS THROUGH SCHOOLS - RESULTS FROM A THREE YEAR EVALUATION IN CROSS RIVER STATE, NIGERIA

Albert Kilian[1], Emmanuel Obi[2], Richmond A. Selby[3], Angela Acosta[4], Kolawole Maxwell[2], Iyam Ugot[5], Matt Lynch[4]

[1]Tropical Health LLP, Montagut, Spain, [2]Malaria Consortium, Abuja, Nigeria, [3]Malaria Consortium, Kampala, Uganda, [4]Centers for Communication Programmes, Baltimore, MD, United States, [5]Office of the Governor, Calabar, Nigeria

Following a phase of mass campaigns for the distribution of Long-lasting Insecticidal Nets (LLIN) for malaria prevention in Africa South of the Sahara most countries now focus on establishing systems for continuous distribution of LLIN to sustain achieved gains. However, the primarily used channels of antenatal care and child immunization services alone are not sufficient to sustain universal coverage with LLIN. Schools have been considered as possible additional distribution channels, but to date no data exist to show the potential of such distributions. A pilot program of school-based LLIN distributions was undertaken in two districts in Cross River State, Nigeria in collaboration between the State Government's health and education departments of and the USAID NetWorks project funded by US President's Malaria Initiative. All students registered in two classes in public primary as well as two classes in public secondary schools were eligible to receive an LLIN. Distribution was organized by the education department of the districts and was undertaken in the second term of the school year. Distribution was sequentially rolled out starting in Obubra district (three distribution rounds in total) in early 2012 and in Ogoja district in 2013 (two rounds). By March 2014 a total of 55,000 LLIN had been distributed in 192 schools with a program efficiency (% of eligible children reached) of over 95%. In March 2014 an evaluation survey was undertaken to assess the impact of the school-based distributions on overall household LLIN ownership. In addition to the two implementation districts, one district which only had received LLIN through ANC services was included as a control site. In each of the districts 510 households (1,530 in total) were sampled in a cluster survey design. Data are currently processed and will be presented focusing on the success

in equitably sustaining LLIN coverage. A second focus of analysis will be the impact of behavior change communication in schools on the net use practices of exposed families.

1032

POTENTIAL IMPLICATIONS OF OUTDOOR-SLEEPING BEHAVIORS AND NIGHTTIME ACTIVITIES FOR MALARIA CONTROL IN THE UPPER WEST AND NORTHERN REGIONS OF GHANA

April Monroe[1], Obed Asamoah[2], Yukyan Lam[3], Matthew Lynch[1], Hannah Koenker[1], Paul Psychas[4], Sureyya E. Hornston[5], Jonathan Akuoku[3], Steven Harvey[3]

[1]Johns Hopkins Center for Communication Programs, Baltimore, MD, United States, [2]Malaria Consortium, Accra, Ghana, [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [4]University of Florida, Gainesville, FL, United States, [5]United States Agency for International Development, Accra, Ghana

Despite large-scale net distribution and indoor residual spraying campaigns, malaria rates in the northern regions of Ghana remain high. According to the Ghana Multiple Indicator Cluster Survey (2011), malaria prevalence rates for children under five were 52% and 48% in the Upper West and Northern Regions respectively. Nighttime activity, including outdoor sleeping, might be a possible explanation. In-depth interviews and nighttime observations were used to document outdoor sleeping and a variety of social, cultural, and economic activities that occur during night time in the Upper West and Northern Regions of Ghana. The study included 48 in-depth interviews with key stakeholders and 24 household observations at night with a total of 175 household members. Outdoor sleeping due to heat was reported and observed frequently among household members of all ages. Long-lasting insecticidal net (LLIN) use was observed to be low irrespective of whether people slept indoors or outdoors, in both regions. In addition to outdoor sleeping, a variety of outdoor nighttime activities were documented including cooking and other household chores, socializing both within the household compound and elsewhere, and studying both within the household compound and at night school classes. Funerals emerged as a common large-scale nighttime event with participants reporting that they attended funerals up to once a week. Nighttime behavior is key to understanding malaria transmission in Ghana and other malaria endemic countries. This presentation will discuss the potential contribution of outdoor sleeping and nighttime activity to increased risk of malaria and its potential implications for malaria control programs in Northern Ghana and beyond.

1033

HIGH ACCEPTABILITY BUT LOW ADHERENCE TO MALARIA PREVENTIVE MEASURES MAY LIMIT THE EFFECTIVENESS OF TOPICAL REPELLENTS

Charlotte Gryseels[1], Sambunny Uk[2], Lies Durnez[1], Vincent Sluydts[1], Tho Sochantha[2], Somony Heng[2], Marc Coosemans[1], Koen Peeters Grietens[1]

[1]Institute of Tropical Medicine Antwerp, Antwerp, Belgium, [2]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia

The effectiveness of topical repellents in addition to long-lasting insecticidal nets has been evaluated at community level by an epidemiological trial in the forested and remote province of Ratanakiri, Cambodia. The introduction of repellents aimed at tackling residual malaria transmission induced by early and outdoor biting vectors. Ancillary to the trial, a mixed methods social science study on the acceptability and use of the topical repellents was conducted, combining ethnographic research, a cross-sectional survey and a structured observation study. In the cross-sectional survey (n=824), self-reported daily use was estimated at 52.4%. As local ethnic minorities have a multiple residence system (depending on the season and agricultural activities they live either in the village, at the forest farm or on the rice field), reported use was differentiated per location. The highest use was reported during deep forest activities (68.0%) such as hunting and logging, and the lowest use while residing in the villages (35.0%). During the structured observation study (n=1495) - relying on a respondent-independent method of collecting data - repellent use was 7,8% on the evening the observation took place. Alternative uses (including spraying on bed nets, on insects, on hair) were reported by the large majority of respondents (>90%), indicating a low consistent and continuous use of the repellent on the skin. The qualitative study, nevertheless, showed a high acceptance of the trial and the product. The perceived inconveniences and risks (bad smell, skin irritation, perceived toxicity), as well as a lack of 'habit' in the daily use of the repellent, contributed to the non-optimal use of repellents.

1034

INVESTIGATING THE POTENTIAL CIRCULAR EFFECT OF BED NET OWNERSHIP ON UNDER-FIVE MORTALITY RISK IN UGANDA

Cameron Taylor, Yazoume Ye

ICF International, Rockville, MD, United States

Numerous studies have demonstrated the impacts of bed net ownership on mortality risk and there is clear agreement that bed nets are an effective malaria prevention intervention. However, it has been challenging to quantify the exact effect due to several factors including the lack of data on malaria specific mortality and the possible circular effect, whereby mortality risk might lead to increase ownership of bed nets. For households that experience the death of a child under five years of age, it can be a traumatic event that shapes uptake of health interventions. This study investigated whether this circular effect should be considered when analyzing the impact of bed net ownership on under five mortality risk. Data from the 2011 Uganda Demographic and Health Survey (DHS) was analyzed using logistic regression to assess whether death is associated with household ownership of bed nets. We hypothesized that households might be more likely to own nets after experiencing a death event in the household after controlling for other possible covariates. Analysis was restricted to households with at least one bed net. These households were retrospectively checked of death in the past 36 months for the survey dates to assess exposure to the predictor. Overall bed net ownership was 74% and of the households with bed nets, 12% experienced a death event. The results of the analysis show no association between death event and bed net ownership (OR=0.96, CI: 0.99;0.78), suggesting that a households decision to own a net is not driven by death. The results suggest that the circular effect is not necessarily affecting the estimate of mortality risk in relation to bed net ownership, however, this relation should be accounted for.

1035

LONGITUDINAL IMPACT OF INDOOR RESIDUAL SPRAYING (IRS) ON MALARIA PARASITAEMIA IN NORTHERN GHANA

Benjamin Abuaku[1], Collins Ahorlu[1], Philip Ricks[2], Paul Psychas[2], Peter Mumba[3], David Mensah[1], Sedzro Mensah[1], William Sackey[1], Achuyt Bhattarai[2], Kwadwo Koram[1]

[1]Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon, Accra, Ghana, [2]Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Ghana Africa IRS Project, Abt Associates, Accra, Ghana

Malaria remains a major public health problem in Ghana, with an endemicity more pronounced in the northern savannah zone. The US PMI and the Ghana Health Services have been implementing an Indoor Residual Spraying (IRS) program in selected districts in the northern savannah zone of Ghana since 2008, as part of an initiative to reduce the disease burden in the country. This study was conducted between November 2010 and November 2013 to assess the impact of the PMI-

IRS program on malaria parasitaemia during the high transmission season in children under five in Bunkpurugu-Yunyoo District in northern Ghana. The district was sprayed with alphacypermethrin (a pyrethroid) in 2011 and 2012 at a rate of 25mg/m^2, and with pirimiphos methyl (an organophosphate), at an application rate of 1g/m^2 in 2013. The change of insecticide was as a result of declining susceptibility of local vectors to pyrethroids. During each household survey, probability proportional to size estimates (PPSE) were used to yield a minimum sample size of 1,311 children under 5 years old. All selected children were tested for malaria parasitaemia by microscopy, and a questionnaire was used to collect demographic, recent fever and bednet ownership and use information from caregivers. Trend analyses of prevalence of malaria parasitaemia showed a marginally significant decline from pre-IRS prevalence of 52.5% (95% CI: 50.1, 54.8) in November 2010 to post-IRS prevalence of 47.7% (95% CI: 45.5, 49.9) in October 2012 (p=0.013). After the change of insecticide in April 2013, malaria parasitaemia significantly declined further to 20.6% (95% CI: 18.4, 22.9) in October 2013 (p<0.001). Similar trends were observed in prevalence of reported fever and measured severe anaemia (hemoglobin < 7 g/dL) in the district. These findings support the policy change from pyrethroid to organophosphate for IRS in the Bunkpurugu Yunyoo District.

1036

PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF EXTENDED-SPECTRUM BETA-LACTAMASES IN *KLEBSIELLA PNEUMONIAE* AND *ESCHERICHIA COLI* ISOLATES IN ACCRA, GHANA

Henry Kwadwo Hackman[1], Kingsley Twum-Danso[2], Charles A. Brown[3], Lawrence Annison[4]

[1]Accra Polytechnic, Accra, Ghana, [2]University of Ghana Medical School, Department of Microbiology, Korle Bu, Accra, Ghana, [3]University of Ghana, School of Allied Health Sciences, Department of Medical Laboratory Technology, Korle Bu, Accra, Ghana, [4]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Extended-spectrum beta-lactamases are plasmid-mediated beta-lactamases capable of hydrolysing beta-lactams except carbapenems and cephamycins. Hence, ESBL-producing isolates limit therapeutic options and contribute to treatment failure. This study determined the occurrence of ESBL genotypes in *Escherichia coli* and *Klebsiella pneumoniae* and their antibiotic resistance profile in Accra, Ghana. Four hundred (400) *K. pneumoniae* and *E. coli* non-duplicate isolates were collected at Korle Bu Teaching Hospital and Advent Clinical Laboratories. The species identification, ESBL detection, MIC and antibiotic sensitivity testing were concurrently determined using Vitek 2 Compact System. The combined disc synergy method was used to confirm ESBL-producing strains. The genotypes of the ESBL-coding genes were determined using standard PCR reaction, agarose gel electrophoresis and bands visualization. The results showed that 202 (50.5%) of the bacterial isolates were ESBL-producers with increased resistance to amoxicillin/clavulanic acid (31.7%), nitrofurantoin (46.5), piperacillin/tazobactam (52.5%), tetracycline (70.8%), ciprofloxacin (79.7%), gentamicin (82.2%) and trimethoprim/sulphamethoxazole (97%). Of the 100 ESBL producers, CTX-M ESBL genes (90%) were dominant and 25% had TEM genes. None of the ESBL producers possesses SHV genes. Twenty (20%) of the isolates had both CTX-M and TEM genes. Of the 100 ESBL phenotypes, 78% and 2% were positive for CTX-M-1 group and CTX-M-9 group ESBL genes respectively. The CTX-M-type and TEM-type ESBL showed co-resistances to piperacillin/tazobactam, amoxicillin/clavulanate, ciprofloxacin, nitrofurantoin, ciprofloxacin, gentamicin, tetracycline and trimethoprim/sulphamethoxazole. Imipenem and amikacin were the *in-vitro* drugs of choice for treating CTX-M and TEM-type ESBL producers. It is vital to routinely detect ESBL-phenotypes in health facilities and implement appropriate antibiotic stewardship programs. There is the need for further studies into the clinical therapeutic outcomes to infections by CTX-M-type and TEM-type ESBL producers in Accra.

1037

MOLECULAR CHARACTERIZATION OF MULTI-DRUG RESISTANCE *STAPHYLOCOCCUS AUREUS* ON CLINICAL AND NON-CLINICAL SOURCES IN NIGERIA

Joseph Omololu-Aso[1], Anthonia O. Oluduro[1], Oluwaseun O. Omololu-Aso[2]

[1]Obafemi Awolowo University, Ile Ife, Nigeria, [2]Family Medicine Department, University College Hospital, Ibadan, Oyo State, Nigeria

Staphylococcus aureus is a major pathogen associated with serious community-acquired and nosocomial diseases in all age group and of such a frequent microorganism obtained from clinical and non-clinical sources. Data on clinical identity, diversity, surveillance and new approaches in the molecular characterization of this pathogen in Nigeria are limited. 850 samples were collected from both clinical and non-clinical sources. The clinical source included the routine specimens of wound swabs, urine, stool, blood and sputum from the University Teaching Hospital Complex. The non- clinical samples were obtained from food handlers and food vendors at OAU campus restaurants and Ile-Ife centre community market respectively. The bacteriological analysis was carried out on the samples which was later cultivated on mannitol salt agar and incubated for 24-48 hours. Gram's reaction, catalase, Coagulase, DNAse tests and antibiotic susceptibility was carried out using disk diffusion technique.Plasmid isolation and detection of mecA,nucA, Panto van luekocidin genes and Random Amplified Polymorphic DNA(RAPD) by PCR amplification were also carried out.A total of 405 *Staphylococcus aureus* isolates comprising 230 (56.8%)from clinical and 175(43.2%) from non-clinical sources were recovered from 770 presumptive staphylococci. Prevalence of S.aureus infections and its incidence rate was higher in clinical than in non-clinical sources(p>0.05). All the 405 clinical and non clinical isolates were resistant to penicillin while 96% were sensitive to vancomycin. Among the clinical isolates, 4% were vancomycin intermediate resistant. All the non-clinical isolates were sensitive to vancomycin.Meanwhile,53% of the food handlers isolates were resistant to oxacillin. (44%) of the 50 representative screened clinical and non clinical isolates contained plasmid genes of varying molecular weight ranging from 562 to 23,490kb. 21(42%) contained nuc gene and 13(26%) mec gene, while 2(4%) clinical isolates had PVL gene. RAPD constituted 4(8%) of the clinical isolates. In conclusion, the study established clonal interrelatedness between the clinical and non-clinical isolates and confirm the importance of phenotypic identification of *S. aureus* by molecular techniques. Improved evaluation scheme should be put in place to combat the ineffectiveness of antimicrobial therapy due to high incidence of resistance among the isolates of *S. aureus* in Nigeria.

1038

BRUCELLA MELITENSIS METHIONYL-TRNA SYNTHETASE: A PROMISING TARGET FOR STRUCTURE-BASED DRUG DEVELOPMENT FOR THE TREATMENT OF BRUCELLOSIS

Heidi Hillesland[1], Janette B. Myers[1], David M. Dranow[2], Kayode K. Ojo[1], Thomas E. Edwards[2], Douglas R. Davies[2], Don Lorimer[2], Zhongsheng Zhang[1], Erkang Fan[1], Wesley C. Van Voorhis[1]

[1]University of Washington, Seattle, WA, United States, [2]Emerald BioStructures, Bainbridge Island, WA, United States

Brucellosis is the most common zoonotic disease and disproportionally afflicts the most impoverished people. Its devastating impact on human lives is a result of both debilitating human illness and the loss of affected livestock. Brucellosis treatment is plagued by high failure and relapse rates despite long durations of therapy. New drugs have not been discovered for treatment of Brucellosis in decades. New and more effective drugs are greatly needed. Aminoacyl-tRNA synthetase (AaRS) enzymes are promising antimicrobial drug targets. These enzyme are necessary for protein synthesis and, therefore, bacterial growth. Bacteria resistance to antimicrobials is an enormous issue and one promising feature of anti-AaRS compounds is that there appears to be a profound

loss of fitness associated with bacteria resistance to the compounds. We have solved the crystal structure of *Brucella melitensis* methionyl-tRNA synthetase (*Bru*MetRS) and this allows for structure-based drug development. Furthermore, we have developed a working assay to screen for compounds that inhibit *Bru*MetRS and have identified a number of promising leads from screening a small compound library. *Brucella* is highly infectious and easily transmitted in the laboratory environment. One of the major barriers to drug development for Brucellosis is the necessary research precautions, including a BSL-3 laboratory. To further facilitate rapid screening of compounds for inhibition of *Brucella* growth via BrumetRS inhibition, we are using genetic manipulation to replace the MetRS gene in a nonpathogenic *E coli* with the structurally unique *Bru*MetRS gene. We are assessing for off-target effects by testing both the wild-type and mutant *E coli.* The dramatic difference of *E. coli* and *Brucella* MetRS will likely be reflected by compounds targeting *Bru*MetRS inhibiting *E. coli* growth of bacteria expressing *Bru*MetRS but not *Ecoli*MetRS. Lead compounds will later be tested directly against *Brucella melitensis* for confirmation of activity. This is a needed first step to developing a new drug for an ancient and neglected disease.

1039

SINGLE DOSAGE OF DOXYCYCLINE FOR PROPHYLAXIS AGAINST LEPTOSPIRAL INFECTION AND LEPTOSPIROSIS DURING URBAN FLOODING IN SOUTHERN THAILAND: A NON-RANDOMIZED CONTROLLED TRIAL

Sarunyou Chusri

Prince of Songkla University, Hat yai, Songkhla, Thailand

Data on doxycycline for prophylaxis against leptospiral infection and leptospirosis during flooding is limited. During October 2010, a study was conducted in Hat Yai City of Southern Thailand, to explore the risk factors for leptospiral infection and leptospirosis among urban flood victims and investigate the protective efficacy of a single dosage of 200-mg doxycycline against the infection and developing disease. Of 641 participants, 600 received doxycycline while 41 did not due to fear of side effects and having a history of drug allergy. Twenty two participants were infected with leptospires and six developed leptospirosis. Having a laceration wound was significantly associated with both leptospiral infection (odds ratio [OR] = 37.20; P < 0.001) and leptospirosis (OR = 18.24; P = 0.003) whereas exposure to flood water more than 3 hours per day was significantly associated with only leptospiral infection (OR = 3.70; P = 0.038). Seventeen participants who received doxycycline were infected with leptospires compared to five who did not receive doxycycline, resulting in a protective efficacy of 76.8 % (95% confidence interval [CI] = 34.3 % - 92.0 %). Among participants with leptospirosis, 4 received doxycycline while 2 did not, a protective efficacy of 86.3% (CI = 9.8% - 98.2%). Among the participants with laceration wound, the protective efficacy for leptospiral infection was 92.0 % (CI = 81.2% - 96.6%) and for leptospirosis was 95.6% (CI = 78.2% - 99.3%). Among the participants exposed to flood water less than or equal to three hours per day, the protective efficacy for leptospiral infection was 89.2 % (95% CI 63.6 % - 96.67 %). Flood victims with laceration wounds and exposed for more than three hours per day should take precautions for leptospiral infection and leptospirosis. A single dosage of doxycycline could be considered as prophylaxis for victims of flooding.

1040

ACUTE UNCOMPLICATED FEBRILE ILLNESS IN CHILDREN AGED 2-59 MONTHS IN ZANZIBAR - ASSESSMENT OF INFECTIOUS DISEASE ETIOLOGIES AND INFECTIONS REQUIRING ANTIBIOTICS

Kristina Elfving[1], Delér Shakely[2], Maria Andersson[3], Abdullah S. Ali[4], Kimberly Baltzell[5], Wei Ping Xu[2], Rahila S. Omar[4], Mwinyi I. Msellem[4], Annika Ljung[1], Max Petzold[6], Birger Trollfors[7], Magnus Lindh[1], Anders Björkman[2], Andreas Mårtensson[2]

[1]Department Infectious Disease, Gothenburg, Sweden, [2]Malaria Research, Department of Microbiology, Tumor and Cell Biology, Stockholm, Sweden, [3]Department Infectious Disease, Institute Biomedicine, University of Gothenburg, Gothenburg, Sweden, [4]Zanzibar Malaria Elimination Programme, Zanzibar, United Republic of Tanzania, [5]Department of Family Health Care Nursing, San Francisco, CA, United States, [6]Akademistatistik, Centre for Applied Biostatistics, Occupational and Environmental Medicine, Gothenburg, Sweden, [7]Department Paediatrics, Institute Medicine, University of Gothenburg, Gothenburg, Sweden

Understanding the causes of fever in areas of sub-Saharan Africa where malaria recently has undergone a rapid decline is key to ensure evidence based fever case management. We therefore studied infectious etiologies of uncomplicated febrile illness in 677 children aged 2-59 months managed according to Integrated Management of Childhood Illness (IMCI) at a rural primary health facility in Zanzibar, using point-of-care tests, radiology and multiplex real-time PCR analyses of nasopharyngeal (NPH) and fecal samples. Some 168 asymptomatic community controls provided NPH and fecal samples for PCR. The IMCI classifications were compared with final reference diagnoses established after study completion based on all available clinical and laboratory data. A majority of patients were classified according to IMCI either as non-bloody diarrhoea 164 (24%), or non-severe pneumonia 387 (57%), of which 42 (11%) were confirmed by chest x-ray. More than one pathogen was detected by NPH PCR in 592 (87%) of patients and 139 (83%) of asymptomatic controls. The most common final reference diagnoses in patients, were Influenza A or B (22%), respiratory syncytial virus (22%), rhinovirus (11%), streptococcal infection (7%), radiological pneumonia according to WHO criteria (6%) and Shigella gastroenteritis (4%). *Plasmodium falciparum* was diagnosed in two cases. Antibiotics were prescribed to 500 (74%) patients, but only 154 (23%) had a final reference diagnosis considered requiring antibiotic treatment of whom 24 (16%) did not receive antibiotics. The detection rate of pathogens in both cases and controls were high, indicating the complexity of determining infectious etiologies in acute uncomplicated febrile illness among children in the new context of low malaria transmission in Zanzibar, and further the difficulty of identifying infections requiring antibiotics.

1041

CONTACT INVESTIGATION OF MELIOIDOSIS CASES REVEALS REGIONAL ENDEMICITY - PUERTO RICO, 2010 AND 2012

Thomas J. Doker

United States Air Force, Fort Detrick, MD, United States

Melioidosis results from infection by percutaneous inoculation, ingestion, or inhalation of the saprophyte *Burkholderia pseudomallei*, and is associated with case-fatality rates up to 40%. Improved survival rates are attributed to early diagnosis and treatment with appropriate antimicrobials. Sporadic cases have been identified in Puerto Rico, where the incidence and epidemiology is unclear. Following identification of one fatal and one non-fatal melioidosis case in 2010 and 2012, respectively, contact investigations were conducted to identify risk factors for infection. Questionnaires were administered and serum specimens were collected from co-workers and persons living within 250 meters of cases' residences (neighborhood contacts) and from injection drug use (IDU) contacts of the 2012 case. Serum specimens were tested for evidence of prior exposure to *B. pseudomallei* by indirect hemagglutination assay (titer

≥1:40). Serum specimens were collected from 51 and 60 individuals associated with the 2010 and 2012 cases, respectively. None of the co-workers were seropositive for anti-*B. pseudomallei* antibody, whereas 2 (5%) of 40 and 12 (23%) of 52 of 2010 and 2012 neighborhood contacts were seropositive, respectively, and 67% (2 of 3) of IDU contacts. Of all seropositive persons, 39% reported no travel outside of Puerto Rico. Characteristics significantly associated with seropositivity were reporting skin wounds, sores, or ulcers (adjusted odds ratio [aOR] = 4.6; 95% confidence interval [CI]: 1.2-17.8) and IDU (aOR=18.0; 95% CI: 1.6-194.0). Sporadic reports of melioidosis and high seropositivity in case contacts suggest at least regional endemicity in Puerto Rico. Increased awareness of melioidosis among clinicians, laboratories, and public health professionals is needed to improve case identification, initiate appropriate antimicrobial therapy, and facilitate case reporting in Puerto Rico.

1042

NOVEL VACCINE CANDIDATES TO PREVENT INFECTION WITH GROUP A STREPTOCOCCUS

Michael Good[1], Manisha Pandey[1], Emma Langshaw[1], Michael Batzloff[1], James McCarthy[2], Tania Rivera-Hernandez[1]

[1]Institute for Glycomics, Gold Coast, Australia, [2]QIMR Berghofer Medical Research Institute, Gold Coast, Australia

Streptococcus pyogenes (group A streptococcus, GAS) infection leads to a wide array of clinical manifestations ranging from uncomplicated self-limiting infections such as pharyngitis and impetigo to severe invasive diseases including deep soft tissue infection, sepsis, and streptococcal toxic shock syndrome. If untreated, streptococcal infection can also lead to post-infection sequelae of rheumatic fever and rheumatic heart disease. We have developed candidate vaccines based on a conserved peptide from the M protein (J8) and on a recombinant fragment of the streptococcal interleukin-8 protease virulence factor, Spy-CEP. Using a novel skin challenge model in mice that we have developed and which closely mimics the human condition, we have been able to show that vaccination with J8 can protect against pyoderma caused by multiple strains of GAS and can also protect against septicemia that develops post skin infection. Long term immunity is dependent on memory B cells, T cells and neutrophils. We were thus concerned that the vaccine might not protect against the virulent CovR/S strains of GAS that up regulate Spy-CEP, thus potentially limiting neutrophil ingress to the site of infection. We show that vaccine-induced immunity to these strains is in fact significantly reduced; however, co-vaccination with a recombinant fragment of SpyCEP induces antibodies that restore IL8 function and which are able to fully restore the level of protection against CovR/S mutants. J8 is currently in a Phase I clinical trial and J8/Spy-CEP is being developed as a second-generation vaccine to prevent infection with hyper-virulent strains of GAS.

1043

CHARACTERIZATION OF BLOODSTREAM INFECTIONS FROM HOSPITALIZED PATIENTS IN IQUITOS, PERU

Drake H. Tilley

U.S. Naval Medical Research Unit - 6, Lima, Peru

Bloodstream infections, especially those associated with central line devices, are associated with increased length of hospital stay and substantially increased morbidity and mortality. The identification and characterization of pathogens associated with such nosocomial infections is critical to establishing control programs, particularly in resource-limited settings. Accordingly, in this study we characterized bloodstream infections in two major hospitals located in Iquitos, a jungle city in the Amazon Basin of Peru. A total of 336 hemocultures were performed on patient samples obtained between June 2011 and March 2014. Of the samples tested, 58% were from adult patients ($p<0.0001$) while the remaining 42% were from children under the age of 18. All samples were cultured and screened according to the guidelines and criteria established by the Centers of Disease Control and Prevention (CDC) in the United States. A total of 11% (36/336) of the samples tested were deemed positive for bacteremia. *Klebsiella pneumoniae* and *Staphylococcus aureus* were the most common bacteria isolated and were present in 10 isolates each, accounting for a combined total of 56% of positive samples. In addition, 9 isolates of *Acinetobacter* (25%), 3 isolates of *Enterobacter* sp. (8.3 %), 2 isolates of *Pseudomonas aeruginosa* (5.6%) and one isolate of *E. coli* and *Serratia marcescens* were also identified. *Klebsiella pneumoniae* showed 100% resistance to ceftriaxone, gentamicin and ticarcillin while 30% of *S. aureus* and 11% of *Acinetobacter spp.* were resistant to methicillin and imipenem, respectively.

1044

SEVERE BUBONIC PLAGUE WITH SEPTIC SHOCK AND ACRAL NECROSIS IN CENTRAL OREGON

Jon E. Lutz[1], John Zachem[1], Jonathan Brewer[1], Michael Feldman[1], Emilio DeBess[2], Christina Nelson[3]

[1]St. Charles Medical Center, Bend, OR, United States, [2]Oregon Department of Human Services, Portland, OR, United States, [3]Centers for Disease Control and Prevention, Fort Collins, CO, United States

A 59 year-old man acquired bubonic plague from a cat bite in central Oregon in June 2012. He developed secondary septicemia, probable plague pneumonia, severe septic shock, disseminated intravascular coagulation, acute renal failure, and acute respiratory failure. His clinical course was complicated by the need for renal replacement therapy, mechanical ventilation, and vasopressor drug support, and he ultimately developed acral gangrene of all of his fingers and toes. Despite renal failure, he was successfully treated with gentamicin and high volume hemofiltration and was discharged after a lengthy hospital course. Save for the loss of essentially all of his digits, he made a good recovery including return of normal renal function. The case highlights options for antibiotic therapy for plague in the context of various clinical conditions, the role of renal replacement therapy in severe sepsis, and challenges with antibiotic dosing during high volume hemofiltration. We also review additional recent plague cases in Oregon and cat-associated plague infections in humans.

1045

CLINICAL CHARACTERISTICS AND ETIOLOGIES OF ACUTE CENTRAL NERVOUS SYSTEM INFECTIONS IN CHILDREN AND ADULTS ADMITTED TO RURAL AND URBAN HOSPITALS IN KENYA: PRELIMINARY FINDING, 2011-2014

Janet A. Awando[1], Martina Oneko[1], Godfrey M. Bigogo[1], Clayton Onyango[2], Ondari D. Mogeni[1], Newton Wamola[1], Allan O. Audi[1], Barry S. Fields[2], Joel M. Montgomery[2], Deron C. Burton[2]

[1]Kenya Medical Research Institute, Kisumu, Kenya, [2]Centers for Disease for Control and Prevention, Nairobi, Kenya

Acute central nervous system (CNS) infection is an important cause of hospital admission of children and adults in Sub-Saharan Africa with high mortality, but diagnostic testing is often limited, hindering treatment and prevention strategies. We investigated etiologies among inpatients with suspected acute CNS infection at one rural (Siaya District Hospital [SDH]/Western) and one urban (Mbagathi District Hospital [MDH]/Nairobi) hospital in Kenya. Clinical data and diagnostic specimens were collected from eligible patients from November 2011 to November 2013 at SDH and from January 2014 to March 2014 at MDH. Cerebrospinal fluid (CSF) and blood samples were tested by routine microbiology, chemistry and cytology/hematology. CSF specimens were examined for antigens of bacterial pathogens and *Cryptococcus neoformans* and were stored for multi-pathogen testing using a real-time polymerase chain reaction (PCR) assay platform. Malaria was ruled out by blood smear. CSF was collected from 340 patients (60% aged <5 years, 54% male) and 145 patients (40% aged <5 years, 50% male) at SDH and MDH, respectively. Of 153 (32%) participants with known HIV status, 92 (60%) were positive. Common presenting symptoms included seizures (66%), neck stiffness/

nuchal rigidity and/or bulging fontanel (51%) and fever (88%). A positive malaria blood smear was obtained from 27% of participants at SDH and 11% at MDH (P<0.0001). Abnormalities of CSF chemistry and/or cytology were observed in 33% and 66% of specimens from SDH and MDH, respectively (P<0.0001). A pathogen was identified from CSF or blood in 14% of participants, including *Cryptococcus neoformans* (7%), *Neisseria meningitidis* (3%, MDH only), non typhoidal salmonella (0.9%), *Haemophilus influenza* (0.2 %) and *Streptococcus pneumonia* (0.2%). Additional clinical and PCR testing is ongoing. These preliminary findings demonstrate a high burden of cryptococcal and bacterial meningitis among patients hospitalized with suspected acute CNS infection in Kenya. Marked rural-urban differences are observed and should be evaluated.

1046

COMPARISON OF TWO MOLECULAR DIAGNOSTIC APPROACHES USING SERUM AND BUFFY COAT FOR RAPID DIAGNOSIS OF ACUTE LEPTOSPIROSIS

Caoimhe Nic Fhogartaigh[1], Sabine Dittrich[1], Anisone Chanthongthip[1], David A. Dance[1], Paul N. Newton[1], Nandini Shetty[2], Catherine Arnold[2], Maria Zambon[2]

[1]Laos-Oxford-Mahosot Hospital-Wellcome Trust-Research Unit, Vientiane, Lao People's Democratic Republic, [2]Public Health England, London, United Kingdom

Leptospirosis (lepto) has a high incidence in the tropics and is often clinically indistinguishable from other causes of fever. Early diagnosis is required to ensure appropriate therapy. Culture and serology (MAT) are slow and insensitive. PCR methods hold promise, but few assays have been evaluated clinically, and the optimal sample is unclear. We compared a newly developed quantitative real-time PCR (qPCR), targeting *rrn* and pathogen-specific *LipL32* (and including an internal control), with a previously described qPCR targeting *rrs*, using serum and buffy coat (BC) DNA from stored admission blood. 59 Lao patients diagnosed with lepto by culture (n=19), qPCR (n=20), and/or MAT (n=20) between 2008-2012 were identified retrospectively. Mean symptom duration was 5.2 days. 83 controls included other infections (n=78), or no diagnosis (n=5). Using culture as gold-standard, *rrn-LipL* was 73.7% sensitive and 98.7% specific with serum, and 57.9% and 97.5% respectively with BC; *rrs* PCR was 89.5% sensitive and 95.2% specific with serum, and 78.9% and 95.2% respectively with BC. Using the 59 lepto cases as gold-standard, *rrn-LipL* was 39% sensitive with serum and 30.5% with BC; *rrs* PCR was 44.1% sensitive with serum and 42.4% with BC. Performance of the PCRs did not differ significantly (using serum, p=0.58; BC, p=0.25), and performance of serum and BC did not differ significantly (for *rrn-LipL*, p=0.44; *rrs* PCR, p=0.86). Mean Ct values were lower for BC (suggesting higher bacterial loads) but not significantly (for *rrn-LipL*, p=0.21; *rrs* PCR, p=0.28), and did not correlate with duration of illness. PCR inhibition was seen in 12.5% of BC extracts (indicated by failed internal control), but never with serum. In conclusion, PCR has the potential to rapidly diagnose acute lepto. There was no significant difference in performance of the 2 PCRs, however, *rrn-LipL* had the advantages of a pathogen-specific probe and internal control. Inhibition seen with BC emphasises the importance of the internal control, and supports use of serum as the optimal sample. Due to the lack of a perfect gold-standard, Bayesian latent class modelling may help determine true performance characteristics of lepto PCRs.

1047

KATRINA COUGH: SYMPTOM COMPATIBLE WITH EARLY DIAGNOSIS OF LEPTOSPIROSIS?

Infanta M. Spence-Lewis

Operation Hope, Atlanta, GA, United States

Katrina Hurricane (2005) victims made extensive complaints regarding symptoms of a cough during 2006 described as the 'Katrina Cough'. Coughing is a symptom compatible with an early diagnosis of leptospirosis. This symptom is important because victims of Katrina were exposed to environmental, occupational and zoonotic conditions favorable to leptospirosis. New Orleans had no integrated surveillance system that included diagnostics for leptospirosis in place prior to hurricane Katrina. .Extreme weather conditions influence patterns of the disease. During the early 50's leptospiral case histories from Grady hospital in Atlanta, Georgia were reviewed. This reflected decades of important growing concern regarding epidemiological and public health problems caused by leptospirosis in the United States. leptospirosis is an acute febrile zoonotic disease first observed clinically by Adolf Weil in 1886. Drs. Inada, Ido, Hoki, Kaneko and Hiroshi in 1916 established 'The Etiology, Mode of Infection, And Specific Therapy' of the disease. Hideyo Noguchi described the morphology and nomenclature of the infection naming it Leptospira icterohemorrhagiae in 1918. The predominant vector for leptospira is found in the urine and feces of rodents. Infections are also transmitted through the bites or scratches of diseased rodents. The infection is also found in: raccoons, foxes, pigs, dogs and other mammals. Evidence based guidance is recommended for early diagnosis of the infection to avoid misdiagnosis and progression to Weil's disease with possible fatal outcomes. We did a select literature search of medical journals between 1922 and 1958 from Pub Med and the archives of Medical Journals for case histories on leptospirosis. Mapping and weather data are used to examine influence on surveillance and progress of the disease. Temperature and humidity are important factors for the survival of Leptospira. We used radiological images to review the systemic progression of the infection. Risk factors for leptospirosis were evaluated in various settings. A network of sentinel surveillance sites are required in order to develop appropriate interventions for populations at higher risk of developing the disease in the United States. Long term funding must be allocated so that active and passive public health programs have funds from all levels of government to foster the demands of the work.

1048

DOES HIGHER MASS ANTIBIOTIC TREATMENT COVERAGE FOR TRACHOMA REDUCE CHLAMYDIAL INFECTION AMONG CHILDREN? RESULTS FROM PRET-NIGER

Sun N. Yu[1], Abdou Amza[2], Boubacar Kadri[2], Baido Nassirou[2], Zhaoxia Zhou[1], Nicole E. Stoller[1], Lina Zhong[1], Stephanie Chin[1], Sheila K. West[3], Robin L. Bailey[4], David C. Mabey[4], Jeremy D. Keenan[1], Travis C. Porco[1], Bruce D. Gaynor[1], Thomas M. Lietman[1]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Programme Nationale des Soins Oculaire, Niamey, Niger, [3]Johns Hopkins University, Baltimore, MD, United States, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom

Programs distribute mass oral azithromycin in an effort to eliminate the ocular strains of *Chlamydia trachomatis* which cause trachoma. In trachoma-endemic areas, WHO guidelines recommend 3 annual mass antibiotic distributions with a target of at least 80% coverage. It is unclear if higher coverage would be more effective and worth the additional effort. Here, we compare a single day treatment aiming for 80% coverage (standard) to subsequent distribution days aiming for greater than 90% coverage (enhanced). 24 communities in Matamaye District, Niger, were randomized to either standard or enhanced coverage and infection was assessed biannually until 36 months. A random sample of up to 100 children (0 - 5 years of age) in each of the 24 communities were swabbed for the presence of conjunctival chlamydia Amplicor PCR. The mean antibiotic coverage was 70.6% per community in the standard arm and 88% in the enhanced arm. At baseline, the prevalence of chlamydial infection among children was 20.2% (95% CI: 9.6% to 30.8%) in the standard arm and 22.1% (95% CI: 11.4% to 32.7%) in the enhanced arm. The clinical activity among children was 27.0% in the standard arm and 28.4% in the enhanced arm. At 36 months, the prevalence of chlamydial infection among children was 4.6% (95% CI: 0.0% to 9.5%) in the standard arm and 7.1% (95% CI: 2.7% to 11.4%) in the enhanced arm. The clinical activity among children was 7.1% in the standard arm and 8.9% in the enhanced arm. Correcting for baseline, we were unable to demonstrate a statistically significant difference between standard

and enhanced treatment (p = 0.41). This study suggests that additional efforts to achieve higher mass antibiotic treatment coverage may not add significant benefit.

1049

PROPHYLAXIS AND DISEASE TRENDS OF TROPICAL DISEASES (ALTITUDE SICKNESS, MALARIA, DIARRHEA) AMONGST TRAVELERS TO PERU

Jasmeet Kaur

University of Texas Medical Branch, Austin, TX, United States

Travelers to foreign countries are subject to country-specific ailments of which many are preventable. This study attempts to determine the current trends in altitude sickness, malaria, and diarrhea prophylaxis amongst travelers to Peru. The participants of the study include students and travelers from around the world that participate in study abroad and tourism in Lima, Tumes, and Cuzco. In particular these sites include the Tropical Medicine Institute in Lima and UPCH-UTMB Collaborative Research Center in Cuzco, which have close relations with the University of Texas Medical Branch. The specific objective of the study is to examine quantitative and qualitative data on the use of prophylaxis for altitude sickness, malaria, and diarrhea amongst the travelers. The study will look at both allopathic and naturopathic remedies, as well as, disease prevention strategies amongst the travelers. Moreover, the frequency of the ailments with and without the various prophylaxis remedies will be determined. Results from the study help dictate travel consultation suggestions to optimize future prophylactic treatment.

1050

PILOT STUDY TO ASSESS THE PREVALENCE OF CHAGAS DISEASE IN PREGNANT WOMEN FROM LATIN AMERICA IN A LOS ANGELES COUNTY HOSPITAL

Marja Paulino, Efrain E. Murillo, Salvador Hernandez, Lissette Rodriguez de Armas, Sheba K. Meymandi, **Mahmoud I. Traina**

Olive View-University of California Los Angeles Medical Center, Sylmar, CA, United States

Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, causes the most important parasitic disease burden in Latin America, where an estimated 8-10 million persons are infected. Approximately 24 million persons born in the countries in which CD is endemic currently reside in the U.S. and roughly 300,000 of these immigrants are thought to have chronic CD. Though transmission by domestic vectors is still the most important route of infection in disease-endemic areas, congenital transmission is the most important transmission route in non-endemic countries. Rates of congenital transmission from untreated infected mothers range from 2-12% in published studies. Previous studies demonstrate considerable information regarding CD prevalence among pregnant women in Latin America, however information about the prevalence of CD in pregnant women immigrants residing in the U.S. is largely lacking. This study of prevalence of CD in pregnant women from Latin America was performed at Olive View-UCLA Medical Center (OV-UCLA MC), a Los Angeles County hospital, from 4/2008 -5/2011 under the auspices of the Center of Excellence for CD. Enrollment criteria for the study were pregnant patients who had lived in a Latin American country for at least 1 year. 300 consecutive patients were screened for Chagas disease using both an immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA). Testing was performed by the Center for Disease Control and Prevention (CDC). Subjects were 15-46 years old with a median age of 32 years old. The countries of origin where subjects spent 1 year or more were Mexico 233 (77.7%), El Salvador 34 (11.3%), Guatemala 20 (6.7%), others 13 (4.3%). The average time of residence in country of origin was 14 years versus average time of residence in US of 17 years. A total of 1 subject was positive (0.33%) by both serological tests. The positive subject was a 36 year old female from rural El Salvador who migrated to US 19 years prior. Our study demonstrates a prevalence of CD in pregnant women immigrants from Latin America residing in the U.S. lower than expected, though the small sample size makes conclusive determination not possible. Further larger scale studies are needed to obtain a better idea of prevalence in this population, especially given the high-risk of congenital transmission.

1051

PRE-TRAVEL HEALTH CARE FOR PEDIATRIC TRAVELERS: EXPERIENCE FROM THE TRAVMIL COHORT

Tahaniyat Lalani[1], David Tribble[2], Jason Maguire[1], Edward Grant[2], Jamie Fraser[2], Mark Johnson[3], Anuradha Ganesan[2], Timothy Burgess[4], Patrick Hickey[4]

[1]IDCRP, Portsmouth, VA, United States, [2]IDCRP, Bethesda, MD, United States, [3]Naval Medical Center San Diego, San Diego, CA, United States, [4]Walter Reed National Military Medical Center, Bethesda, MD, United States

Practice guidelines and prospective data regarding pediatric travel medicine are limited. We investigated the pre-travel health care and illnesses encountered by children enrolled in TravMil between 1/2010 and 7/2013. 64 children were enrolled at 3 military travel clinics (5% of all enrollees [n=1379]): 61 were enrolled pre-travel, and 3 enrolled post-travel (due to a travel-related illness). Pediatric travelers were visiting friends and relatives more frequently than adults (31% vs 14%; p< 0.05). The median trip duration for children was 17 days (IQR: 12-25) and the most common destination was South/Central America & Caribbean (n=26). Coverage rates for travel related vaccines (for those at risk) were similar among children and adults: Japanese encephalitis 71% vs 55%; yellow fever: 65% vs 85%; meningococcus: 100% vs 96%; typhoid: 95% vs 96% (p>0.05 for all). Malarone was the most common antimalarial prescribed (56%; n=22/39) regardless of the duration or location of travel. Rates of partial or non-compliance with chemoprophylaxis were similar among children and adults (13.3% vs 23%; p=0.54). Children < 10 years of age were less likely to be prescribed antibiotics (69% vs 97%; RR: 0.07 [95% CI: 0.01-0.61]) or antidiarrheals (7% vs 78%; RR 0.09 [95% CI: 0.02-0.34]) for self-treatment of traveler's diarrhea (TD) compared to children ≥10 years. Rates of travel-related illness were similar among children and adults: TD: 15% vs 24%; undifferentiated fever 3% vs 2%; influenza-like illness 12% vs 17% (p> 0.05). Three patients were enrolled post travel: 1 each with malaria, influenza-like-illness, and suspected dengue. None had been seen for a pre-travel evaluation. Significant differences in travel characteristics and pre-travel health care exist based on the age of travelers. Children < 10 years were less likely to receive medications for TD self-treatment despite similar rates of TD among pediatric and adult travelers. Strategies to improve utilization and standardization of pre-travel healthcare in pediatric travelers are needed.

1052

A SYSTEMATIC REVIEW OF THE EFFECTS OF ARTEMETHER-LUMEFANTRINE ON GAMETOCYTE CARRIAGE AND DISEASE TRANSMISSION

Michael Makanga

EDCTP, Tygerberg, South Africa

Despite significant advances made in the prevention and treatment of malaria in recent years, the current success still falls short of the World Health Organisation (WHO) goals for malaria control and elimination. For elimination strategies to be effective, all parasite lifecycle targets including disease transmission have to be addressed. Rapid and effective reduction of infectious parasite reservoir and gametocyte carriage is therefore critical. Currently, artemisinin-based combination therapies (ACTs) form the cornerstone of WHO-recommended treatment for uncomplicated *P. falciparum* malaria, and in combination with other control intervention measures will play a pivotal role in elimination programmes due to their gametocytocidal properties. There is irrefutable epidemiological evidence of reductions in malaria incidence and transmission in African

regions since the introduction of these agents. A systematic review of 62 articles published between 1998 and January 2014 was done which compares effects of the ACT artemether-lumefantrine (AL) on gametocyte carriage and malaria transmission with other ACTs and non-ACTs. AL was assessed based on its widespread usage as 'gold standard' treatment for uncomplicated *P. falciparum* malaria in several African countries and the high number of clinical trials that have evaluated the product. The impact of AL on population gametocyte carriage and the potential future role of AL in malaria elimination initiatives are also considered. Despite the inherent difficulties in comparing data from a range of studies that utilised different diagnostic approaches to assess baseline gametocyte counts and differences in study designs, the gametocytocidal effect of AL was proportionately consistent across the studies reviewed, suggesting that AL will potentially play a vital role in treatment and elimination of malaria. However, the specific place of AL is the subject of ongoing research and will be dependent on its use in combination with other intervention measures, rational use of the product, interaction with other medicinal agents *in situ*ations of malaria co-infections and comorbidities, as well as demographic differences. Use of ACTs like AL in malaria elimination strategies will therefore require balancing potential increased roll out with rationale use and protection against resistance development.

1053

THE EFFECT OF AN ENHANCED ANTENATAL CARE PACKAGE FOR PREVENTION OF MALARIA AND ANEMIA IN PREGNANCY IN GHANA

Gifty D. Antwi[1], Harry K. Tagbor[1], Imelda Bates[2]

[1]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Efficacious antenatal care (ANC) interventions for malaria and anemia have been implemented for over two decades. However, the prevalence of malaria and anemia remains high among pregnant women due to sub-optimal uptake of these interventions. Subject participation in their own health care can improve health outcomes by improving adherence to treatment. We hypothesized that if pregnant women participated in their ANC, this would improve their adherence to ANC recommendations and promote better health outcomes. A cluster randomized, controlled trial was conducted to assess the effect of pregnant women's participation on the risk of anaemia and parasitemia 4-8 weeks after enrolment. We also assessed the feasibility and acceptability of the intervention. In the intervention group, ANC staff showed women their rapid malaria test (RDT) and Hb colour scale (HCS) results and explained their significance; the control group received only routine care. The overall mean age, gestational age and Hb concentration at baseline were 26.4 years, 17.3 weeks and 110 g/l respectively and similar in each group; 10.7% had asymptomatic parasitaemia; 74.6% owned an ITN, 48.8% slept under it the night prior to enrolment. The prevalence of anemia after 8 weeks was 51%, (95%CI: 37.2-64.7) in the control group and 52%, (95%CI: 42.8-61.5) in the intervention group. The corresponding figures for parasitemia were 6.5%, (95% CI: 3.1-9.9) and. 6.2%, (95%CI: 4.0-8.2). Integration of the HCS and the RDT into the ANC system was feasible and acceptable. Pregnant women who saw their own blood being tested believed the results and felt motivated to act to improve their health. Although ANC staff and pregnant women perceived some improvement in pregnant women's adherence to ANC recommendations, their enhanced participation in ANC did not have any effect on the prevalence of malaria or anemia. The introduction of the use of RDT into routine ANC in Ghana at the time of the study and implementation challenges may have contributed to the lack of impact of the intervention on anemia or parasitemia.

1054

FACTORS ASSOCIATED WITH COMPLICATED MALARIA IN COLOMBIA

Sandra Martinez

Fundación Santa Fe de Bogotá, Bogotá, Colombia

Colombia is an area of low transmission for malaria but with environmental and social conditions that favor its transmission principally in the Pacific coast. Between 2009 and 2013 2.099 cases of complicated malaria were reported. The incidence of complicated malaria increased from 14.68/1.000 in 2012 to 21.19/1.000 in 2013. A retrospective case-control study in a 1:2 proportion was conducted, in Tumaco, Cali and Buenaventura. The sample size was 60 cases and 120 controls, patients with positive thick blood smear between the period 2009 -2013 were included. The cases were selected base on the national clinical malaria attention guideline, adding transminases greater than 80 as a severity criteria to asses hepatic disfunction, controls were patients without severity (complication) criteria. 88.3% (159) of patients originated from Tumaco, 5.0% (9) from Buenaventura, and 6.67% (12) from Cali. 50.0% (30) of the cases were female. In the controls 46.6% (56) corresponded to women and the remaining percentage to men; regarding the distribution of parasite specie, 43 cases corresponded to P.falciparum, 16 to P.vivax, and 1 was mixed. With respect to the controls, 85 were P.falciparum, 32 were P.vivax and 3 were mixed malaria. In the bivariate analysis identified risk factors were chills OR 3,44 (IC 95%: 1,14; 10,42), coluria OR 3,49 (IC 95%: 1,02; 11,95), jaundice OR 4,00 (IC 95%: 1,72; 9,24), thrombocytopenia less than 100.000 platelets/mm3 OR 10,77 (IC 95%: 3,73; 31,10), and transfer from an institution of low medical complexity OR 3,79 (IC 95%: 1,75; 8,21). In the multivariate analysis continued as risk factors chills OR 7,24 (IC 95%: 1,00;52,45), janduice OR 4,86 (IC 95%: 1,75;13,40) and thrombocytopenia OR 5,95 (IC 95%: 1,84; 19,22). Even though jaundice was identified as a risk factor we did not establish any associations with laboratory studies like bilirubin o transaminase values due to lack of information in the medical records, which was the principal limitation of the study due to its retrospective nature.

1055

OPPORTUNISTIC INTESTINAL PARASITES DURING CHRONIC MALNUTRITION IN URBAN AND RURAL AREA OF MADAGASCAR: A PROBLEM IN TROPICAL AREA?

Guillaume Roux[1], Fabiola Gosinary[1], Rindra Randremanana[1], Sarah Delshadi[1], Gisele Raherinampinaina[2], Sandrine Soloniando[1], Elisoa Hariniaina[1], Annie Robinson[2], **Ronan Jambou**[3]

[1]Institut Pasteur de Madagascar, Antananarivo, Madagascar, [2]Tsaralalana Children's Hospital, Antananarivo, Madagascar, [3]Institut Pasteur, Paris, France

In low income countries, malnutrition-related immunosuppression is suspected to pave the way of opportunistic protozoan infection. A study was conducted to better understand the burden of intestinal parasite carriage in malnourished versus control children in Madagascar. Children hospitalized with severe acute malnourished (SAM) and diarrhoeal were enrolled in hospital as well as children from rural area attending dispensary for diarrhoea. These last children (n=508) were enrolled for a two years follow up. Anthropometric measurements were registered. Stools analysis was done using specific stainings for detection of opportunits in microscopy and pathogens were typed by PCR methods. In hospital 246 children were treated for malnutrition out of which 43 children had diarrhoea. Opportunistic pathogens were frequent with mainly yeast in abnormal quantity (51%), microsporidia (21%), *Cryptosporidium hominis* (7%) and mixed co-infestations. In rural area, 273 children attended dispensary for diarrhea. Out of which 138 malnourished children and controls were selected for further analysis. Children between 12 and 24 months were the most affected by chronic malnutrition (45.7% of children). Wasting did not differ according to age, gender, but differs between villages (p=0.02). Opportunists where found in 24.6% of chronic

malnutrition and 14.4% in controls. Opportunistic carriage (i.e. yeasts, microsporidias, cryptosporidia) was linked to brachial perimeter under 105 mm (p=0.029). In multivariate analysis, factors associated with this carriage were i) growth retardation (5x increase in risk p=0.004), non-exclusive breast-feeding, and hygiene (absence of soap). *Cyclospora cayetenensis* and *Isospora beli* were also found in these children at low rate. Overall, this study described for the first time opportunistic intestinal infections in children with malnutrition in Madagascar. It highlights the role of chronic malnutrition in carriage of cryptosporidies, whereas microsporidies were more frequent during SAM. Implication of these findings must be discussed.

1056

A RURAL FOCUS OF LEPTOSPIROSIS IN MADAGASCAR?

Ronan Jambou[1], Verosoa Fomenjanahary[1], Vonimbola Tahina[1], Domohina Rakotomanana[2], Maherisoa Ratsitorahina[1], Arthur Randriamanantena[1], Sandrine Soloniando[1], Felana Ihantamalala[1], Fanjasoa Rakotomanana[1], Pascale Bourhy[3], Benoit Garin[1], Rasamoelina Harentsoaniaina[2]

[1]Institut Pasteur de Madagascar, Antananarivo, Madagascar, [2]Veterinary Department, Faculty of Medicine, Antananarivo, Madagascar, [3]Institut Pasteur, Paris, France

Leptospirosis is due to bacteria belonging to more than 20 species. All animals especially rats can be chronic carriers of the bacteria and the human disease is often due to professional exposure and/or to contact with contaminated fresh water. Clinical expression can be mild which could explain under-estimation of the burden. The Mascareignes islands are endemic areas but in Madagascar only a couple of human cases were reported mostly in travelers. In highlands of Madagascar, the use of zebus for rice cultivation could be associated with transmission in an Asian like setting. To explore this setting 111 livestock in 28 villages of the district of Moramanga were investigated. In the same time, farmers' families were blood sampled, as well as an equivalent number of peoples randomly selected in households of the vicinity but without cattle. All the cattle and humans were geolocalized for cluster analysis. 670 zebus were sampled, out of which 81 (12% from 43 livestock) were ELISA IgG positive for leptospirosis. Additionally, 25 zebus suffering from symptoms related to leptospirosis were lepto-IgG negative. 70 positive serums (out of 81) were tested in microagglutination technic (MAT) and 10% were positive. In the same time 21 urines from the 81 IgG positive zebus and 11 blood samples from the 25 sick animal were obtained and tested with a Leptospirae sp 16S-PCR. Respectively 10 and 3 samples were positive from urines and blood samples. Sequencing of two PCR products from urines confirmed Leptospira interrogans. In households 530 subjects were enrolled (329 farmers/201 controls) with an overall lepto-IgG prevalence of 4.5%. Subjects from households with positive zebu had higher IgG prevalence than those with negative ones (7.2% vs 4.2%, p=0.3) but even more than those without zebus (7.2% vs 1.5%, p=0.004). In this setting zebus may not be the unique source of contamination of humans and in deep analysis of risks factors for leptospirosis is now in process. However this study highlights the circulation of the bacteria in rural area of Madagascar and the presence of L interrogans in cattle.

1057

NON-INVASIVE MANAGEMENT OF MADURA FOOT WITH ORAL POSACONAZOLE

Amit Sharma, Namita Sharma, Amritpal Singh, Timothy Endy

SUNY Upstate Medical University, Syracuse, NY, United States

Madura Foot is a chronic granulomatous subcutaneous fungal (eumycetoma) or bacterial(actinomycetoma) infection with high disease burden. Estimation of the global burden of madura foot is difficult but most cases have been reported in the "Mycetoma belt" i.e. from countries between 30N and 60 S of the Equator. Traditionally,maduramycosis of the foot has been treated with surgical debridement, which leads to permanent disfigurement of the limb. Non-invasive management with long-term antimicrobials alone has been reported in the past but, almost always the cost of antimicrobials, increased rates of non-compliance and resistance against antifungals have discouraged physicians from this approach. We report a case of biopsy proven eumycetoma of the foot in a young Somali refugee presented with right foot pain and swelling for 7 years and "tiny watermelon seeds" extruding out of the "puncture wounds" on the sole of the right foot, which was successfully managed without the need for any kind of surgical intervention with oral posaconazole and ciprofloxacin.

1058

SENSITIVE REAL-TIME PCR DETECTION OF PATHOGENIC *LEPTOSPIR*A SPP. AND A COMPARISON OF MOLECULAR METHODS FOR THE DIAGNOSIS OF LEPTOSPIROSIS

Jesse J. Waggoner[1], Ilana Balassiano[2], Janaki Abeynayake[1], Malaya K. Sahoo[1], Yuanyuan Liu[1], Mia Mattioli[1], Alexandria Boehm[1], Juliana M. Vital-Brazil[2], Benjamin A. Pinsky[1]

[1]Stanford University, Stanford, CA, United States, [2]Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil

Leptospirosis remains under-diagnosed due to a non-specific clinical presentation and limitations of available diagnostics. Bacteria of the genus *Leptospira*, the causative agents of leptospirosis, are categorized into pathogenic and saprophytic species, though the clinical import of making this distinction remains unclear. Our group recently developed a real-time PCR (rtPCR) for the detection of all Leptospira species, which is included in a multiplex diagnostic for an undifferentiated febrile illness (the UFI assay). While the UFI assay proved more sensitive than conventional PCRs for *Leptospira*, it was not evaluated against another rtPCR and it was unclear if similar results could be obtained with an assay for pathogenic Leptospira. In this study, we present the development and evaluation of an rtPCR for the detection of pathogenic *Leptospira* species (the pathogenic rtPCR) that targets the same region of the 16S gene as the UFI assay. The linear range of the pathogenic rtPCR extended from 7.0 to 2.0 log10 copies/µL, with a lower limit of 95% detection of 29 copies/µL. Thirty-nine cultured Leptospira isolates, representing 7 species and 23 serovars, were tested. All isolates were detected using the pathogenic rtPCR except for two strains of L. biflexa, which produced no signal. Clinical samples from 65 patients with suspected leptospirosis were tested using the pathogenic rtPCR and a reference 16S rtPCR originally reported by Smythe, et al, 2002. All 65 samples had previously tested positive using the UFI assay; 62 (95.4%) samples tested positive using the pathogenic rtPCR (p=0.24). Twenty-four (36.9%) samples tested positive in the reference 16S rtPCR, which was significantly less sensitive than the UFI assay or pathogenic rtPCR (p<0.0001 for both comparisons). In conclusion, both the pathogenic Leptospira rtPCR and the UFI assay proved significantly more sensitive than the rtPCR used for comparison. Future studies are needed to determine the impact of more sensitive Leptospira detection on both patient care and epidemiologic surveillance.

1059

VITAMIN D AND OSTEOARTHRITIS IN IRAQI WOMEN

Sami Salman, Zahraa Al Jalili

Rheumatology Unit, College of Medicine, Baghdad University, Baghdad, Iraq

Osteoarthritis (OA) is a biomechanical process whereby joints respond pathologically to mechanical stress, resulting in cartilage degradation and changes in subchondral bone. OA has many known risk factors including gender, obesity and aging. Low levels of vitamin D may exert its effect on OA through synergistic bone density loss (osteopenia) and impairment of the ability of bone to respond optimally to insults. Hypovitaminosis D is a global medical problem even in regions where oral intake is sufficient and sunlight is abundant because subtle degrees of malabsorption of vitamin D can occur independent of adequate vitamin D intake. To explore

the role of vitamin D in Iraqi women with knee OA, we enrolled 45 females with the diagnosis of OA and an equal number of age matched healthy controls. OA was diagnosed according to American College of Rheumatology (ACR) criteria for OA and subject body mass indexes (BMI) were recorded according to the WHO classification of BMI in 3 groups: normal weight, overweight and obese. Radiological grading was assigned according to Kellegren and Laurence radiological classification for OA. Vitamin D levels were measured using an ELISA assay (normal range > 30 ng/ml). Our study found that the vitamin D levels in OA patients ranged from 0.309 - 0.641 whereas the range in controls was 0.261 - 0.386. Vitamin D deficiency was prevalent among both OA cases and controls, but Vitamin D levels were inversely proportional to BMI. Vitamin D deficiency was more severe among females in the obese and overweight groups than those with normal BMI. Lower levels of vitamin D were observed in OA subjects with radiological grades III and IV disease compared to those with grade II. In summary because vitamin D deficiency was so prevalent among almost all study subjects, it was difficult to establish a clear association between vitamin D deficiency and OA. Also, there is debate about the possible variability of different methods used to determine vitamin D levels. However, the magnitude of the observed hypovitaminosis D problem in Iraqi women warrants considerable attention to all possible causes and treatment options.

1060

AN UNUSUAL PRESENTATION OF AMEBIC LIVER ABSCESSES IN A RETURNING TRAVELER

Rapeephan R. Maude[1], Richard J. Maude[2], Richard E. Bessette[3]

[1]Department of Medicine, Saint Vincent Hospital, Worcester, MA, United States, [2]Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, [3]Department of Infectious Disease, Saint Vincent Hospital, Worcester, MA, United States

Amebic liver abscess (ALA) is uncommon in the USA. Clinical presentation is usually with fever and abdominal pain and is predominantly in men. Rupture of ALA is a rare but life threatening complication if left untreated. Patients usually have leukocytosis and deranged liver function tests. Serology can be positive in >90% 7 days after exposure. Needle aspiration is indicated only for impending rupture or to aid diagnosis and reveals trophozoites in 20%. We present an unusual case in a female returned traveler initially thought to have only community-acquired pneumonia (CAP). A 53-year-old female presented with cough and fever for 3 weeks having spent 4 weeks in India. She had low-grade fever, wheeze and cough for 1 week in India where she was treated unsuccessfully with azithromycin. Following her return to the US she also developed vomiting, anorexia, malaise and vague right upper quadrant abdominal pain. She was markedly distressed with fever of 102ºF, tachycardia, tachypnea and right upper quadrant tenderness. Her white blood count was 19,000/µl (neutrophils 82%) and hematocrit 27.9%. Liver function tests were normal. Chest X-ray showed minor linear patchy density in the left lung base. Ceftriaxone and azithromycin were initiated for possible CAP and she was admitted. Ultrasound abdomen subsequently demonstrated an 8 cm hypoechoic, septated, thick walled mas in the left lobe of liver. Computed tomography (CT) revealed 3 cm and 8 cm abscesses. Thick pus were drained under CT guidance. Microscopy of the pus showed no trophozoites or bacteria and culture was negative. Around 700 cc of pus were drained over the next 2 weeks. She was given metronidazole and ciprofloxacin. Antibody to *Entamoeba histolytica* was positive so ciprofloxacin was stopped. A follow-up CT scan showed marked reduction in size of the abscesses and the patient fully recovered. ALA in this patient was not initially suspected due to the respiratory presentation and normal liver function. Without a low threshold for imaging, this potentially life threatening diagnosis could have been overlooked.

1061

DETECTING APPROXIMATE REGIONS OF LUNG CANCER AND FLUID USING CHEST X-RAY SCREENING IMAGES

Sabah N. Mazhar, **Alyaa H. Ali**, Marow Al Majed

Department of Physics, College of Science for Women, Baghdad University, Baghdad, Iraq

Lung cancer is a leading cause of death in Iraq and despite active research in computer-aided diagnostic tools, development of an automated screening process for lung cancer is extremely challenging. The mortality rate for lung cancer is high in part because early detection is difficult, along with technical factors in capturing images or experience in x-ray interpretation all contribute to this problem. Therefore, the aim of our research is to develop an image-based coarse level texture descriptor to distinguish between cancerous regions and fluid filled regions in chest radiograph (x-ray) images of the lung. The presence of a significant amount of fluid in the lung is also associated with a high mortality rate. We present a computer-based approach for automatic detection of cancerous and fluid regions in the lung using chest radiographs. The image morphology approach using Marker-Controlled Watershed Segmentation method is used to isolate the cancerous and fluid regions within the lung tissue boundary. Different methods were used to enhance the X-ray images prior to computing the first order texture analysis histogram used for feature extraction. Our textural analysis method is shown to be capable of distinguishing between normal and cancerous cases, normal and fluid cases, as well as between cancerous and fluid cases. Such approaches will be useful for developing image-based automated lung cancer screening systems.

1062

ULTRASOUND AND CHEST X-RAY IN AN ADULT PATIENT WITH DENGUE

Tun-Linn Thein, Gervais Wansaicheong, Victor C. Gan, Frederico Dimatatac, Chi-Jong Go, David C. Lye, Yee-Sin Leo

Tan Tock Seng Hospital, Singapore, Singapore

The pathognomonic feature of dengue hemorrhagic fever (DHF) is a transient increase in vascular permeability resulting in plasma leakage. Several studies assessed ultrasound as a tool in gauging disease severity in detecting plasma leakage into tissues and body cavities. Most of these studies were done in children and information on ultrasound study on adults was limited. We conducted a prospective study to recruit adult patients above 18 years presenting with acute fever from August 2011 to September 2012 to Communicable Disease Center, Singapore. Demographic, clinical and laboratory data were collected. Ultrasound assessment included pericardial, pleural and abdominal cavities including gall bladder wall thickness, liver, spleen and ascites on enrolment visit, recovery (4-7 days from enrolment) and convalescent visit (3-4 weeks). Postero-anterior chest X-ray (CXR) together with right lateral decubitus view was done on the same day as ultrasound. Plasma leakage (clinically detected fluid accumulation, hematocrit [Hct] change>20% and hypoproteinemia) and DHF were defined using 1997 World Health Organization criteria. Of 110 recruited patients, 74 had laboratory confirmed dengue, 11 probable dengue and 25 were dengue negative. Among confirmed dengue cases, median age was 34 years, 98% were males, and 24% were hospitalized. Median duration of fever on enrolment was 6 days (range: 2-10). Fluid was detected by ultrasound in 2 cases and CXR in 10 cases. There were 19 cases with plasma leakage (0 clinical fluid accumulation, 1 HCT change and 19 hypoproteinemia) and 11 with DHF. One ultrasound and 6 CXR detected fluid in those with hypoproteinemia; one patient with HCT change and hypoproteinemia had fluid on CXR. Of 11 DHF cases, fluid was detected by CXR in 4 cases (3 on visit 1, 1 on visit 2) and ultrasound in 1 case (on visit 2). Detection of fluid by imaging was uncommon in our adult dengue cohort. Its utility in severe adult dengue needs further study.

1063

SERUM BIOCHEMICAL AND HEMATOLOGY VALUES IN *ONCHOCERCA VOLVULUS* INFECTED PEOPLE IN NORTHEAST DRC, LIBERIA AND GHANA DETERMINED PRE-TREATMENT IN A CLINICAL STUDY COMPARING THE EFFECT OF MOXIDECTIN AND IVERMECTIN

Hayford Howard[1], Germain Mambandu[2], Kambale Kasonia[3], Nicholas O. Opoku[4], Eric Kanza[5], Didier Bakajika[6], Michel Vaillant[7], Christine Halleux[8], Piero L. Olliaro[8], **Annette C. Kuesel**[8]

[1]The Liberian Institute for Biomedical Research, Clinical Research Center Bolahun, Monrovia, Liberia, [2]Centre de Recherche en Maladies Tropicales de l'Ituri, C.R.M.T-ITURI, Hôpital Général de Référence de Rethy, Rethy, Province Orientale, Democratic Republic of the Congo, [3]Centre de Recherche Clinique de Butembo, Université Catholique du Graben, Butembo, Democratic Republic of the Congo, [4]Onchocerciasis Chemotherapy Research Center, Hohoe, Ghana, [5]Centre de Recherche Clinique de Butembo, Université Catholique du Graben, Butembo, Province du Nord Kivu, Democratic Republic of the Congo World Health Organization, Butembo, Democratic Republic of the Congo, [6]Centre de Recherche en Maladies Tropicales de l'Ituri, C.R.M.T-ITURI, Hôpital Général de Référence de Rethy, World Health Organization, Rethy, Province Orientale, Democratic Republic of the Congo, [7]CCMS CRP-Santé, Luxembourg, Luxembourg, [8]World Health Organization, Geneva, Switzerland

For a Phase 3 study comparing the effects of a single dose of moxidectin and of ivermectin in 941 males and 531 females, including 79 children 12-17 years, 1499 people underwent baseline serum biochemical and haematological examinations. All had ≥10 *Onchocerca volvulus* microfilaria/mg skin and 876/1465 (60%) who underwent stool examination with a single sample Kato Katz test were infected with at least 1 type of intestinal helminth. None of the people suffered from an acute disease at the time of testing. The interquartile range of the laboratory values (i.e. the range within which the values for 50% people screened lay on either sides of the median) were: Albumin: 37.2-43.0 g/L, Alkaline phosphatase (>20yrs): 72.4-108.2 U/L, Bilirubin: 5.2-11.0 umol/L, Creatinine: 60.3-78.6 umol/L, GGT: 18.2-41.7 U/L, Glucose: 4.5-5.3 mmol/L, LDH: 210.0-278.0 U/L, Protein: 77.3-88.0 g/L, SGOT-AST: 23.1-35.1 U/L, SGPT-ALT: 18.0-31.1 U/L, Urea: 1.8-3.1 mmol/L, WBC: 5.70-8.62, x10E9/L Basophiles: 0.01-0.03 x10E9/L, Eosinophiles: 0.44-1.47 x10E9/L, Lymphocytes: 1.90-3.17 x10E9/L, Monocytes: 0.39-0.75 x10E9/L, Neutrophils: 2.64-7.15 x10E9/L, Platelets: 206-316 x10E9/L, Hematocrit Female: 36-43 %, Male: 40-48 %, Hemoglobin Female: 12-14 g/dL, Male: 14-16 g/dL. There were no substantial differences between the values obtained in the four different areas in which participants were recruited: Nord-Ituri and Nord-Kivu in DRC, Lofa county in Liberia and Nkwanta district in Ghana. These values in an essentially adult population are within generally accepted 'normal ranges' for 'healthy subjects'. Given that 'chronic' or temporary infections are frequent in many African populations, the data obtained can contribute to defining more locally-relevant 'normal laboratory values'.

1064

A TAQMAN ARRAY CARD (TAC) FOR DETECTION OF CENTRAL NERVOUS SYSTEM INFECTIONS IN KENYA

Vladimir Loparev[1], Clayton O. Onyango[2], Vinod Bhullar[1], Scott Schmid[1], Kay Radford[1], Michael Lo[1], Leonard Mayer[1], Susanna E. Schmink[1], Paul Rota[1], Barbara W. Johnson[3], Robert Lanciatto[3], Jorge Munoz[4], Bernard Beall[1], Maria da Gloria Carvalho[1], Jonas Winchell[1], Kathleen Thurman[1], Jamie Posey[1], Deron Burton[2], John Neatherlin[2], Joel M. Montgomery[2], Carolyn Black[1], **Barry Fields**[5]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Division of Global Health Protection, Global Disease Detection, Centers for Disease Control and Prevention/Kenya Medical Research Institute, Kisumu, Kenya, [3]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [4]Centers for Disease Control and Prevention, San Juan, PR, United States, [5]Centers for Disease Control and Prevention/KEMRI, Nairobi, Kenya

Infections of the CNS are often associated with meningitis, encephalitis or meningoencephalitis and may result in significant morbidity and mortality. In Kenya, the Ministry of Health reviewed hospital-based surveillance and concluded that hospitals in Kenya failed to diagnose large numbers of meningitis cases, probably due to technical challenges of diagnosis. To mitigate diagnostic challenges, we developed encephalitis TaqMan Array Card (TAC) which enables simultaneous detection of 21 pathogens associated CNS infections. These targets includetwo amoebae, 13 virus, and 6 bacteria. An intrinsic control (RNAase P*)* was included to monitor the extraction and amplification efficiency and assure the validity of testing results. Nucleic acid was extracted and assayed on TAC using TaqMan Fast Virus 1-Step kit (Life Technologies, Carlsbad, CA) on a ViiA 7 Real time system. Validation of the pathogen specific assays included evaluation of the linearity and range, limit of detection, sensitivity and specificity of each assay with pathogen specific nucleic acids. Transcripts of recombinant plasmids were developed to serve as positive controls for the assays. Each assay displayed linear amplification of the target nucleic acid, detected 5 copies or less of target genomes, and was specific for its target pathogen. This TAC will be deployed and evaluated as a rapid screen for organisms associated with CNS infection in surveillance and/or clinical care settings.

1065

ETIOLOGIES OF ACUTE FEBRILE ILLNESS AMONG ADULTS ATTENDING OUTPATIENT CLINICS IN DAR ES SALAAM, TANZANIA

Noémie Boillat[1], Samaka Josephine[2], Zainabu Mbarack[3], Tarsis Mlaganile[4], Teckla Kazimoto[5], Blaise Genton[6], Valérie D'Acremont[6]

[1]University Hospital of Lausanne, Lausanne, Switzerland, [2]Amana District Hospital, Dar es Salaam, United Republic of Tanzania, [3]Mwananyamala District Hospital, Dar es Salaam, United Republic of Tanzania, [4]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [5]Ifakara Health Institute, Bagamoyo, United Republic of Tanzania, [6]Swiss Tropical and Public Health Institute, Basel, Switzerland

Fever is one of the most frequent cause of attendance at outpatient level. Beyond malaria, little is known about etiologies of fever in adults which urge clinicians to overprescribe antimicrobials. We aimed at investigating precise causes of acute febrile episodes in adults attending outpatient clinics in urban Tanzania. Consecutive patients >18 years with tympanic temperature >37.5°C were recruited. Detailed medical history and clinical examination were done and blood taken to perform rapid tests for malaria, Dengue, Typhoid, HIV, Cryptococcus, Tuberculosis, Streptococcus A, Syphilis and rota/adenovirus, as well as blood cultures, serological and molecular analyses. All had nasal/throat swabs taken for PCR. Chest X-rays were performed when WHO criteria for clinical pneumonia were met. Urine culture, stool culture and other investigations were performed according to pre-defined algorithms. All final diagnoses were based on pre-defined criteria. 400 patients out of a total of 500 have been recruited up to April 2014. Preliminary results showed that 36% were HIV infected

(prevalence in the general population: 12%). Causes of fever (prior to serologies/PCR results) were: 41% acute respiratory infections (16% URTI, 10% tuberculosis, 7% radiological pneumonia, 3% Pneumocystis jiroveci (PCP), 3% tonsillitis, 2% COPD exacerbation), 9% typhoid, 7% occult bacteremia, 4% malaria, 4% urinary tract infection, 3% diarrhea, 3% sexually transmitted infections (STI), 2% chickenpox, 2% Cryptococcus, 1% skin infection, and 24% unknown at this stage. In the subgroup of patients with severe illness (of which 47% were HIV infected), radiological pneumonia, diarrhea and occult bacteremia were overrepresented, while in the subgroup of HIV infected patients with illness of any level of severity, it was the case for tuberculosis, PCP, STI and Cryptococcus. These results provide for the first time an accurate picture of the diversity of causes of fever in African adults. Results of molecular analyses will provide further insight on respective contribution of bacteria versus viruses, a critical issue to design decision charts for the appropriate management of fever and rational use of antibiotics.

1066

CHILDHOOD MORTALITY IN COMMUNITIES TREATED WITH AZITHROMYCIN FOR TRACHOMA CONTROL IN NIGER

Nicole E. Stoller[1], Abdou Amza[2], Kadri Boubacar[2], Nassirou Beido[2], Sun N. Yu[1], Jeremy D. Keenan[1], Bruce D. Gaynor[1], Sheila West[3], Thomas M. Lietman[1]

[1]University of California San Francisco, San Francisco, CA, United States, [2]PNSO, Niamey, Niger, [3]Johns Hopkins University, Baltimore, MD, United States

Childhood mortality in communities treated with azithromycin for trachoma control in Niger Trachoma control programs utilize mass azithromycin distributions to treat ocular *Chlamydia trachomatis* as part of an effort to eliminate this disease world-wide. Antibiotics are provided to target the ocular strains of chlamydia that cause trachoma, but may also have some effect against diarrhea, upper respiratory infections, and malaria_frequent causes of childhood mortality in trachoma-endemic areas. One previous trial in Ethiopia suggested that childhood mortality was reduced in communities treated with oral azithromycin. Here we monitor childhood mortality in a large mass treatment trial in Niger. We conducted a community randomized clinical trial for trachoma from April 2010 - August 2013. Communities were selected from 6 health centers (Centre de Santé Intégrée or CSIs) and were eligible for inclusion if they had an estimated total population of 250 to 600 persons, generally encompassing 50 to 100 children. 48 communities were randomly assigned to annual treatment of the entire community or biannual treatment of children only. Deaths of children under age 5 were identified and trained interviewers conducted verbal autopsies in Hausa, the local language, with the next of kin using the 2007 WHO standard verbal autopsy questionnaire in order to determine the cause of death. A total of 351 of 2632 children under age 5 (13.4%, 95% CI 11.6% - 15.3%) died in the communities treated annually; whereas 287 of 2493 children (11.4%, 95% CI 10.1% - 12.7%) died in the communities treated biannually (p=0.07). Thus, fewer deaths were reported in the biannually treated communities, although the difference was not significant. Verbal autopsies have been conducted and data from the two treatment arms will be compared during Summer 2014.

1067

INCIDENCE OF NON-MALARIA FEVERS IN A HIGH MALARIA ENDEMIC AREA OF GHANA

Kwaku P. Asante[1], Seth Owusu-Agyei[1], Mathew Cairns[2], Daniel Dodoo[3], Ellen A. Boamah[1], Grace Manu[1], Mieks Twumasi[1], Richard Gyasi[4], George Adjei[1], Ben Gyan[3], Emmanuel Mahama[1], Seeba Amenga-Etego[1], David K. Dosoo[1], Kwadwo Koram[3], Brian Greenwood[2], Daniel Chandramohan[2]

[1]Kintampo Health Research Centre, Brong Ahafo Region, Ghana, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana, [4]Department of Pathology, University of Ghana Medical School, College of Health Sciences, Accra, Ghana

With the role out of diagnostic tests that allow the rapid diagnosis of malaria in febrile patients, the need for a better understanding of the epidemiology of non malaria fevers (NMF) is now increasingly important. In this study, we investigated some of the risk factors associated with NMF in a malaria endemic area of Ghana. A prospective birth cohort study was carried out among 1855 newborns between 2008 and 2011; 4028 episodes of NMF were detected during one year of follow up. Diagnoses in infants with NMF included illnesses associated with respiratory (43.9%), gastrointestinal (32.6%), or cutaneous (12.8%) systems. The incidence of all episodes of NMF (first and subsequent) was 1.55 per child- year (95% CI 1.51, 1.60). Infants born in households of lower socio-economic status experienced a higher incidence of NMF compared with those from households of higher socio-economic status [adjusted hazard ratio 1.33 (95% CI 1.14, 1.56), [P<0.01]. The incidence of NMF was higher in infants from rural communities compared to those from urban areas [adjusted hazard ratio 1.33 (95% CI 1.02, 1.32), [P=0.02]. Similarly, the incidence of NMF was higher among infants living further from health facilities compared with those living close to health facilities [adjusted hazard ratio 1.24 (95% CI 1.12, 1.38), P<0.01]. Placental malaria was not associated with the incidence of NMF [adjusted hazard ratio 0.97 (95% CI 0.88, 1.07), P=0.49]. The incidence of NMF is high in this region of Ghana, especially in poor, rural communities.

1068

EFFECT OF ALENDRONATE ON SERUM GHRELIN LEVEL IN OSTEOPOROTIC POSTMENOPAUSAL WOMEN

Zahraa W. Al-Nuiemi, **Nahla O. Tawfik**

Department of Dental Basic Sciences, College of Dentistry, University of Mosul, Mosul, Iraq

Osteoporosis is a systemic skeletal disorder where net bone resorption exceeds bone formation and is characterized by low bone mass and density and micro-architectural deterioration of bone tissue. Osteoporosis leads to increased bone fragility with a consequent increase in fracture risk. Ghrelin a 28-amino acid peptide hormone that is synthesized primarily in the stomach and released during fasting, is an osteoblast mitogen that may regulate the activity of human bone cells. This study was designed to evaluate the effect of an anti-resorptive drug alendronate, a nitrogen containing bisphosphonate, on serum ghrelin levels. Twenty-three postmenopausal women with mean age, 64±8.3 years and diagnosed as osteoporotic patients based on bone mineral density (BMD) measurements using Dual X-ray Absorptiometry (DXA). The study was conducted at the Ibn Seena teaching hospitals in Mosul, Iraq. Patients were treated with alendronate tablets (PMS-Alendronate, Canada) 70mg once weekly and followed for three months between November 2011 and March 2012. Serum ghrelin hormone concentration was measured pre- and post-treatment using a commercially available Enzyme-Linked Immune Sorbent Assay (ELISA) diagnostic kit (MyBiosour, USA). The results showed that three months post treatment with the drug alendronate led to a statistically significant (p>0.05) increase of 21.4% in the basal serum level of ghrelin peptide hormone and that there was a significant inverse

association of ghrelin with body mass index (BMI). The implication of increased serum ghrelin levels on osteoporosis in postmenopausal women is being further investigated.

1069

BAYESIAN GEOSTATISTICAL MODEL-BASED ESTIMATES OF GEOSPATIAL DISTRIBUTION OF SOIL-TRANSMITTED HELMINTHIASIS AND ALBENDAZOLE TREATMENT REQUIREMENTS IN NIGERIA

Uwemedimo F. Ekpo[1], Akinola S. Oluwole[1], Dimitrios-Alexios Karagiannis-Voules[2], Eniola M. Abe[3], Franca Olamiju[4], Sunday Isiyaku[5], Chukwu Okoronkwo[6], Yisa Saka[6], Obiageli J. Nebe[7], Ekanem I. Braide[3], Chiedu F. Mafiana[8], Jurg Utzinger[2], Penelope Vounatsou[2]

[1]Federal University of Agriculture, Abeokuta, Nigeria, [2]Swiss Tropical and Public Health Institute, Basel, Switzerland, [3]Federal University, Lafia, Lafia, Nigeria, [4]Mission to Save the Helpless (MITOSATH), Jos, Nigeria, [5]Sightsavers, Kaduna, Nigeria, [6]Federal Ministry of Health, Abuja, Nigeria, [7]Federal Ministry of Health, Abeokuta, Nigeria, [8]National Universities Commission, Abuja, Nigeria

The control of soil-transmitted helminthiasis (STH) in Nigeria, using preventive chemotherapy, has become imperative in the light of global fight against Neglected Tropical Diseases (NTD). We provide for the first time Bayesian model-based risk maps to facilitate planning, targeting of control activities and surveillance. Disease prevalence data were derived from National STH surveys carried out in 2011. The data were geo-referenced and collated in a geographical information system (GIS) database for the generation of STH point prevalence maps. Bayesian geostatistics methods using advanced variable selection with remotely sensed environmental covariates, was used to model the spatial risk of STH for Nigeria. STH is currently endemic in 20 of 36 states of Nigeria including Abuja. Infections were found in 513 unique locations out of 555 different survey locations. Hookworm infection was found in 482 (86.8%) locations covering 20 states, ascariasis is endemic in 305 (55%) locations in 16 states and trichiuriasis is endemic in 55 (9.9%) locations in 12 states. ascariasis and hookworm infection are co-endemic in 16 states, while the three species are co-endemic in 12 states. The prevalence range for ascariasis was 1.6% to 77.8%, 1.7% to 51.7% for hookworm and 1.0% to 25.5% for trichiursis. Model-based predicted prevalence of ascariasis, ranged from 0.1% to 82.6% with a mean prevalence of 2.9% (95% confidence interval (CI): 2.90-2.93%), while hookworm infection ranges from 0.7 to 51.0% with a mean prevalence of 7.9% (95% confidence interval (CI): 7.86-7.91). Land surface temperature and dense vegetation are the significant covariate influencing the spatial distribution of STH in Nigeria. Prevalence estimates adjusted for school-aged children in 2011, showed that ascariasis is <10% in all the 36 states including Abuja, while hookworm infection is >10% in 8 states and <10% in 29 states. The model estimated that 40.1 million school-aged children are at risk of STH in Nigeria, requiring 80.2 million albendazole tablets annually for treatment. These maps and its associated predictions would help in accelerating the control of STH in Nigeria.

1070

PODOCONIOSIS PATIENTS' WILLINGNESS TO PAY FOR TREATMENT SERVICES IN NORTHWEST ETHIOPIA: POTENTIAL FOR COST RECOVERY

Abreham Tamiru Alemayehu[1], Girmay Tsegay[2], Moges Wubie[2], Molla Gedefaw[3], Sara Tomczyk[4], Fasil Tekola-Ayele[5]

[1]International Orthodox Christian Charity, Debre Markos, Ethiopia, [2]School of Public Health, College of Medicine and Health Sciences, Debre Markos University, Debre Markos, Ethiopia, [3]GAMBY Medical Sciences College, Bahir Dar, Ethiopia, [4]Institute of Tropical Medicine, Antwerp, Belgium, [5]Center for Research on Genomics and Global Health, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, United States

Podoconiosis is non-filarial elephantiasis (neglected tropical disease) of the lower legs. It is more commonly found in tropical Africa, Central and South America, and northwest India. In Ethiopia, a few non-governmental organizations provide free treatment to podoconiosis patients, but sustainability of free treatment and scale-up of services to reach the huge unmet need is challenged by resource limitations. We aimed to determine podoconiosis patient's willingness to pay (WTP) for a treatment package (composed of deep cleaning of limbs with diluted antiseptic solution, soap, and water, bandaging, application of emollient on the skin, and provision of shoes), and factors associated with WTP in northwestern Ethiopia. A cross-sectional study was conducted among randomly selected untreated podoconiosis patients (n=393) in Baso Liben woreda, northwestern Ethiopia. The contingent valuation method was used with a pre-tested interviewer-administered questionnaire. The majority of podoconiosis patients (72.8%) were willing to pay for treatment services. The median WTP amount was 64 Birr (US$ 3.28) per person per year. More than one-third of patients (36.7%) were willing to pay at least half of the full treatment cost and 76.2% were willing to pay at least half of the cost of shoes. A multivariate analysis showed that having a higher monthly income, being a woman, older age, being aware of the role of shoes to prevent podoconiosis, and possession of a functional radio were significantly associated with higher odds of WTP. The considerable WTP estimates showed that podoconiosis treatment could improve sustainability and service utilization. A subsidized cost recovery scheme could reduce treatment costs and more feasibility integrate podoconiosis treatment service with other NTDs and the government's primary health care system.

1071

PREDICTORS OF NON-COMPLIANCE WITH MASS DRUG ADMINISTRATION FOR SCHISTOSOMIASIS CONTROL IN WESTERN KENYA - THE SCORE PROJECT

Martin O. Omedo[1], Michael Ogutu[1], Rosemary Musuva[1], Isaac O. Onkanga[1], Alphonce Awiti[1], David Sang[2], Susan P. Montgomery[3], Evan W. Secor[3], Pauline N. Mwinzi[1]

[1]KEMRI/Centers for Disease Control and Prevention, Kisumu, Kenya, [2]Maseno University, Kisumu, Kenya, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

Mass drug administration (MDA) is being used to control schistosomiasis and other neglected tropical diseases of public health significance. However, achieving optimal community participation during implementation remains challenging. When a critical proportion of the population fails to participate in MDA, a potential reservoir for the parasite is left untreated, opening the opportunity for resurgence of the infection and reducing the probability of successful transmission control. This study was designed to identify predictors of non-compliance with MDA in western Kenya in villages with ≥25% prevalence of schistosomiasis to develop more effective health educational and delivery strategies. We used a population based unmatched case-control study design nested within a cross sectional household survey employing a structured questionnaire administered to 550 heads of households. Both univariate and multivariate analyses were used to identify the independent predictors of non-

compliance. Two hundred and forty respondents (44.9%) reported being non-compliant. By univariate analysis, non-compliance was significantly associated with the household head not asking the community health workers (CHWs) questions about the program, crude odds ratio (COR) 10.3, 95% CI [6.61-15.93], not having heard about the program COR 2.4 [1.6-3.6], and low schistosomiasis risk perception COR 3.1 [1.89-5.03]. In a logistic regression model, the odds of being non-compliant significantly increased amongst household heads who perceived their CHW not to be doing good work during the MDA exercise; adjusted odds ratio (AOR) 4.9, 95% CI [1.82-13.74], heads of households who lacked knowledge about schistosomiasis control methods AOR 7.5 [3.3-16.8], and those who did not know how the CHW was selected AOR 2.5 [1.3-5.0]. In order to improve compliance with praziquantel MDAs, effective strategies should be identified to ensure CHWs are well-trained and supervised to ensure quality service provision. Health education is also necessary to increase the knowledge levels of the disease in the community.

1072

THESE PEOPLE HAVE USED US AS RUBBER STAMPS: QUALITATIVE DESCRIPTION OF COMMUNITY PARTICIPATION IN WATER AND SANITATION ACTIVITIES IN THE CONTROL OF BILHARZIA IN NYALENDA B, AN INFORMAL SETTLEMENT IN KISUMU CITY, WESTERN KENYA

Rosemary M. Musuva

Maseno University, Kisumu, Kenya

The burden of disease caused by schistosome and soil-transmitted helminthes (STHs) infections is recognized as a major public health problem .A key ingredient to the success of the control interventions is community participation, which not only enhances the control efforts, but also guarantees their sustenance. Despite evidence from Community Directed Intervention (CDI) studies that community participation has brought great successes many policy makers and implementers have often neglected this aspect. Eight key informant interviews (KIIs) and eight focus group discussions (FGDs) categorized by gender and age were conducted. In addition, data on NGOs dealing with water and sanitation activities in Kisumu was collected from the NGO registration Board (Kisumu office). Qualitative data was organized into themes and concepts and analyzed using Atlas.ti. Most participants felt that project implementers did not involve them in key levels of project implementation. This in turn led to unsustainable projects and unacceptance from the community. Participants identified structures in the community that could be used as avenues of engaging the community in improving water and sanitation, for instance use of organized groups such as youth groups, gender based groups, farmers groups, merry-go rounds, and HIV support groups. Factors mentioned that hinder community participation included negative attitude from community members, poor monitoring and evaluation strategies, limited disclosure of project details to community members, and over-dependence from the community. Poor drainage systems, low latrine coverage, broken pipes and leakage of the sewerage systems were the leading factors associated with poor water and sanitation conditions. For effective community participation in water and sanitation activities, a multi-pronged paradigm is required that incorporates change of attitude from the community, information sharing and consultation, improved monitoring and evaluation, transparency and accountability.

1073

DETERMINING THE RISK OF TRANSMISSION OF MALARIA AND LYMPHATIC FILARIASIS IN A POST-MASS DRUG ADMINISTRATION SETTING IN CHIKWAWA DISTRICT, RURAL SOUTHERN MALAWI

Justin Kumala[1], Themba Mzilahowa[2], Square Mkwanda[3], Louise Kelly-Hope[1], Lisa J. Reimer[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Malaria Alert Centre, Blantyre, Malawi, [3]Ministry of Health, Lilongwe, Malawi

Malawi is a lymphatic filariasis (LF) and malaria co-endemic country where these diseases also share the same vectors *Anopheles gambiae* and *Anopheles* funestus. A high transmission focus for LF occurs in the rural southern part of the country, Chikwawa district with a microfilaria (MF) baseline prevalence of 35.9%. Here, five annual rounds of mass drug administration (MDA) of anthelminthic drugs were completed in 2012, a WHO recommended strategy to interrupt LF transmission. Long lasting insecticide-treated bednets (LLINs) for malaria prevention were also rolled out by the ministry of health. In our first study, we did a survey of LF prevalence, LF-associated morbidity, MDA and LLIN coverage in a small geographical area in Chikwawa district ten months after the fifth round of MDA was distributed by the Ministry of Health. 795 individuals were surveyed from six villages with an antigen prevalence of 15% (12.4% in females, 20% in males, p = 0.006). Prevalence of adult filarial worm antigen measured using rapid ICT cards ranged 4.1 - 38.5% and the prevalence of night blood microfilaria (MF) was estimated to range 0-7.5%. Median MF density was 4 MF/20 µL (range: 0.3-58.5 MF/20 µL). Self-reported MDA coverage in the fifth round ranged 69.2-90.2% and household LLIN coverage ranged 74.5 - 92.2%. 99% of 665 people that owned at least a net reporting usage the previous night before the survey. MF prevalence dropped from baseline 35.9% to range 0-7.5% after MDA,attributed to MDA and LLIN scale up. It is unknown whether the relatively low MF densities observed are sufficient to sustain transmission. To investigate this, in May 2014, we shall randomly sample 40 households for a questionnaire survey and mosquito surveys using CDC light trap and human landing catch methods to check the prevalence of LF and malaria in mosquitoes using PCR. The questionnaire will allow us to determine any gaps in intervention coverage, identify reasons for non-compliance and identify associated household risk factors for mosquito biting and LF and malaria transmission. Results will be due end of July 2014.

1074

DIGITIZED DATA FROM RURAL AFRICA: A FIELD APPLICATION OF OPEN DATA KIT (ODK) TO COLLECT BASELINE HEALTH DATA IN EASTERN SUDAN, NEW HALFA LOCALITY

Mohammed Jalaleldeen Abdulgadir, Tomonori Hoshi, Kenji Hirayama, Satoshi Kaneko

Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki, Japan

The collection of health survey data is often a challenge in developing countries. Although there are some of available health data in these countries, many are often not enough for research purposes or useful as a basis for health interventions. Hence, usually, when researchers or organizations grasp of health needs in target areas, health survey data need to be collected before meaningful intervention. Recently, a free and open-source set of tools named Open Data Kit (ODK) developed by a research team in the University of Washington is available. In brief, the ODK simplifies three steps of data collections: designing a questionnaire form, data collections with Android devices, and aggregating collected data. Herein we present our field application of ODK in Sudan to collect baseline health data for Schistosomiasis, Leishmaniasis and human immunodeficiency virus and the acquired immune deficiency syndrome (HIV/AIDS). We conducted the study in New Halfa, Eastern Sudan, and randomly selected 10 villages. Basically, 10 households (HH) per village

were randomly selected using the expanded programme of immunization (EPI) sampling method developed by the World Health Organization. We administrate questionnaire to members in each HH. The study was conducted between 22 February and 3 March 2014. We visited 100 HHs, and administrated 485 questionnaires. Majority of HHs had pit latrines or better toilet facilities (n=83), but 17 HHs did not have any such facilities. Self-reported Schistosomiasis prevalence was 21.6% (n=105), and 16 members experienced blood urine. Self-reported Leishmaniasis cases were few (n=1) although only 17.3% of residents used bednet during the previous night of the survey for malaria and Leishmaniasis prevention. Regarding HIV, many participants were unaware of Voluntary Counseling and Testing (VCT) cervices in their area (89.6%, n=435). We showed the results of health data collections using the ODK and EPI sampling method. This combination made it easy to collect data in a short period. We believe that ODK is a quite useful tool for data collections in a country with a poor health system such as Sudan.

1075

OVERALL PERFORMANCE OF THE INTERNATIONAL TRACHOMA INITIATIVE 1999-2012

Huub C. Gelderblom, Rebecca Mann-Flueckiger, Colin L. Beckwith, Paul M. Emerson

Emory University, Atlanta, GA, United States

Trachoma is the world's leading infectious cause of blindness. The WHO recommended approach for trachoma control is the comprehensive SAFE strategy (Surgery-Antibiotics-Facial cleanliness-Environmental improvements, e.g., access to safe water, latrine building), coordinated by the WHO Alliance for Global Elimination of blinding Trachoma by the year 2020 (GET 2020). More than 20 organizations in the International Coalition for Trachoma Control (ICTC) work together to assist countries. The International Trachoma Initiative (ITI) coordinates the 'A' component of SAFE, the donation of azithromycin (Zithromax®, Pfizer) to countries for annual mass drug administration (MDA). We reviewed ITI's performance since the start of the program. From 1999 to 2012, 24 countries conducted MDA with Zithromax® for ≥1 years, increasing from 2 countries in 1999, to 20 in 2012. In total, 291,105,608 doses of Zithromax® were distributed, increasing from 677,401 in 1999 to 47,911,058 in 2012. Collection of 2013 distribution data is ongoing, 6 additional countries were scheduled to start MDA in 2013 (Central African Republic, Chad, Guatemala, Guinea, Mozambique, Solomon Islands). 7 countries (Ghana, Iran, Morocco, Myanmar, Oman, the Gambia and Vietnam) have reported to WHO the achievement of the ultimate intervention goals. ITI has been working on fine-tuning and streamlining procedures, to increase transparency, and make it easier to apply for and distribute Zithromax®, while maintaining quality standards and controls. We anticipate a shift from reporting process to reporting impact, with more detail down to the district level. Challenges encountered include conflict and post-conflict situations, resource limitations in endemic countries, uncertainty about disease burden (currently being assessed through the Global Trachoma Mapping Project), costs and need-to-treat. The trachoma community is developing diagnostics and strategies for MDA impact surveys, MDA stopping decisions, and post-MDA surveillance. Coordination with the 'S', 'F' and 'E' components of SAFE is needed. ITI has become a successful drug donation program, and a partner among partners in the ICTC. The progress towards GET 2020 is encouraging, but further upscaling is needed to eliminate blinding trachoma by 2020.

1076

NEGLECTED TROPICAL DISEASE (NTD) COMMUNICATIONS WORKSHOPS FOR HEALTH PROFESSIONALS AND JOURNALISTS IN NTD ENDEMIC COUNTRIES, TACKLING FEAR AND NEGLECT, SHARING KNOWLEDGE, BUILDING BRIDGES

Elizabeth Kurylo[1], Geoffrey Knox[2], Lisa Rotondo[3], Mawo Fall[3], P. J. Hooper[1], Phil Downs[3], Danny Haddad[1], **Huub C. Gelderblom**[1]

[1]Emory University, Atlanta, GA, United States, [2]Geoffrey Knox & Associates, New York, NY, United States, [3]RTI International, Washington, DC, United States

Informing the public and policy makers about NTDs is a task shared by health professionals and the media. Interaction between these groups is essential, yet often limited and difficult. Not enough and/or incorrect information reaches target audiences. NTD programs have been challenged by misinformation and rumors. We addressed these issues by organizing dedicated NTD communications workshops for health professionals and journalists in NTD endemic countries. The workshops were meant to 1) help health professionals build skills needed to effectively engage with and inform the media, the public and policy makers about NTDs, and 2) to inform journalists about NTDs and the health professionals that work on NTDs, and explain how, why, and on what the public should be informed. The aim is to build rapport and trust, increase awareness and support, reduce misinformation, and reduce the fear that many health professionals have of engaging with the media. From 2010 to 2013, we conducted 5 workshops in 4 NTD endemic countries. Local communications companies and NGOs were involved in planning and organizing. Health professionals and journalists first worked separately, reviewing their own communications, advocacy and/or reporting work, to identify and develop key messages, and identify key audiences (public, donors, media). Health professionals practiced media interviews that were videotaped and played back with critiques. The 2 groups then engaged with each other, and went on a joint field visit. Health professionals and journalists began to appreciate each other as partners. The workshops and field visits led to several local publications about NTDs. Health professionals said that learning how to speak simply and clearly about NTDs was very useful. We are planning NTD communications workshops in additional NTD endemic countries. This model, and the lessons we learned during development, may be useful for other disease programs. We will create a toolkit that countries can use for advocacy and NTD communications plans, combined with training and/or remote technical assistance.

1077

ASSESSMENT OF SUPPLY CHAIN MANAGEMENT (SCM) SYSTEMS FOR NEGLECTED TROPICAL DISEASE (NTD) DRUGS IN CAMEROON, MALI, TANZANIA AND UGANDA

Gregory G. Simon, Gabriel Daniel, Kwesi Eghan

Management Sciences for Health, Arlington, VA, United States

The rapid expansion of NTD control activities has not been without pharmaceutical and health system challenges. The purpose of the four country assessment of the pharmaceutical and supply chain management of NTD control programs was to identify the gaps and make recommendations for strengthening the system. Qualitative and quantitative information concerning NTD medicine availability and management were collected using structured assessment tools, interviews of healthcare workers, and direct observations at sample sites at national, regional, districts and peripheral facilities. The questionnaires focused on availability of treatment guidelines, procurement, distribution, and stock management practices, as well as the adverse drug reaction reporting, disposal of expired products, reverse logistics challenges following MDA and integration with relevant national services and structures. Along the supply chain several NTD medicines were not documented in certain depots due to poor stock management practices. Donated NTD medicines sourced from the manufacturers makes quality issues less of a concern. However, there are concerns on the downstream quality of NTD medicines

as the medicines come in loose pills and are distributed from containers. The outdoor nature of the MDA's increases the chances of exposure of the medicines to humidity, heat, dust, and other unhygienic situations. NTD programs had difficulty getting information on the actual amounts of medicines distributed by the national medical store to the districts. Likewise, information on persons treated, doses dispensed and balance of stock medicine from MDA sites and districts is not always submitted on time and the quality of data not reliable, making planning and forecasting difficult. The assessment identified both strengths and weaknesses in the different aspects of NTD pharmaceutical management. Following the assessment, two post-assessment workshops were conducted for the purpose of disseminating the assessment report and to reach consensus with key stakeholders and partners on the way forward.

1078

SIGNIFICANT VARIATION OF SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHS PREVALENCE ACROSS ECOLOGICAL AREAS IN NORTHERN BENIN: CALL FOR GENDER FOCUS AND LOCALIZED INTERVENTIONS

Moudachirou Ibikounlé[1], **Jean Jacques Tougoue**[2], Wilfrid Batcho[3], Mariam Sani- Lamine[4], Yolande Sissinto-Savi de Tovè[5], Abdoulaye Daré[3], Dorothee Kindé-Gazard[5], Achille Kabore[2]

[1]Département de Zoologie, Faculté des Sciences et Techniques, Université d'Abomey-Calavi, Cotonou, Benin, [2]RTI International, Washington, DC, United States, [3]Programme National de Lutte contre les Maladies Transmissibles, Ministère de la Santé, Cotonou, Benin, [4]Ministère de la Santé Publique, République du Niger, Niamey, Niger, [5]Laboratoire de Parasitologie-Mycologie, Faculté des Sciences de la Santé, Cotonou, Benin

Schistosomiasis and soil-transmitted helminthiasis (STH) infections are widespread in sub-Saharan Africa and constitute a public health problem in Benin, but limited data are available on prevalence among vulnerable populations. To address this gap, a survey was conducted to determine the prevalence of schistosomiasis and STH among schoolchildren from eight communes in northern Benin. Parasitological investigations were conducted in May 2013. Urine and stool samples were taken from 850 schoolchildren ages 8 to 14 years in 17 selected primary schools from eight communes within three ecological areas (Departments), using appropriate techniques, i.e. urine filtration and Kato-Katz. The results showed that *S. haematobium* was the most prevalent schistosome species (42.59%), followed by *Schistosoma mansoni* (2.24%). Hookworm was the most prevalent STH, with a mean prevalence of 20.71%, followed by *Ascaris lumbricoides* (3.76%) and *Enterobius vermicularis* (0.59%). There was a significant variation of schistosomiasis and STH prevalence across schools, and ecological areas. *S. haematobium* and hookworm were found in all communes surveyed, however *S. mansoni* was present in four communes with the highest prevalence in N'Dali (15.00%); *A. lumbricoides* was present in 3 communes and *E. vermicularis* was found only in the commune of Ouaké. Hookworm prevalence for males and females was high in the commune of Ouake (46.40% and 27.20% respectively) which also harbors a high prevalence of *S. haematobium* (58.40%). The moderate-to-high hookworm prevalence indicates a need for vigorous deworming campaigns with particular focus on women of child bearing age. Variation of schistosomiasis prevalence across villages in the same ecological zone (department) indicates the presence of highly focal schistosomiasis infection. This underscores the importance of localized interventions based on village-specific findings as opposed to extrapolating results from one village to the whole ecological zone.

1079

MULTIPLEX SEROSURVEYS AS A TOOL FOR INTEGRATED NEGLECTED TROPICAL DISEASES (NTD) SURVEILLANCE

Sammy Njenga[1], Henry Kanyi[1], Faith Mwende[1], Ryan Wiegand[2], E. Brook Goodhew[2], Jeffrey Priest[2], Vitaliano Cama[2], Yeuk-Mui Lee[2], W. Evan Secor[2], Patrick Lammie[2], Maurice Odiere[3], Kimberly Won[2]

[1]Kenya Medical Research Institute, Nairobi, Kenya, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Kenya Medical Research Institute, Kisumu, Kenya

A key strategy for control and elimination of five Neglected Tropical Diseases (NTDs): lymphatic filariasis (LF), onchocerciasis, schistosomiasis, soil-transmitted helminthiasis (STH), and trachoma is preventive chemotherapy (PC) through mass drug administration (MDA). The impact of MDA is usually monitored by parasitological assessments or clinical examination. There is often geographic overlap of NTDs, and populations in these areas are regularly exposed to a variety of other diseases. Disease control programs require routine monitoring and evaluation (M&E), but coordinated M&E is rarely conducted and can be a challenge. Antibody-based tests may facilitate integrated M&E. A multiplex bead assay (MBA) that detects antibody against multiple antigens using a single blood sample has been developed. A total of 935 serum samples collected from individuals (1-85 years) living in communities in Mbita district, western Kenya were tested for antibody responses to 36 antigens covering a variety of diseases including all five PC NTDs. This area in western Kenya is highly endemic for *Schistosoma mansoni* and at variable risk for STH, but is not believed to be at risk for LF, onchocerciasis or trachoma. It is also an area of intense malaria transmission. Antibody responses to two *Schistosoma* spp. antigens were significantly associated with intensity of *S. mansoni* infection assessed by stool examination ($p<0.001$). Although antibody responses to a *Plasmodium falciparum* antigen were not associated with blood film results, MBA results indicated high levels of infection or exposure to *P. falciparum*, but very low levels of antibody to *P. vivax*. Minimal levels of antibodies were detected for LF, trachoma and onchocerciasis. Additionally, antibody responses to tetanus toxoid were low, indicating insufficient coverage of routine vaccinations. Preliminary multiplex results are consistent with traditional measures such as stool examination and indicate the use of MBAs has the potential to provide an integrated platform for M&E in complex public health settings.

1080

HEALTH SYSTEMS MODELLING - DEMONSTRATING THE POTENTIAL IMPACT OF DIAGNOSTIC AND TREATMENT INTEGRATION OF HUMAN AFRICAN TRYPANOSOMIASIS USING DIFFERENT HEALTH SYSTEM STRUCTURES

Claudette S. Sutherland, Chris Stone, Fabrizio Tediosi

Swiss Tropical and Public Health Institute, Basel, Switzerland

Human African trypanosomiasis (HAT) is a Neglected Tropical Disease targeted for elimination. The declining prevalence of infection will change the demands on health systems to effectively detect cases. Detection of HAT currently relies on vertical surveillance programs where patients are identified in their villages and then required to travel long distances to HAT treatment centres (HTC). New diagnostics and interventions could change the future of service delivery of case detection and treatment; as local services would reduce out-of-pocket (OOP) expenditures and the inconvenience of travelling long distances. It is proposed that the integration of programs into the local health centres (LHC) could be modelled to forecast outcomes related to service delivery, patient accessibility, time spent in the system and resources used with current and new interventions. A discrete-event simulation (DES) health systems model has been developed using SIMUL8®. The model simulates patients' movement through the health system within a specified area. Different health system structures of both integrated (E.g. inclusion of local health centres) and non-integrated (E.g. vertical surveillance programs)

approaches were constructed in the model. Data from current and new diagnostic and treatments have been simulated through the model in order to measure the impact of switching from a non-integrated to integrated health system. Preliminary results suggest that integrated systems with new technologies will increase accessibility, decrease patient wait times but also require additional costs for training and for improving health infrastructures at the local level. An integrated health system could lead to improvements in coverage of treatment and reducing inequity in access to HAT treatment. While the initial additional costs of these interventions could be offset by savings in OOP payments, affordability to health systems should be carefully assessed. The analysis shows that health systems' modelling is an informative tool for investment decisions regarding an integrated approach.

1081

CO-ENDEMICITY OF *SCHISTOSOMA HAEMATOBIUM* WITH *S. MANSONI* AND SOIL TRANSMITTED HELMINTHS IN SOUTH NYANZA, WESTERN KENYA

Huldah Sang

Kenya Medical Research Institute, Kisumu, Kenya

Multiple parasite infections are believed to increase susceptibility to coinfection with other parasites, playing a vital role in the development of morbidity. There is a paucity of information on *Schistosoma haematobium* and polyparasitism in south Nyanza region, western Kenya. This cross-sectional survey determined the co-endemicity and distribution of *S. haematobium* with *S. mansoni* and STHs (*Ascaris lumbricoides* and *Trichuris trichiura*) among 3,487 children aged 7-18 years in 95 primary schools in south Nyanza, western Kenya. Helminth eggs were analyzed from single urine (for *S. haematobium*) and stool (for *S. mansoni* and STHs) samples by centrifugation and Kato-Katz, respectively. Schools and water bodies were mapped using Geographical information system and prevalence maps generated using ArcView GIS Software. Overall, 91.7% (95% CI = 89.9-93.2%) children were infected with a single parasite species, while 7.9% (95% CI = 6.4-9.6%) and 0.5% (95% CI = 0.2-1.1%) children harbored dual and triple species infection respectively. Prevalence of *S. haematobium* and *S. mansoni* dual coinfection with any other parasites was 16.9% (95% CI =13. 3-21.4%) and 19.1% (95% CI =15.8-23.1%) respectively. Of the four parasite species, only *A. lumbricoides* infections were positively associated with both *S. mansoni* (P = 0. 0026) and *S. haematobium* (P = 0. 0295) infections. *S. haematobium* - *S. mansoni* coinfections occurred near Kayuka pond and Kamenya dam in Rachuonyo district, Katumo, Osani and Wachara pond in Homabay district, and along the Ongoche River in Migori district. The prevalence of *S. haematobium* monoinfection (7.2%, 95% CI = 6.4-8%), *S. mansoni* monoinfection (12.3 %, 95% CI = 10.4-12.5%) and *S. haematobium*- *S. mansoni* coinfection (1.2%, 95% CI = 0.9-1.6%) was highly skewed, with less than 10% prevalence was observed in 78%, 63% and 97% of all the schools respectively. There was no significant difference in infection intensity between mono and coinfections. Although the overall distribution and prevalence of *S. haematobium* - *S. mansoni* coinfections was generally low, understanding their geographical distribution is important especially for disease control programs.

1082

DEVELOPMENT OF A MARKOV TRANSITION PROBABILITY MODEL TO PREDICT CHANGES IN SCHISTOSOMIASIS INFECTION FOLLOWING TREATMENT

Arminder K. Deol[1], Michael French[1], Deirdre Hollingsworth[2], Antonio Montresor[3]

[1]Schistosomiasis Control Initiative, London, United Kingdom, [2]University of Warwick, Coventry, United Kingdom, [3]World Health Organization, Geneva, Switzerland

The last decade has seen significant progress in the large-scale control of schistosomiasis and soil-transmitted helminth (STH) infections. Even greater expansion is required to achieve the coverage and morbidity reduction targets set for 2020. A crucial tool in this scale-up will be the ability to monitor the impact of control programmes. Specifically, being able to identify areas not responding to treatment as expected will allow adjustments to be made to the programme design and help ensure their longer-term success. However, to date, there are very few tools available that would allow the identification of such areas whilst at the same time being user-friendly. An STH Markov model developed recently at the World Health Organization (WHO) used data from Vietnam to predict changes in STH prevalence following successive rounds of deworming treatment. In addition, a user-friendly interface was also developed to help ensure the model is used as widely as possible by programme managers. Data collected by the Schistosomiasis Control Initiative and its country partners from several countries in sub-Saharan Africa have enabled the validation of this model for STH infection, its extension to include schistosomiasis infection, and the addition of robust confidence intervals around the predicted changes in prevalence. It is hoped that the output of this model could potentially provide an early warning of where treatment campaigns are not achieving their aims (for example due to poor coverage, adherence, or putative resistance) and enable programme managers to make the necessary changes to meet the expected targets. The performance of the model will be discussed, with particular reference to the utility of stratifying the model outputs by parasite species, location, underlying endemicity, and host age. In addition, we will discuss the results of a model comparison exercise between the predictive capacity of the Markov model and other models currently available.

1083

FEASIBILITY AND ACCEPTABILITY OF INTEGRATING FACE WASHING MESSAGES INTO ONGOING HAND WASHING CAMPAIGN FOR THE CONTROL OF NTDS: LESSONS FROM A SCHOOL-BASED PROGRAM IN TURKANA, KENYA

Laura Senyonjo[1], Edward Woods[2], Richard Wright[3], Katie Greenland[4], Anna Gerrard[1], Elena Schmidt[1]

[1]Sightsavers, Haywards Heath, United Kingdom, [2]Sightsavers, New Orleans, LA, United States, [3]Unilever, Wirral, United Kingdom, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom

Hand-washing is a key intervention in the prevention of a number of infectious diseases, including some Neglected Tropical Diseases (NTD). Improvements in facial cleanliness are also an important aspect of trachoma control, as outlined in the WHO endorsed SAFE strategy and both need to be delivered at scale in order to achieve the elimination of blinding trachoma by 2020. However, little is currently known about the best approach to improve face-washing practices and whether it is practical to integrate face-washing messages into current large scale hand-washing campaigns. This presentation aims to highlight the lessons learnt from the design and testing of an integrated face and hand-washing school based behaviour change campaign implemented in a water poor environment in Loima County, Turkana, Kenya. The intervention is an interactive school-based behaviour change programme integrating face washing messages into an on-going hand-washing campaign, delivered jointly by Sightsavers, Unilever and the London School of Hygiene and Tropical Medicine. The presentation will focus on the use of formative research in the design of face-washing messages and materials and on the assessment of their feasibility and acceptability to the local communities. The assessment of the pilot project will apply qualitative methodologies, including observations of hygiene practices both in school and in the community, participatory focus group discussions with children, caregivers and teachers and semi-structured interviews with key stakeholders. We will also present how the findings from this pilot work will be used to modify and scale up the proposed intervention across other NTD-endemic regions of Kenya and how we will evaluate its impact on improving and sustaining face-washing practices in these communities.

1084

EVALUATION OF INTEGRATED MASS DRUG ADMINISTRATIONS FOR NEGLECTED TROPICAL DISEASES IN MADAOUA DISTRICT, NIGER

Megan Klingler[1], Alaine K. Knipes[2], Salissou Adamou Bathiri[3], Mawuli Nyaku[2], Els Mathieu[2]

[1]Rollins School of Public Health, Emory University, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Niger Ministry of Health, Niamey, Niger

Neglected Tropical Diseases (NTDs) are a group of debilitating illnesses that affect the lives of more than one-sixth of the world's population. Preventive chemotherapy partnered with health education is the primary strategy in the control and elimination of NTDs. In December 2012, the Niger Ministry of Health carried out an integrated mass drug administration (MDA) with the goal of eliminating lymphatic filariasis (LF) and trachoma and controlling soil-transmitted helminthiasis (STH) and schistosomiasis. Six months after the MDA, a coverage and knowledge, attitudes and practices (KAP) survey was carried out in the Madaoua district, Niger using the WHO-recommended two staged probability cluster survey design. In each of the selected households, coverage data were collected from all persons, while the KAP survey was administered to one randomly-selected adult (≥14 years). A total of 293 households in 30 villages participated in the surveys, with 1711 persons interviewed for coverage and 291 adults for KAP. Overall, 80.2% (95% CI: 78.2-82.1) of those surveyed reported taking at least one medication; 75.5% of men and 83.7% of women reported taking a medication during the 2012 MDA. Surveyed coverage of Ivermectin (60.0%; 95% CI: 57.1-62.9), albendazole (71.3%; 95% CI: 69.0-74.3) and praziquantel (65.6%; 95% CI: 62.8-68.4) was significantly lower than the reported coverage (96.9%, 96.9%, and 86.0%, respectively), while the reported coverage for azithromycin (72.2%) was confirmed by the survey (71.8%, 95 CI: 69.3-74.1). KAP respondents reported that they had heard of LF (66.0%), STH (93.8%), schistosomiasis (72.2%) and trachoma (86.6%), but only 24.0%, 51.8%, 56.2% and 57.9%, respectively, knew at least one symptom. Of 46 respondents who had heard of LF, only 2 respondents (4.3%) knew it was transmitted via a mosquito, and of those who had heard of schistosomiasis, 70.9% believed that one is infected by the sun or heat. There was no significant association between participation in the MDA and knowledge of the NTDs. Knowledge of someone with a NTD did increase the odds of participation: men who knew someone with hydrocele were 4.3 times (95% CI: 4.1-4.4) more likely to take medication. MDA participation appeared not to be affected by the low level of knowledge. With the exception of Ivermectin, drug-specific coverage was adequate.

1085

DETERMINING WHERE TO MAP FOR TRACHOMA: LESSONS FROM UGANDA'S CLASSIFICATION TOOL

Harriet Namwanje[1], Patrick Turyaguma[2], Anne Heggen[3], Edridah Muheki Tukahebwa[4], Stella Agunyo[1], Ambrose Onapa[1]

[1]RTI International, Kampala, Uganda, [2]Ministry of Health Uganda, Kampala, Uganda, [3]RTI International, Washington, DC, United States, [4]Vector Control Division, Ministry of Health Uganda, Kampala, Uganda

Trachoma (Chlamydia trachomatis) is the leading cause of preventable infectious blindness. Ten million people are at risk in 36 of Uganda's 112 districts, with 1.1 million people estimated to be infected. After mapping all highly-suspected districts, the National NTD Control Program turned their focus on prevalence surveys in districts that shared a border with known endemic districts. Population-based prevalence survey (PBPS) is the WHO-recommended methodology for trachoma baseline surveys, but cost, time, and logistics prohibit execution of these surveys. In Uganda, the estimated cost of a two-week PBPS is $11,000 per district. Instead of immediately conducting PBPS, the national program used a trachoma classification tool in 8 districts in Eastern Region in order to prioritize where a PBPS should be conducted. Open-ended interview guides were developed to conduct key informant interviews at regional and district health facilities. A tool was developed in line with the Health Management Information System (HMIS) health unit database to collect clinical record data. Districts were classified as suspected if they met each of the following criteria: more than 10 cases of TT reported per year for two or more years; at least 3 health staff at 5 or more health facilities identifying TT as a problem in the catchment area; a shared border with an endemic district (TF in children 1-9 years old ≥10%). In each district, the classification tool cost $1000 per district and took 3 days for a team of 4 people to complete the methodology. Two districts met all three criteria and were classified as suspected; these districts were prioritized for PBPS. Two more districts showed evidence of being endemic for trachoma. A recommendation was made to conduct PBPS in these four districts to validate the methods used in the classification tool. Final analysis is expected in May 2014. In districts with suspected low TF prevalence, this trachoma classification tool offers programs a simple way to prioritize districts in which to conduct the more resource-intensive PBPS.

1086

MARKED CHANGES IN MATERNAL PARASITIC INFECTIONS IN KWALE COUNTY (2006-2014)

Francis M. Mutuku[1], Indu Malhotra[2], A. Desiree LaBeaud[3], Jackson Muinde[4], Elton Mzungu[4], Peter L. Mungai[2], Dunstan Mukoko[4], Christopher L. King[2], Charles H. King[2]

[1]Technical University of Mombasa, Mombasa, Kenya, [2]Case Western Reserve University, Cleveland, OH, United States, [3]Childrens Hospital Oakland Research Institute, Oakland, CA, United States, [4]Division of Vector Borne and Neglected Tropical Diseases, Nairobi, Kenya

In the developing world, maternal pre-natal parasitic infections can place a high burden on pregnancy outcomes, including potential adverse effects on neonates. We evaluated changes in malaria, *Schistosoma haematobium*, hookworm, and *Trichuris trichiura* prevalence in two serial cohorts in the same population. One cohort was recruited in between 2006 and 2009 and the other between 2013 and 2014. Pregnant women were recruited from the antenatal clinic (ANC) at Msambweni District Hospital in Kwale County. For both cohorts, maternal venous blood was drawn at first ANC visit and maternal peripheral, cord, and placental blood were examined at delivery for malaria parasites by light microscopy. Maternal stool and urine samples were collected at the first ANC visit and at delivery. Stool samples were tested for hookworm and *T. trichiura* infections using Ritchie's concentration method. Urine was evaluated for presence of *S. haematobium* using Nuclepore filtration. Among the 4 infections evaluated, only malaria prevalence was observed to have increased both at ANC enrollment (60%) and at delivery (50%) during the interval between studies. By 2013-14, *T. trichiura* infection prevalence had increased at ANC enrollment (36%) but was decreased at delivery (72%). *S. haematobium* and hookworm prevalence decreased by 30% and 80%, and by 60% and 30%, respectively, at ANC enrollment and at delivery. The increased prenatal malaria burden was unexpected and may be an indicator of operational failures of the current malaria control efforts. On the contrary, the current soil transmitted helminths (STH) control efforts seem to be bearing fruit. This decline STH infection may be attributable to the ongoing national school-based de-worming programme. Similar population-based studies on parasitic infections are necessary for monitoring and evaluation of the current control measures.

1087

ANTIBODY-BASED MULTIPLEX TESTING AS A PLATFORM FOR INTEGRATED SURVEILLANCE OF NEGLECTED TROPICAL DISEASES AND OTHER INFECTIONS OF PUBLIC HEALTH INTEREST

Sonia Pelletreau[1], Amanda Barry[2], E. Brook Goodhew[1], Gretchen Cooley[2], Olga Amiel[3], Pedro Manuel Maulana[4], Augusto Magubeia[4], Patrick J. Lammie[1], Ricardo Thompson[4]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Lifesource Biomedical, Juneau, AK, United States, [3]Ministerio de Saude, Maputo, Mozambique, [4]Instituto Nacional de Saude, Maputo, Mozambique

Neglected Tropical Diseases (NTDs) are debilitating parasitic and bacterial diseases that affect 1.4 billion of the world's poorest people. Several NTDs are targeted for global elimination and control through programs centered on mass drug administration (MDA). An integrated surveillance strategy would be valuable to assess infection prevalence and program impact in areas of geographical overlap of NTDs. To investigate the utility of multiplex antibody assays for integrated disease and program surveillance, sentinel sites were established in two NTD-endemic, treatment-naïve communities in Morrupula district, Mozambique. Baseline serologic, parasitological and clinical indicators were assessed in a sample of individuals aged >12 months (n=1423) in 2013 prior to the first NTD treatment. Preliminary analysis has been conducted on 302 serologic samples that were tested by multiplex for antibody responses to a panel of 36 antigens for NTDs, malaria, water-borne and vector-borne parasites. Multiplex results demonstrate antibody responses against multiple NTDs and malaria, confirming the high prevalence of lymphatic filariasis (LF), *Schistosoma haematobium*, trachoma, *Ascaris lumbricoides* and *Plasmodium falciparum*. Antibody responses to antigens for LF (Wb123, Bm14, Bm33NS), trachoma (pgp3, CT694), and malaria (Pf MSP-1) were correlated with results from conventional diagnostic tests. Antibody responses in children to LF antigens were detected before ICT and microfilaria (Mf) responses, indicating that antibody provides a more sensitive measure of exposure. Multiplex analysis additionally provided novel observations on diseases that were not assessed using conventional tests and on which little or no data exist. High levels of reactivity to a number of vector-borne or water-borne pathogenswere detected. From these preliminary baseline analyses, we believe that antibody-based multiplex tests can provide important epidemiologic information about disease burden and actionable disease surveillance data for national health programs that includes but is not limited to data on NTDs.

1088

A SENSITIVE AND REPRODUCIBLE *IN VIVO* IMAGING MODEL IN MICE FOR LATE STAGE HUMAN AFRICAN TRYPANOSOMIASIS FOR EVALUATION OF ANTI-TRYPANOSOMAL DRUGS

Hollie Burrell-Saward

London School of Hygiene & Tropical Medicine, London, United Kingdom

In the past decade 3 pre-clinical murine models have been used for the evaluation of new drugs for stage II human African trypanosomiasis (HAT) involving the parasites *Trypansoma brucei brucei* GVR35 and AnTat1 and *T. brucei rhodesiense*. Although there are currently two novel drugs in clinical trials, there remains a need for proven treatments with potency, pharmacokinetic and safety profiles that are required for new HAT chemotherapy, not to mention a short treatment regimen. Incomplete knowledge of the metabolic status and drug sensitivity of trypanosomes in the central nervous system (CNS), and the long murine CNS model has hindered drug development. Here we report the generation of highly bioluminescent parasites and their use in an *in vivo* imaging model of stage II African trypanosomiasis. Bloodstream forms of the chronic model strain GVR35 were transformed with a construct designed to express "red-shifted" luciferase. Using the standard 21 day treatment model in CD1 mice, we were able to identify CNS infection after treatment with berenil. By using the bioluminescence model in a drug relapse experiment with a stage II drug, early relapse could be identified when no peripheral blood parasitaemia could be detected. We provide evidence that the model can be used to reduce the current 180-day experiment and also provide dose-response data.

1089

THE EFFECT OF CIVIL WAR ON CUTANEOUS LEISHMANIASIS ("ALEPPO BUTTON") IN ALEPPO CITY, SYRIA

Abdulnasser Kaadan

University of Aleppo, Aleppo, Syrian Arab Republic

In the ancient northern Syrian city of Aleppo, CL has been present for hundreds of years (if not longer), where it is known as the "Aleppo evil", "Aleppo ulcer", "Aleppo boil", or "Aleppo button" which is Cutaneous Leishmaniasis. Aleppo ulcer is a disfiguring condition that disproportionately occurs on the face, especially of young people. It typically lasts one or 2 years before the lesion heals spontaneously, and is often known locally as "one-year sore". However, in many cases specific anti-parasitic chemotherapy can hasten the healing process and improve clinical and cosmetic outcomes. A major problem with one-year sore is that the scar can produce permanent disfigurement of the face. It is well known about the rise and fall and then a rise again in the incidence of the disease in the city of Aleppo. During the 1950s the number of cases of CL fell after an insecticide campaign aimed at controlling malaria, but it then rose again during the 1960s. However, CL was mostly controlled during the 1980s.There is no doubt that the areas of Syria affected by the civil war are experiencing an increase in cutaneous leishmaniasis, and this will also be seen in the refugee camps in Jordan and Turkey. This is due to garbage collection, open sewage, and poverty which promote the habitats of Phlebotomus_ sandflies that transmit CL. Interestingly, a clinical trial conducted prior to the current civil conflict found that use of insecticide-treated bednets (ITNs) could prevent CL in Aleppo. Recently, WHO reports out of Syria indicate the emergence of epidemic cutaneous leishmaniasis in the besieged city of Aleppo, adding further to the misery there, perhaps the international community needs to focus on refugees and refugee encampments to ensure local control and patient access to treatments.

1090

TRIPLEX METHODOLOGY (FC-TRIPLEX-IGG1) FOR THE DIFFERENTIAL DIAGNOSIS OF CHAGAS DISEASE, LOCALIZED CUTANEOUS LEISHMANIASIS AND VISCERAL LEISHMANIASIS

Fernanda F. de Araujo[1], Fernanda F. Nunes[1], Roberta D. Rocha[1], Carolina R. Freitas[1], Stefan M. Geiger[2], Anna Barbara F. Proietti[3], Claudia L. Oliveira[3], Ester C. Sabino[4], Elenice M. Lemos[5], Andrea Teixeira-Carvalho[1], Olindo A. Martins-Filho[1]

[1]CPqRR-FIOCRUZ, Belo Horizonte, Brazil, [2]UFMG, Belo Horizonte, Brazil, [3]Hemominas, Belo Horizonte, Brazil, [4]Fundacao Pro-Sangue/Hemocentro de Sao Paulo, Sao Paulo, Brazil, [5]UFES, Vila Velha, Brazil

Conventional serological tests for Chagas disease (CD) are routinely used for blood banks screening. However, non-negative result for CD screening in blood banks does not guarantee the presence of *Trypanosoma cruzi* infection, since other infectious diseases, such as Leishmaniasis (LEISH) can contribute to the occurrence of false positives results. In this work, we present a nonconventional serological approach for the simultaneous detection of anti-*T. cruzi,* anti-*Leishmania chagasi* and anti-*L. braziliensis* IgG1, using a differential Alexafluor647-fluorescent parasite staining in a single flow cytometry platform. The method, named FC-TRIPLEX Chagas/Leish IgG1, uses the percentage of Phycoeritrin-fluorescent positive parasites (PPFP) as indicative of seropositivity upon the use of specific cut-off edges. The method is based on an inverted detuned algorithm applied starting with the analyses of anti-*L. chagasi* positivity at 1:32,000 (PPFP>60%) to define the diagnosis of Visceral Leishmaniasis (VL). Samples

with anti-*L. chagasi* reactivity <60% guide to the next algorithm step consisting of the analysis of anti- *T. cruzi* positivity at 1:2,000 (PPFP>50%) defining the diagnosis CD. Samples with negative anti- *T. cruzi* reactivity (PPFP<50%) are forwarded to the final algorithm step with the analysis of anti-*L. braziliensis* reactivity at 1:1,000 with PPFP>60% defining the Localized Cutaneous Leishmaniasis (LCL) diagnosis and PPFP<60% excluding these three Trypanosomatidae infections. A proof concept carried out using a range of well characterized sera samples from VL, CD and LCL showed correct results in 76 out of 80 tested samples reaching outstanding global accuracy and 95% of overall performance of FC-TRIPLEX Chagas/Leish IgG1 array. The occurrence of 5% of incorrect diagnosis (false negative results in 1/20 CD and 1/20 LCL and false positive results in 2/20 non-infected carriers) underscore the remarkable performance of the methodology. Alexafluor-647 differential brightness as well as the parasite antigenicity were preserved up to one year at distinct storage conditions (RT, 4°C and -20°C). In conclusion, our data suggest that the outstanding performance of FC-TRIPLEX Chagas/Leish IgG1 array for the differential diagnosis of CD and LEISH shall contribute to the elucidate the false positives results frequently observed in conventional tests currently used for serological screening in blood banks.

1091

DEVELOPMENT OF ORAL EFLORNITHINE CHEMOTHERAPY FOR HUMAN AFRICAN TRYPANOSOMIASIS

Michael Zhuo Wang, Sihyung Yang

University of Kansas, Lawrence, KS, United States

Human African trypanosomiasis (HAT), a neglected tropical disease, threatens millions of people in sub-Saharan Africa. Without treatment, sleeping sickness is fatal. The majority of cases are diagnosed during the second stage of disease, after parasites (*Trypanosoma brucei gambiense* and *T. b. rhodesiense*) have crossed the blood-brain barrier and infected the central nervous system. Current eflornithine-based chemotherapies for second stage HAT are unsatisfactory, due to a complex regimen of intravenous infusions required to overcome inadequate oral bioavailability and poor BBB permeation. We hypothesized that an intercellular junction-modulating peptide ECP1 can improve the BBB permeation and gut absorption of eflornithine. ECP1 was relatively stable ($t_{1/2}$>5.5 h) after a 4-h incubation at 37°C in the simulated gastric fluid with pepsin, in the rat small intestinal mucosal scrapings and in the rat plasma. Using MDCK cell monolayers, ECP1 (1.0 mM) increased eflornithine permeability by 5-fold, whereas a scrambled peptide (ECP1scr) did not have any effect. In rats, co-administration of ECP1 (50 mg/kg) with eflornithine (100 mg/kg) orally increased plasma C_{max} and AUC of eflornithine by 40-60%, compared to vehicle or ECP1scr. Furthermore, using *in situ* rat brain perfusion, ECP1 (1.0 mM) increased the concentrations of eflornithine in various parts of the brain (e.g., cerebellum, hippocampus, frontal cortex and choroid plexus) by 85-390%. Further experiments are currently underway to determine eflornithine brain concentrations after co-administration with ECP1 orally in rats and to demonstrate that co-administration of ECP1 could improve the oral efficacy of eflornithine in rodent models of first and second stage HAT. If successful, oral ECP1-eflornithine formulation could positively impact the elimination of HAT.

1092

THE EFFICACY OF THREE COMPOUNDS ISOLATED FROM CVP005B LEAVES AGAINST LABORATORY AND FIELD STRAINS OF *LEISHMANIA* SPP.

Michael Amoa-Bosompem[1], Mitsuko Suzuki[2], Kofi D. Kwofie[1], Mba T. Mosore[1], Richard Adegle[3], Nguyen H. Tung[4], William K. Anyan[1], Shoji Yamaoka[2], Kwadwo Koram[1], Nobuo Ohta[2], Dominic Edoh[3], Naiki Puplampu[1], Godwin K. Nuako[5], Paul Bates[5], Irene Ayi[1], Daniel A. Boakye[1]

[1]Noguchi Memorial Institute For Medical Research, Accra, Ghana, [2]Tokyo Medical and Dental University, Tokyo, Japan, [3]Centre For Scientific Research into Plant Medicine, Mampong, Ghana, [4]Nagasaki International University, Nagasaki, Japan, [5]Lancaster University, Lancaster, United Kingdom

Leishmania spp. are parasitic protozoans that cause Leishmaniasis which is characterized by disfigurement, morbidity and mortality. Chemotherapy, the main form of control is undermined by toxic effects of available drugs and emerging drug resistance. The use of traditional medicine to treat infections is very common in Africa. CVP005B is one of the popular medicinal plants in West Africa. Although several research groups have reported on CVP005B to have anti-protozoa (e.g. Trypanosome and Leishmania) properties, no compound(s) have been assigned the responsibility for this activity. Our research group first identified novel compounds, ML-2-2, ML-2-3 and ML-F52, active against trypanosome by *in vitro* assay-guided purification from CVP005B leaves. These compounds share the same side chain but two of them, ML-2-2 and ML-F52, have the same functional group. This study was therefore aimed at finding out if these anti-Trypanosoma active compounds also possess anti-Leishmania properties. ML-2-2 and ML-F52 were found to have anti-Leishmania activities, by microscopic observation, with Minimum Inhibition Concentration (MIC) values of 2.87 µM and 2.87 µM respectively, while ML-2-3 had no activity on Leishmania culture for up to 72 hrs incubation. Comparison of the efficacies of these compounds against field strains of *Leishmania* showed MIC values of 4.17 µM and 2.60 µM for ML-2-2 and ML-F52 respectively. These data suggest that the functional group of ML-2-2 and ML-F52 might be crucial for their activity against *Leishmania*, and that could be effective against diverse *Leishmania* field strains. Investigations are underway to determine the molecular mechanisms of the activities using FACS analysis, Immunohistochemistry and Western Blotting.

1093

NOVEL COMPOUNDS ISOLATED FROM CVP005B LEAF EXTACT SHOW STRONG ANTI-TRYPANOSOMAL ACTIVITY *IN VITRO*

Kofi D. Kwofie[1], Mitsuko Suzuki[1], Nguyen H. Tung[2], Naa O. Ammah[1], Kwadwo K. Frempong[1], William K. Anyan[1], T. Uto[2], Morinaga Osamu[2], Frederick Aboagye[3], Regina Appiah-Opong[1], Alexander K. Nyarko[1], Shoji Yamaoka[4], T. Shoyama[2], Dominic Edoh[3], Kwadwo Koram[1], Nobuo Ohta[4], Daniel A. Boakye[1], Irene Ayi[1]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Nagasaki International University, Japan, Nagasaki, Japan, [3]Centre for Scientific Research into Plant Medicine, Mampong, Ghana, [4]Tokyo Medical and Dental University, Tokyo, Japan

African Trypanosomiasis, a devastating disease in Africa, is caused by the kinetoplastid parasite, *Trypanosoma brucei* sp. Due to drug inefficacy against the late stage of Human African Trypanosomiasis, toxicity and resistant parasites, development of novel chemotherapy is urgently needed. Africa has a long history of the use of traditional medicinal plants and the WHO reports about 80% of people relying on traditional medicines as their first-line treatment. CVP005B, a famous and one of the main medicinal plants in use in West-Africa, has previously been reported to have anti-trypanosomal activity by several research groups, though

active compounds were still unknown. This study identified compounds responsible for anti-*Trypanosoma* activity in CVP005B. Bioassay-guided fractionation and purification of CVP005B crude extract led to the isolation of 3 novel active compounds, "ML-2-2", "ML-2-3" and "ML-F52". They shared the same side chain and two of them had same functional group. FACS analysis of the Nexin assay revealed that ML-2-3 and ML-F52 induced apoptosis in *T. b. brucei* (GUTat3.1 strain), whereas ML-2-2 did not. FACS analysis of Multi-caspase assay in ML-2-3-treated trypanosomes also indicated the involvement of the caspase cascade in apoptosis signaling in trypanosomes. Cell cycle assay revealed alteration in G2/M phase in ML-2-3-treated parasites. Further investigation into the phenotypic differences in ML-2-2-, ML-2-3- and ML-F52-treated trypansomes by immunohistochemistry and western blot analysis using α-tubulin antibodies and flagellum marker, PFR-a, showed: nucleus fragmentation only in ML-2-3-treated trypanosomes which is a marker for apoptosis and could be a confirmation of the Nexin assay results. ML-2-3 and ML-F52 suppressed the expression of both α-tubulin and PFR-a in parasites, while ML-2-2 showed no effect. ML-2-3 and ML-F52, so far show very promising prospects for development of new anti-trypanosomal drugs whilst, ML-2-2 may be investigated for its usefulness for other scientific purpose(s).

1094

IN VITRO ACTIVITY OF NATURAL PRODUCTS AGAINST *LEISHMANIA* AND *TRYPANOSOMA CRUZI*

Luiza G. Tunes[1], Policarpo A. Junior[1], Caryne M. Bertollo[2], Tânia M. Alves[1], Carlos L. Zani[1], Alvaro J. Romanha[3]

[1]Oswaldo Cruz Foundation, Belo Horizonte, Brazil, [2]Federal University of Minas Gerais, Belo Horizonte, Brazil, [3]Federal University of Santa Catarina, Florianópolis, Brazil

In Brazil, the tropical neglected diseases leishmaniasis and Chaga's disease are causes of great impact on public health. The drugs currently used to treat these conditions have limitations concerning cost, efficacy and safety, making the search for new therapeutic approaches urgent. Natural products (NP) and their derivatives are important sources of new molecules that can be used for drug development. In the search for new leishmanicidal and tripanocidal drugs, we have carried out a screening for *in vitro* activity of various NP and two naphthoquinones stood out: eleutherine and naphthothiophenquinone. To evaluate the leishmanicidal effect we used THP-1-derived macrophages infected with *Leishmania amazonensis*. After incubation, infected cells were stained with hematologic dye and macrophages were counted to assess the percentage of infection. Amphotericin at 1.08μM was used as control. For the tripanocidal assay we used cultured mouse fibroblasts infected with *Trypanosoma cruzi* Tulahuen strain transfected with the β-galactosidase gene. After incubation, the enzyme substrate was added and the absorbance was measured at λ_{570}nm. Benznidazole was used as control at 3.81μM. The toxicity of the compounds was evaluated in THP-1 cells using alamarBlue™. Eleutherine reduced *Leishmania* infection to 4.5% at 70μM and *Trypanosoma* infection to 49% at 140μM. It decreased cell viability by 2% at 140μM. Naphthothiophenquinone showed a strong leishmanicidal activity, reducing the infection to 2.5% at 93μM. The tripanocidal activity was already described. It decreased cell viability by 16% at 196μM. In conclusion, eleutherine and naphthothiophenquinone are better leishmanicidal than tripanocidal agents. Moreover both compounds showed low toxicity to the THP-1 cells. The next steps are to investigate the cellular targets and mechanisms of action of these NP.

1095

PCR-BASED DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN TWO ENDEMIC VILLAGES FROM PERU AND BOLIVIA: AN ARTICULATED STRATEGY FOUNDED ON LOCAL HEALTH PROMOTERS AND CONVENTIONAL SAMPLE SHIPPING

Braulio M. Valencia[1], Lineth Garcia[2], Ernesto Rojas[2], Milena Alba[1], Nair Montaño[2], Cecilia Angulo[2], Aleida Verduguez[2], Ana M. Montalvo[3], Jorge Fraga[3], Jorge Arevalo[1], Gert Van der Auwera[4], Andrea K. Boggild[5], Alejandro Llanos-Cuentas[1], Vanessa Adaui[1]

[1]Institute of Tropical Medicine Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Instituto de Investigaciones Biomedicas e Interaccion Social, Universidad Mayor de San Simon, Cochabamba, Plurinational State of Bolivia, [3]Instituto de Medicina Tropical Pedro Kourí, Havana, Cuba, [4]Institute of Tropical Medicine, Antwerp, Belgium, [5]Tropical Disease Unit, Division of Infectious Diseases, Department of Medicine, University Health Network-Toronto General Hospital, University of Toronto, Toronto, ON, Canada

Diagnosis of cutaneous leishmaniasis (CL) is critical for appropriate management since confirmatory diagnosis and identification of causative species are recommended due to differences in parasite pathogenicity and drug sensitivity. Smear examination has a poor sensitivity even under expert examiners whereas identification of species is nowadays impossible in rural conditions. We implemented an articulated strategy for PCR diagnosis based on samples obtained and shipped by local health promoters (LHP) in two villages from Peru and Bolivia. LHP normally obtaining smears were trained to obtain samples using cytology brushes and apply and interpret Leishmanin skin test (LST). Smear examination was performed following local procedures whereas two clinical samples (brushes and lancet scrapings) were collected, preserved in absolute ethanol and shipped at environmental temperatures to reference centers for further PCR. A lesion was defined as CL if two of the following procedures were positive: smear, LST, or PCR on lancets. One hundred and twelve individuals were enrolled from whom 115 lesions were sampled. Sixty nine lesions fulfilled definitive CL criteria whereas 44 were positive by smear examination, 61 by LST, 63 by PCR of lancets, and 67 by PCR of cytology brushes. Sensitivity and specificity under ROC curve analysis were 64% and 89% for smear, 91% and 80% for LST, 91% and 93% for PCR on lancets, and 97% and 85% for PCR on cytology brushes. PCR in non-invasive samples was the diagnostic procedure with the highest sensitivity and specificity in comparison to smear examination and LST. This procedure has been demonstrated to be a valid diagnostic tool in controlled and real conditions with the additional potential to be easily performed by minimally trained personnel. Our strategy including LHP can be an interesting alternative to replace conventional diagnostic methods with the additional advantage of identifying causative species and establishing the geographic distribution of Leishmania parasites in endemic regions of Latin America.

1096

NATURAL PRODUCTS DRUG DISCOVERY FOR TREATMENT OF HUMAN AFRICAN TRYPANOSOMIASIS: NEW DRUG LEADS FROM A NATURAL PRODUCTS FRACTIONS LIBRARY

Surendra K. Jain[1], Melissa Jacob[2], Cynthia Jeffries[3], Anang Shelat[3], Julie Bryan[3], Ying Tu[3], Larry Walker[1], Muhammad Ilias[2], Babu Tekwani[1]

[1]National Center for Natural Products Research, Department of Pharmacology, School of Pharmacy, University of Mississippi, University, MS, United States, [2]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS, United States, [3]Department of Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, Memphis, TN, United States

Human African Trypanosomiasis (HAT) or sleeping sickness is caused due to infection with Trypanosoma brucei. Current drugs for treatment of HAT suffer from severe toxicities and require intramuscular or intravenous

administrations. Situation is further aggravated due to emergence of drug resistance. There is an urgent need of new drugs effective orally against both stages of HAT. Natural products offer an unmatched source for bioactive molecules with new chemotypes. However, natural product extracts present several challenges with respect to modern drug discovery programs. Polyphenols (vegetable tannins), often present in considerable quantities in ethanol extracts of plants, can cause false-positive results in both enzymatic and cellular screening procedures due to non-selective enzyme inhibition and changes in cellular redox potential. The chemical diversity found in a single extract may represent several different classes of molecules that exhibit different (and sometimes opposing) biological activities. The biologically active compounds may be present in crude extracts at extremely low concentrations, below the detection threshold for bioactivity screening. A high throughput fractionation of natural product extracts was done to encounter these problems. A library of >60,000 natural product fractions has been generated through a high throughput fractionation paradigm. Total 7379 fractions from 510 plant extracts were screened *in vitro* in both Trypanosoma brucei assay and cytotoxicity assay using differentiated THP1 cell lines. 454 active fractions were identified with more than 50% inhibition and subjected to dose-response evaluation. 285 anti-trypanosomal fractions were confirmed with IC50 values of <10 µg/mL: 22 with IC50 < 2µg/mL, 105 with IC50 between 2 and 5µg/mL and 158 with IC50 between 5 and 10µg/mL. Only 13 fractions showed toxicity against THP1 cells. The most active fractions namely Ledum groenlandicum c2 (0.75 µg/ml), Hippeastrum reticulatum c3 (0.9 µg/ml), Psychotria berteroana c4(0.98 µg/ml), Muntingia calabura c7(0.57 µg/ml), Eucalyptus robusta c3(0.92 µg/ml) and Myrsine coriacea c14(0.84 µg/ml) were further analyzed for QC-UPLC-MS/MS data and showed several new antitrypanosomal drug leads with novel pharmacophores.

1097

LEISHMANIASIS IN SURINAME - NEW INSIGHTS INTO A NEGLECTED DISEASE

Henk Schallig[1], Alida Kent[2], Sahienshadebie Ramdas[3], Ricardo Hu[4], Dennis Mans[2], Sjaak van der Geest[3], Henry de Vries[5], Lai a Fat Rudy[6]

[1]Royal Tropical Institute, Amsterdam, Netherlands, [2]Anton de Kom University, Paramaribo, Suriname, [3]University of Amsterdam, Amsterdam, Netherlands, [4]Dermatological Service, Paramaribo, Suriname, [5]Academic Medical Centre, Amsterdam, Netherlands, [6]Academic Hospital Paramaribo, Paramaribo, Suriname

According to text books, leishmaniasis manifests in Suriname as cutaneous leishmaniasis (CL) caused by *Leishmania guyanensis*, which can be adequately treated with pentamidine. Sand fly vectors and possible reservoir are not well known. A Suriname - Netherlands research consortium studies in an integrated approach several aspects of the disease in order to gain more in depth knowledge of CL in the country and to contribute to a control programme for leishmaniasis in Suriname, which is currently not available. The research programme comprises three projects: 1) Biological aspects of parasite and vector; 2) Clinical aspects of CL, and 3) Medical anthropology. Biological research has revealed that, next to *L. guyanensis*, at least three other species are present in Suriname, including the muco-cutaneous leishmaniasis-(MCL-)causing *L. braziliensis*. Medical doctors (also in non-endemic countries!) treating cases from Suriname for CL must be aware that next to CL, also MCL could be contracted. This finding has therapeutic implications since the first-line recommended treatment for *L. braziliensis* infections is not standard in Suriname. Furthermore, at least three, for Suriname new, sand fly species have been identified, and molecular analysis revealed that these can be infected with *Leishmania*. Reservoir studies on dogs are on-going. Clinical research has demonstrated that pentamidine may not be efficacious for all cases of CL, as around 25% cases of treatment failure are observed, and alternative treatment regimens are being explored. Effect of treatment can be well monitored over time by using a recently developed RT-PCR method that can predict treatment outcome. There is an excellent correlation between parasite load at week 6 and treatment outcome at week 12 after initiation of treatment. Medical anthropology revealed that stigmatization of infected individuals may not be a major problem in the social acceptability of the disease in Suriname, in contrast to other countries. Many non-conventional methods, including the use of dangerous chemicals, are practiced to treat CL, in particular in the interior of Suriname. Reasons for failing treatment adherence are being studied and will be presented.

1098

A FULLY INTEGRATED PARTNERSHIP PERFORMING DRUG DISCOVERY TOWARDS VISCERAL LEISHMANIASIS: PART 2

Tim Miles[1], Paul Wyatt[2]

[1]GlaxoSmithKline, Madrid, Spain, [2]University of Dundee, Dundee, Scotland, United Kingdom

GSK Kinetoplastid DPU and University of Dundee with support from the Wellcome Trust have formed a 5 year partnership to conduct drug discovery within kinetoplastid diseases (Visceral Leishmaniasis, Chagas disease and Human African Trypanosomiasis). This collaboration has made significant progress in the first 3 years, which has resulted in the identification of a lead optimisation series for Visceral Leishmaniasis through phenotypic screening. It is estimated that Visceral Leishmaniasis causes over 20,000 deaths per year world-wide. Current drugs suffer from multiple issues such as lack of efficacy and unacceptable levels of toxicity. Part 1, by Paul Wyatt from Dundee University, will describe work that resulted in the identification of a series that fulfils lead optimisation criteria for Visceral Leishmaniasis. This novel series is one of the few reported globally to show oral efficacy in an acute *in vivo* mouse model against Visceral Leishmaniasis. Part 2, by Tim Miles from GSK, will concentrate on the lead optimisation and progression of this series. As a number of issues were highlighted through critical path screening that have been overcome (*i.e.* solubility and exposure). Hence a discussion of medicinal chemistry strategies to solve these issues within a phenotypic screening setting will be discussed. The current set lead compounds within this series are being evaluated for pre-candidate selection.

1099

SPECIES DISCRIMINATION BETWEEN *LEISHMANIA PANAMENSIS* AND *L. GUYANENSIS* FROM CLINICAL SAMPLES OF COLOMBIAN PATIENTS VIA PCR-RFLP

Clemencia Ovalle-Bracho, Carolina Camargo-Paredes

Centro Dermatologico Federico Lleras Acosta, Bogotá, Colombia

The discrimination between two genetically related *Leishmania* species, e. i. *L. panamensis* and *L. guyanensis*, has been accomplished by the amplification of a region of the gene coding for the heat shock protein 70kDa (Hsp70), followed by restriction fragment length polymorphism (RFLP) assays using the BccI enzyme. Here we determined the efficiency of this experimental approach for gene amplification and species discrimination, from isolated parasites as well as clinical samples. We included reference strains, 30 parasites isolates from patients and 30 clinical samples from needle aspirates, dermal scrapings or skin biopsies obtained from patients diagnosed for cutaneous or mucocutaneous leishmaniasis. *Leishmania* parasites isolated from patients and clinical samples were previously identified as belonging to the *L. guyanensis* complex (*L. panamensis*/*L. guyanensis*), by a PCR-RFLP assay using the gene encoding mini-exon sequence and the restriction enzyme HaeIII. The 30 isolated *Leishmania* were further identified by isoenzyme analysis. A 1422bp sequence within the Hsp70 gene was amplified using the following primers: (Forward 5'-GACGGTGCCTGCCTACTTCAA-3') (Reverse 5'-CCGCCCATGCTCTGGTACATC-3'). Amplification reaction mixtures where then treated with BccI restriction enzyme for RFLP analysis. The positive reaction was obtained in 100% of the cultured parasites and in 30% of the clinical samples. Each amplicon showed either *L. panamensis* or *L. guyanensis* RFLP pattern and there was a 100% correspondence

between isoenzyme and PCR-RFLP analyses. Even though these results confirm the ability of this technique to discriminate between the two species, it also suggests that the amplification efficiency from clinical samples is limited. This could be due to the low parasite load typical of clinical samples especially in cases of mucocutaneous Leishmaniasis or the size of the amplicon. Given the clinical importance of these two *Leishmania* species discrimination, further optimization of the technique should be pursued.

1100

ACCURATE DIAGNOSIS OF SLEEPING SICKNESS BY TARGETING THE TRYPANOSOME'S SPLICED LEADER RNA

Pablo Gonzalez Andrade[1], Mamady Camara[2], Hamidou Ilboudo[3], Bruno Bucheton[4], Vincent Jamonneau[3], **Stijn Deborggraeve**[1]

[1]Institute of Tropical Medicine, Antwerpen, Belgium, [2]Ministry of Health and Public Hygiene, Conakry, Guinea, [3]Centre International de Recherche Développement sur l'Elevage en zones Subhumides, Bobo-Dioulasso, Burkina Faso, [4]Institut de Recherche pour le Développement, Unité Mixte de Recherche IRD-CIRAD 177, Montpellier, France

Trypanosomatids transcribe their genes in large polycistronic clusters that are further processed into mature mRNA molecules by trans-splicing. During this maturation process a conserved spliced leader RNA (SL-RNA) sequence of 39 bp is physically linked to the 5-prime end of the pre-mRNA molecules. Among the trypanosomatids are three major human pathogens: *Trypanosoma brucei*, *T. cruzi* and *Leishmania*. Their trans-splicing mechanisms have been extensively investigated for understanding eukaryotic cell biology but the SL-RNA has never been explored as a diagnostic target, although this molecule has several attracting diagnostic features. It is a short non-coding RNA sequence that is conserved but unique for each species and is present in each mRNA molecule in the cell. Importantly, mRNA is considered as the best surrogate marker for viable organisms. In this study, we investigated this SL-RNA molecule for its diagnostic potential using reverse transcription followed by real-time PCR. As a model we used *T. b. gambiense* that causes sleeping sickness in west and central Africa. We showed that the copy number of the SL-RNA molecule in one single parasite is at least 8600. The lower detection limit of the SL-RNA assay in spiked blood samples was 100 trypanosomes per mL of blood and in the same range as for DNA based tests. We also showed that we can detect the trypanosome's SL-RNA in the blood of sleeping sickness patients recruited in Guinee with a sensitivity of 92% (95% CI: 78%-97%) and a specificity of 96% (95% CI: 86%-99%). For the first time, we explored the SL-RNA as a molecular target for next-generation diagnostics in diseases caused by trypanosomatids. Evaluation of the assay for the assessment of cure after treatment of sleeping sickness is ongoing. We will present the design of the SL-RNA assay, the experimental proof of concept and the accuracy of the test for sleeping sickness diagnosis and cure assessment.

1101

RETINAL CHANGES IN VISCERAL LEISHMANIASIS

Richard J. Maude[1], B. U. M. Wahid Ahmed[2], Abu Hayat Md Waliur Rahman[2], Ridwanur Rahman[3], Eshaque Majumder[3], Darryl Braganza Menezes[4], Abdullah Abu Sayeed[5], Laura Hughes[6], Thomas MacGillivray[7], Shyamanga Borooah[6], Baljean Dhillon[6], Arjen M. Dondorp[1], M Abul Faiz[2]

[1]Wellcome Trust Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, [2]Sir Salimullah Medical College, Dhaka, Bangladesh, [3]Shaheed Surhawardy Medical College and Hospital, Dhaka, Bangladesh, [4]Queen Elizabeth Hospital, Birmingham, United Kingdom, [5]Chittagong Medical College Hospital, Chittagong, Bangladesh, [6]College of Medicine and Veterinary Medicine, Edinburgh, United Kingdom, [7]Clinical Research Imaging Centre, Queen's Medical Research Institute, Edinburgh, United Kingdom

In visceral leishmaniasis (VL), retinal changes have previously been noted but not described in detail and their clinical and pathological significance are unknown. A prospective observational study was undertaken in Mymensingh, Bangladesh aiming to describe in detail visible changes in the retina in unselected patients with visceral leishmaniasis. Patients underwent assessment of visual function, indirect and direct ophthalmoscopy and portable retinal photography. The photographs were assessed by masked observers including assessment for vessel tortuosity using a semi-automated system. 30 patients with VL were enrolled, of whom 6 (20%) had abnormalities. These included 5 with focal retinal whitening, 2 with cotton wool spots, 2 with haemorrhages, as well as increased vessel tortuosity. Visual function was preserved. These changes suggest a previously unrecognized retinal vasculopathy. An inflammatory aetiology is plausible such as a subclinical retinal vasculitis, possibly with altered local microvascular autoregulation, and warrants further investigation.

1102

EVALUATION OF THE POINT OF CARE TEST INBIOS CHAGAS DETECT PLUS IN BOLIVIA

Vishal Shah[1], Emi Okamoto[2], Jacqueline Sherbuk[2], Gerson Galdos Cardenas[3], Eva H. Clark[4], Lisbeth Ferrufino[5], Ricardo Bozo[6], Jorge Flores[7], Robert H. Gilman[3], Caryn Bern[8]

[1]St. Louis University School of Medicine, St. Louis, MO, United States, [2]New York University School of Medicine, New York, NY, United States, [3]Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States, [4]Baylor College of Medicine, Houston, TX, United States, [5]Universidad Catolica Boliviana, Santa Cruz, Plurinational State of Bolivia, [6]Hospital Municipal Camiri, Camiri, Plurinational State of Bolivia, [7]San Juan De Dios Hospital, Santa Cruz, Plurinational State of Bolivia, [8]University of California San Francisco, San Franciscio, CA, United States

Trypanosoma cruzi causes Chagas disease, which affects an estimated 7-8 million people. Chagas disease is endemic throughout Latin America, with the highest prevalence in Bolivia. Conventional diagnosis requires an experienced laboratory. We evaluated the Chagas Detect Plus (CDP) kit (InBios, Seattle WA), a rapid immunochromatographic assay for antibodies to T. *cruzi*, in both hospital and community settings. Performance of CDP was compared to conventional diagnostic tests including indirect hemagglutination assay (IHA), immunofluorescent antibody test (IFA), and enzyme-linked immunosorbent assay (ELISA). Confirmed infection required positive results by at least 2 conventional assays. Specimens from 3 studies in Bolivia were used: prospectively evaluated specimens from a study of congenital Chagas disease in Camiri Municipal Hospital (n=277) and a hospital-based study of cardiac biomarkers in Santa Cruz (n=108), and archived specimens from a community study in endemic villages of Gutierrez municipality (n=200). CDP was performed in finger stick blood and serum from 385 individuals, and in 200 archived serum specimens from the community study. CDP showed sensitivity / specificity of 96.2% [92.7-98.4] / 98.8% [95.9-99.9] in whole blood, and 99.3%

[97.5-99.9] / 96.9% [94.2-98.6] in serum. There were no differences by sex, age group or study population. For comparison, recombinant ELISA showed sensitivity / specificity of 94.8% [90.7-97.5] / 99.4% [96.6-100.0]. Lysate ELISA showed sensitivity / specificity of 100% [97.9-100] / 100% [97.9-100]. CDP demonstrated excellent sensitivity and specificity in our study population. Sensitivity was higher and specificity slightly lower in serum than in whole blood. The CDP is simple to use and reliable for both hospital settings and field sites. These rapid tests may also be used for practical, accurate maternal screening to identify neonates at risk of congenital transmission.

1103

IMPACT OF PEDIATRIC AND ADULT ACUTE MALNUTRITION ON VISCERAL LEISHMANIASIS RK39 DIAGNOSTIC TEST RESULTS AND CLINICAL OUTCOME IN THE SUDAN

Gabriel Alcoba[1], Atia Abdallah Atia[2], Ann Mumina[2], Esther Sterk[2], Annick Antierens[2], Francois Chappuis[3]

[1]Geneva University Hospitals; Médecins sans Frontières, Geneva, Switzerland, [2]Médecins sans Frontières, Geneva, Switzerland, [3]Geneva University Hospitals, Geneva, Switzerland

In the Sudanese town of Tabarakallah, Gedaref state, Médecins sans Frontières and the Ministry of Health of Sudan treated 2053 patients with primary Visceral Leishmaniasis (VL) between January 2010 and January 2014. Given the high rates of malnutrition, we questioned its impact on performance of rK39 rapid diagnostic tests (RDT) and on clinical outcomes (relapse and case-fatality rates) of primary VL patients. The rK39 RDT was used as first-line test, but due to its limited sensitivity in this region, patients with negative rK39 RDT also had a Direct Agglutination Test (DAT) performed, with lymph node aspirate (LN) restricted to borderline DAT results. Patients were treated with sodium stibogluconate, plus paromomycin since 2011, and liposomal Amphotericin B for severe cases, and followed-up for 6 months. We calculated global acute malnutrition (GAM) using WHO standards adapted to age: under 5 years (n=362) GAM was 53% (95%CI: 49.1-56.9%) weight-for-height <-2SD, including 23.3% (19.9-26.6%) of severe acute malnutrition (SAM); age 6-59 months: GAM 16.0% using middle-upper-arm-circumference (MUAC) <125mm, (including 4.7% SAM using MUAC<115mm); age 5-19 years (n=1202): 60.6% (58-63.1%) using BMI-for-age <2SD; adults (n=489): 27.3% using BMI<17 kg/m^2. Out of 2053 primary VL patients, 1880 (91.6%) were diagnosed by rK39 RDT, and 173 (8.4%) with negative rK39 were confirmed by DAT or LN. For ages 6-59 months (best age for MUAC homogeneity), rK39 was negative in 7.4% of GAM and 8.6% non-GAM children (p=0.78). For adults, rK39 was negative in 11.20% of GAM vs. 8.48% of non-GAM (p=0.37). In the pediatric population GAM or SAM based on MUAC at admission was not significantly associated with higher case-fatality rates (OR: 0.71(95%CI: 0.16-3.28), p=0.66), despite a slight trend in SAM (8.33%) vs. non-SAM (6.64%, p=0.82), unlike descriptions from conflict regions. In contrast, adult case-fatality rates were strongly increased from 5.7 to 18.6% (OR: 3.79 (1.71-8.41)) in GAM patients, independently of HIV-positivity which was low (1.34% of 1657 HIV tested). Odds of relapse with GAM were comparable in adults (OR: 0.97(0.38-2.54)) and children (OR: 0.96(0.21-4.41), p=0.96). In conclusion, no significant rK39 false-negative difference was detected in malnourished patient, so it is safe to rely on rK39 RDT for primary VL field diagnosis. Secondly, acute malnutrition increases mortality nearly fourfold in adults with VL in this stable setting.

1104

EFFECT OF ZINC, COPPER AND IRON *IN VITRO* ON TH1 AND TH2 CYTOKINE RESPONSE OF PERIPHERAL BLOOD MONONUCLEAR CELLS TO *LEISHMANIA* SP. INFECTION

Amilcar Alejandro Flores Leon, Ernesto Rojas Cabrera, Marisol Cordova Rojas, Mary Cruz Torrico Rojas

University Mayor of San Simon, Cochabamba, Plurinational State of Bolivia

An appropriate balance between pro-inflammatory and anti-inflammatory cytokines that mediate innate and adaptive immune responses is required for effective protection against human leishmaniasis and to avoid immunopathology, this immunological balance may be influenced by micronutrient deficiencies. We investigated the effect of zinc, copper and iron, in the production of Th1 and Th2 cytokines *in vitro* by Peripheral Blood Mononuclear Cells (PBMC) from patients with cutaneous leishmaniasis (CL) with therapeutic failure (Resistant) or patients who responded successfully to treatment (Sensitive), and if these trace elements might be involved in the immune response towards the parasite.Patients with CL living in Bolivia, an area highly endemic for Leishmania sp. were enrolled into the Resistant and Sensitive groups mentioned about. Measurement parameters of the immune response were: production of IFN-γ and IL-13 as markers of Th1 and Th2 response respectively, by peripherical blood mononuclear cells (PBMCs) stimulated with Antigen soluble leishmania (SLA) under different conditions of nutrients (Zn, Cu and Fe). The data obtained indicate that zinc, copper and iron are associated with a significant decrease in INF-γ response by PBMC the patients Resistant and Sensitive, as compared to PBMC stimulated only with SLA ($P < 0.05$). Production of IL-13 remained low and similar in both groups. These results show that: i) the specific immune response of Resistant and Sensitive patients is polarized toward TH1. ii) It necessary to know more about this elements trace how possible therapeutic administration in this pathology *in vivo*. iii) Environmentally or genetically determined increase in Cu, Zn and Iron levels might augment susceptibility to infection with intracellular pathogens, by causing decrease in INF-gamma production.

1105

NON-SEROLOGICAL DETECTION OF *TRYPANOSOMA CRUZI* BIOMARKERS IN MURINE DRUG DISCOVERY MODELS OF CHAGAS DISEASE

Rana Nagarkatti[1], Fernanda F. de Araujo[2], Charu Gupta[1], Alain Debrabant[1]

[1]Food and Drug Administration, Bethesda, MD, United States, [2]Centro de Pesquisa Rene Rachou, Fiocruz, Belo Horizonte, Brazil

Trypanosoma cruzi, a bloodborne parasite, is the etiological agent of Chagas disease. Following an infection in the host, patent parasitemia is detectable in the blood, and is termed as the acute phase. This is followed by a chronic phase where parasite levels in the blood are difficult to estimate. For the treatment of Chagas disease, currently prescribed drugs, Benznidazole (Bz) and Nifurtimox display multiple side effects and led to early termination of the treatment regimen by patients. Additionally, these drugs have not been show to result in sterile cure in patients. Thus new drugs are needed for disease mitigation. However, a lack of standardized universally acceptable assays that demonstrate parasitological or sterile cure in animal models have hampered development of new drugs. To address these issues, we envisaged the detection of antigens secreted by parasites (*T. cruzi* Excreted Secreted Antigens or TESA), as a diagnostic for Chagas disease. We have demonstrated recently that aptamers (short RNA molecules) can detect parasite antigens circulating the blood of infected mice using an Enzyme Linked Aptamer (ELA) assay. Here we show the ability of the ELA assay to detect these TESA biomarkers in *T. cruzi* infected mice, treated with the drug (Bz). Of the 7 aptamers tested, Apt-29 was able to detect circulating biomarkers in infected mice treated with Bz during the acute phase and in mice treated during the chronic phase of disease. For the acute phase Apt-29 ELA assay (100% +ve) was as good

as Blood PCR (100% +ve) but significantly better than tissue PCR (11% +ve in heart tissue and 89% +ve in skeletal muscles by PCR), to establish treatment failure in mice. In the chronic phase, Apt-29 ELA assay (100% +ve) was significantly better than blood PCR (46% +ve), heart tissue PCR (38% +ve) or even skeletal muscle PCR (92% +ve), to identify treatment failure in mice. These results indicate that ELA assays may be useful in Chagas drug discovery and can provide longitudinal data indicating reduction in parasite load upon treatment in mice models of Chagas disease. Disclaimer: "The findings and conclusions in this abstract have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy"

1106

NANOTECHNOLOGY TO DNA DETECTION OF *TRYPANOSMA CRUZI* FROM URINE BY REAL TIME PCR

Martha Helena Jahuira Arias[1], Yagahira E. Castro[1], Robert H. Gilman[2], A. Luchini[3], Lance Liotta[3]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Johns Hopkins University, Baltimore, MD, United States, [3]George Mason University, Manassas, VA, United States

In recent years there has been an explosion of interest in developing applications involving nanotechnology. Chagas' disease is a neglected tropical disease caused by *Trypanosoma cruzi* and constitutes a serious public health problem for Latin America. Accurate diagnostics are research priorities and the polymerase chain reaction has been proposed as a sensitive laboratory tool for detection of *T. cruzi* infection and monitoring of parasitological treatment outcome, the urine is a valuable noninvasive sample and some studies reported the presence of DNA fragments in urine. In our work we explored the application of microparticles for DNA detection of *T. cruzi* from urine by RT-pcr. The NIPAm/allylamine microparticles showed efficient results in DNA capture in urine samples. We infected urine samples with DNA and applied the NIPAm/ allylamine microparticles and continued with DNA extraction from micronanoparticles, we recovered 93.5 % of DNA from parasites. Finally we tested in 10 urine samples from guinea pig infected with *T. cruzi*. Our results using Rt-pcr from microparticles showed sensitivity values of 87.5 % and specifity 100%. We consider the use of microparticles could be as biomarker through DNA in urine samples

1107

IS LEPTOSPIROSIS AN OCCUPATIONAL DISEASE IN THAILAND?

Wirichada Pan-ngum[1], Sarin Suwanpakdee[2], Jaranit Kaewkungwal[1], Norberto Asensio[3], Parntep Ratanakorn[2], Pratap Singhasivanon[1], Nicholas John Day[4], Lisa Jane White[4]

[1]Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [2]The Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals, Faculty of Veterinary Science, Mahidol University, Bangkok, Thailand, [3]Faculty of Environment and Resource Studies, Mahidol University, Bangkok, Thailand, [4]Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

The temporal and spatial trend of human leptospirosis in Thailand were explored using statistical models. Several potential factors related to environment, human-animal interaction and human behaviors which are traditionally associated with exposure to the pathogen were investigated. Multiple data sources were deployed including: spatially stratified surveillance of cases between 2010 and 2012; satellite images on flooding; number of potential animal reservoirs spatially explicit livestock population density. We combined this to novel data collected for this study on human contact pattern, level of knowledge about the disease and self-protection practices. We found no significant temporal trend of leptospirosis over the study period. Spatially, leptospirosis occurred repeatedly and predominately in northeastern Thailand. Flooding has previously been assumed to have a significant influence on leptospirosis incidence but for Thailand this was not the case. Statistical analysis showed inconsistent patterns of incidence rate ratio (IRR) of flooding across years and regions. However, the number of buffaloes per district was significantlyassociated with human leptospirosis. From the survey, we found that 75% of population in the study area was farmers who routinely have close contacts with their animals. Their contact with the environment was also extremely high during the rice season when they spent, on average, 6 hours per day in water with only 50% wearing protective footwear. Indeed the seasonal rise and fall of reported leptospirosis cases over time can be explained by differences in seasonal exposure as many rice farming activities and correlates with temporal flooding events. We conclude that there is strong evidence to support leptospirosis being an occupational disease in Thailand rather than one associated with flooding and environmental factors alone. This information will have implications for public health policy to control and treat this disease in Thailand.

1108

DETECTION OF VIRULENCE GENES ASSOCIATED WITH *SALMONELLA* SPP. ISOLATED FROM RAW ANIMAL FOOD AND HUMANS

Yemisi O. Adesiji[1], V. K. Deekshit[2], Indarani Karunasagar[3]

[1]Ladoke Akintla University of Technology, Osogbo, Nigeria, [2]Karnataka Veterinry Animal Science and Fisheries University, Mangalore, India, [3]Department of Microbiology, College of Fisheries, Karnataka Veterinary, Animal and Fisheries Science, Mangalore, India

Disease-causing potential in *Salmonella* requires the coordinated expression of complex arrays of virulence factors that allow the bacterium to evade the host's immune system. The TTSS encoded by SPI-2 is an important component of the virulence strategy of *Salmonella* and contributes to systemic infection and replication within macrophages. The presence of virulence encoding genes factors was determined in a total of 30 *Salmonella* isolates by PCR targeting (ssaT, sseB, sseF, sseG, , sseD, sseC) of SPI-2 and invH (SP1-1) virulence determing genes. The presence of ssaT, sseB, sseG, sseC genes was (100%) and (93.3%) sseF, sseD, while sseG were not detected in any of the isolates tested. This study reports detection of virulence genes in *Salmonella* isolated from clinical, raw food samples, including chicken and seafood from Nigeria and India.

1109

THE EFFECT OF HUMAN MOVEMENT PATTERNS ON EXPOSURE TO *PLASMODIUM KNOWLESI* IN SABAH, MALAYSIA

Kimberly M. Fornace[1], Julian Drewe[2], Martha Betson[2], Matthew J. Grigg[3], Timothy William[4], Chris Drakeley[1], Jonathan Cox[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Royal Veterinary College, London, United Kingdom, [3]Menzies School of Health Research, Darwin, Australia, [4]Sabah Department of Health, Kota Kinabalu, Malaysia

The zoonotic malaria parasite *Plasmodium knowlesi* is the most common cause of human malaria in the heavily deforested region of Kudat in Sabah, Malaysia. Although human movements into macaque and mosquito habitats determine the risk of exposure to *P. knowlesi* and cases have been reported in men, women and children, little is known about individual mobility patterns relative to different land cover types. As part of an integrated programme, this study is investigating the role of individual local movements in the transmission of *P. knowlesi* and exploring the hypothesis that deforestation and resulting habitat fragmentation have led to increased contact between people, mosquito vectors and primate hosts at forest edges. This paper reports data from an ongoing study using GPS tracking devices to map movement patterns of individuals living in two areas where *P. knowlesi* transmission is occurring: a highly deforested area (Matunggong, Kudat) and a less disturbed area (Limbuak, Pulau Banggi). The study incorporates a seasonal, cross-sectional design. During pre-

defined two-week periods randomly selected individuals are asked to carry a tracking device at all times and to record when macaques are sighted. Over the same period, longitudinal mosquito sampling (using human landing catches) is being carried out within representative land cover types. This paper presents data from the first 6-months of human movement monitoring and describes the methods that will be used to combine metrics for mobility with entomological data to assess the probability of exposure to *P. knowlesi* and identify characteristics of individuals and land use types associated with an increased probability of exposure.

1110

RISK FACTORS FOR OUTBREAKS OF ANTHRAX IN LIVESTOCK IN BANGLADESH

Najmul Haider[1], Emily S. Gurley[1], Farhana Haque[1], Md. Saiful Islam[1], Sean V. Shadomy[2], Henry Walke[2], Muhammad Belal Hossain[1], Kamal Hossain[1], Ashraful Alam[3], Mahmudur Mahmudur Rahman[3], Nordin S. Zeidner[1]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Institute of Epidemiology, Disease Control and Research, Dhaka, Bangladesh

Anthrax is endemic and annually causes outbreaks in Bangladesh. The purpose of this study was to identify risk factors for animal anthrax to guide measures to prevent outbreaks among livestock. Between October 2012 and October 2013, we conducted case-control studies in cattle and goats in four districts of Bangladesh where human cutaneous anthrax outbreaks were identified by government officials. Case-animals were defined as ruminants with an epidemiological link to a human cutaneous anthrax case with evidence of anthrax by either Gram stain and McFadyean reaction and/or characteristic appearance of colonies growing on blood agar medium. We enrolled as controls all ruminants on the 10 farms closest to the case-farm that reported no illness in their animals in the three days before the outbreak at the case-farm. We interviewed owners of all enrolled ruminants using a structured questionnaire to record their feeding, grazing and vaccination histories. We estimated the association between animal exposures and anthrax infection with 95% confidence intervals (CI) using bivariate and multivariate logistic regression, accounting for farm-level clustering. We enrolled 47 case-animals from 33 farms and 403 controls from 180 farms. Compared to controls, infected animals were more likely to be <24 months of age (57% vs. 37%, p=0.02), feed on green grass cut or pulled up from agricultural lands (69% vs.33%, p=0.003), and graze on agricultural lands for a longer period of time in the 24 hours preceding onset of the animal outbreaks (4.3 vs. 1.3 hours, p<0.001). Case-animals were less likely to be vaccinated against anthrax during the past year compared to controls (15% vs. 53%, p<0.001). On multivariate analysis, being fed green grass cut or pulled-up from agricultural lands was independently associated with anthrax infection in ruminants (adjusted odds ratio [AOR]=3.0, 95% CI: 1.1-8.3) and anthrax vaccination in the past year was protective (AOR=0.15, 95% CI: 0.05-0.39). Cut or pulled up grass from agricultural lands can be contaminated with soil containing anthrax spores and this is a potential source of anthrax infection for ruminants. Considering the challenges of avoiding the use of green grass mixed with soil contaminated with spores, we suggest identifying and addressing barriers to increased vaccine coverage.

1111

KNOWLEDGE, ATTITUDES AND PRACTICES ABOUT RABIES MANAGEMENT AMONG HUMAN AND ANIMAL HEALTH PROFESSIONALS IN MBALE DISTRICT, UGANDA

Fred Monje

Makerere University, Kampala, Uganda

For the past five years (2007-2011), Mbale District registered the highest number of suspected and confirmed rabies cases in Eastern part of Uganda (587 animal bites and 19 deaths in humans on average annually) and many factors could have contributed to this. The aim of this study was to assess the knowledge, attitudes and practices (KAP) of animal and human health professionals and also to establish the level of relationship within KAP towards rabies management so as to inform responsible authorities to effectively mitigate the problem. A cross-sectional study was conducted between December 2012 and March 2013 among 147 randomly selected animal and human health professionals in Mbale District. Of these, only16 were animal health professionals. Quantitative data was obtained using a semi-structured questionnaire while qualitative data was obtained from 4 Focus Group Discussions (FGDs) using an FGD interview guide and 2 Key Informant (KI) interviews using a KI interview guide. Quantitative data was entered into Epiinfo version 3.5.1 and proportions computed while qualitative data was summarised into themes and sub-themes. Of all the respondents, only 44.22% (n=65) had sufficient knowledge about rabies while 25.2% (n= 37) had positive attitudes towards rabies management. Nearly half of the respondents (49.7%, n= 73) had limited good practices. Respondents knowledgeable about rabies were more likely to have positive attitude towards rabies management (OR=3.65; 95% CI: 1.60-8.3) while respondents with positive attitudes, were more likely to have good practices towards rabies management (OR: 2.22; 95% CI: 1.01- 4.86). Respondents had low knowledge, negative attitude and limited good practices of rabies management in the District. Regular refresher trainings about rabies to broaden staff knowledge and improve their attitudes and hence practices of rabies management should be conducted by the District leaders. Adoption of "One Health" approach for rabies control should be instituted to reduce the incidence of the disease in the District.

1112

PREVALENCE AND RISK FACTORS TO HUMAN AND ANIMAL BRUCELLOSIS IN MUBENDE DISTRICT-UGANDA, 2013

Grace K. Asiimwe

Makerere, Kampala, Uganda

Brucellosis is a highly contagious bacterial zoonosis and annually affects more than 500,000 people globally. In Africa the prevalence is 16.2% and 18-24% in Uganda. Humans contract Brucellosis by direct contact with infected animals and consumption of unpasteurized milk and its products. Brucellosis is endemic in Uganda and specifically Mubende district. Increase in number of cases reported to ministry of health between May to November 2011 from Mubende district with no comprehensive report on risk factors to explain the increase in confirmed cases initiated a study to examine prevalence and main risk factors for both human and animal brucellosis in the district. Unmatched 1:1 case control study done. We extracted Brucellosis cases biodata from laboratory records and actively searched for them in communities where 52 cases out of the target 100 were enrolled. Fifty two Controls enrolled from neighborhood considering age and sex. A structured questionnaire administered to both cases and controls. Adult cases and controls interviewed in person and children, their parents responded to questionnaires after consent. Cattle above two years and goats above one year were randomly selected and bled. Seroprevalence determined using cELISA test. Data entered in Epi info version 5.3.1 and exported to Stata Version 9.0 for analysis. Human Brucellosis seroprevalence was 31%. Seroprevalence of Brucellosis in cattle was 11% at animal level and 38% at herd level. In goats, prevalence was 36% and 58% at animal and herd level respectively. Significant risk factor associated with brucellosis in humans, consumption of undercooked meats [OR=8.3, 95%CI: (1.4-48.1)]. Use of protective wear while handling animals and products was protective [OR=0.04, 95%CI: (0.01-0.84)]. History of animal abortions on farm [OR=7.9, 95% CI: (1.4-45.7)] was found a significant animal risk factor to Brucellosis. The increase in number of human cases in Mubende district was due to consumption of undercooked meat and failure to use protective wear while handling animals. The high prevalence in animals associated with history of abortions on farms. Government should strengthen sensitization on Brucellosis, regular testing of herds and Brucellosis considered a differential in animal handling communities.

1113

SEROPREVALENCE OF RIFT VALLEY FEVER, Q-FEVER AND BRUCELLOSIS IN RUMINANTS ON THE SOUTHEAST SHORE OF LAKE CHAD

Fayiz M. Abakar

Institut de Recherche en Elevage pour le Développement, N'Djamena, Chad

The seroprevalence of Rift Valley Fever (RVF), brucellosis and Q-fever among domestic ruminants on the southeast shore of Lake Chad was studied. The study area consisted of two parts including mainland and islands. On the mainland, the study was conducted in 9 randomly selected villages and camps. On the islands, samples were collected from all four available sites. A total of 985 serum samples were collected and 924 were analyzed using ELISA for RVF. A total of 561 samples collected from islands were analyzed using ELISA for Q-fever and both ELISA and Rose Bengal Tests (RBT) for brucellosis. The apparent seroprevalence by species was 37.8% (95% C.I: 34.2 - 41.3) in cattle, 18.8% (95% C.I: 12.3 - 25.2) in goats and 10.8% (95% C.I: 3.0 - 18.5) in sheep. For brucellosis and Q-fever, only cattle samples from islands were analyzed. For Q-fever, the apparent seroprevalence was 7.8% (95% C.I: 5.6 - 10.1). For brucellosis, the RBT showed a prevalence of 5.7% (95% C.I: 3.8 - 7.6) and ELISA showed 11.9% (95% C.I: 9.3 - 14.6) with κ value of 5.3 showing a moderate agreement between the two tests. This study confirms the presence of the three diseases in the study area. More research is required to assess the importance for public health and conservation of the Kouri cattle breed.

1114

SOURCE TRACKING *MYCOBACTERIUM ULCERANS* INFECTIONS IN THE ASHANTI REGION, GHANA

Charles A. Narh[1], Lydia Mosi[2], Charles Quaye[1], Christelle Dassi[2], Danielle Konan[2], Mathurin Koffi[2], Dziedzom de Souza[1], Daniel Boakye[1], Bassirou Bonfoh[2]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Côte D'Ivoire

Although several studies have associated *Mycobacterium ulcerans* (MU) infection, Buruli ulcer (BU), with slow moving water bodies, there is still no definite mode of transmission. Ecological and transmission studies suggest variable number tandem repeat (VNTR) typing as useful tool to differentiate MU strains from other Mycolactone Producing Mycobacteria (MPM).Determining the genetic relatedness of clinical and environmental isolates is seminal to determining reservoirs, vectors and transmission route. This study source-tracked MU infections to specific water bodies by matching VNTR profiles of human isolates to those in the environment. Environmental samples were collected from 10 water bodies in four BU endemic communities in the Ashanti region, Ghana. Animal trapping identified 5 mice with lesions characteristic of BU. Four VNTR markers in MU Agy99 genome, were used to genotype environmental isolates and those from 15 confirmed BU patients within the same study area. Length polymorphism was confirmed with sequencing. MU was present in the 3 different types of water body but significantly higher in biofilm samples. Four MU genotypes designated, W, X, Y and Z were typed in both human and environmental isolates. Other reported genotypes were only found in water bodies. Our findings suggest that patients were infected from community associated water bodies. Further, we present evidence that small mammals within endemic communities could be susceptible to MU infections and may be acting as reservoirs.

1115

INFLUENZA A AMONG SWINE AND DUCK POPULATIONS IN RURAL BACKYARDS WITHIN TROPICAL WETLANDS IN GUATEMALA, 2013

Celia Cordon-Rosales[1], Danilo Alvarez[1], María L. Müller[1], Lucía Ortiz[1], Ana L. Barrios[1], Ana S. Gonzalez[1], Eduardo Azziz-Baumgartner[2], Jorge Jara[1]

[1]Universidad del Valle de Guatemala, Guatemala, Guatemala, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States

Some ecosystems may serve as hotspots for the emergence of influenza A virus, and surveillance in such areas may detect novel strains before these become a substantial threat to human populations. In the present project, we searched for Influenza A among duck and swine raised in rural backyards in wetlands where aquatic migratory birds have previously tested positive for influenza A. We conducted monthly surveys of domestic animals to in the villages of Candelaria and Monterrico, Municipality of Taxisco, on the pacific coast of Guatemala. Serial samples were collected from ducks and swine to detect current or past infections of Influenza A. At each visit, we collected nasal swabs and sera from each swine and traqueal, cloacal and sera from each duck. Viral antigens were initially screened by rRT-PCR in swab samples, and positive samples were subsequently sub-typified by HI. Total antibodies against Influenza A were measured by ELISA in sera. In February 2013, we conducted a census of all backyard animals in the two villages, and identified 102 swine and 123 ducks which we followed monthly during April-August 2013 for a total of 377 swine- and 449 duck-visits. . A total of 754 swine and 1,635 duck samples were tested through rRT-PCR and/or ELISA. We identified Influenza A among 3% (12/377) of swine nasal swabs and 0.5% (2/377) of their sera; HI testing indicates reactivity to pH1N1 subtype. We identified Influenza A among 2% (10/546) of duck trachea-, 0.7% (4/546) of cloaca-, and 2.9% (16/543) sera-samples. Influenza A antigen and antibody positivity in swine ranged from 0.0 to 5.9% and 0.0 to 2.0 %, and in ducks from 1.0 to 6.2 % and 0.0 to 4.3%, respectively. The antigen positivity in ducks seems to show an increasing trend from April to August 2013, while no trend is evident in the case of swine. Additionally 18 animals, with undifferentiated signs, suggestive of respiratory disease, were tested and none were positive for Influenza A This is the first longitudinal study of Influenza A in backyard animals, and although the percent of infected backyard animals is low it may represents a public health risk for the householders specially those in charge of their husbandry. Additional studies are needed to better define seasonal fluctuations and their association with environmental and ecological variables. Moreover, viral isolation and subsequent nucleic acid Posequencing could reveal characteristics of their pathogenicity, relationship and origin.

1116

INCREASED MORBIDITY AND MORTALITY IN DOMESTIC ANIMALS FED GROUND AND BITTEN FRUIT IN BANGLADESHI VILLAGES AND THE IMPLICATIONS FOR BAT BORNE ZOONOTIC DISEASE TRANSMISSION

John J. Openshaw[1], Sonia Hegde[2], Jahangir Hossain[2], Salah U. Khan[2], Hossain M. Sazzad[2], Jon Epstein[3], Peter Daszak[3], Emily S. Gurley[2], Stephen P. Luby[2]

[1]Stanford University, Stanford, CA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]EcoHealth Alliance, New York, NY, United States

Consumption of bat bitten fruit by domestic animals is a proposed pathway for the introduction of new zoonotic pathogens and is the most likely explanation in a 1998 Nipah virus outbreak on Malaysian pig farms. We hypothesized that consumption of fruits bitten by bats and dropped to the ground leads to increased morbidity and mortality in domestic animals due to bat related pathogens. As part of a study on Nipah virus, teams collected data on animal health and feeding practices from

randomly selected Bangladeshi households in villages inside and outside Nipah endemic areas from 2011 to 2013. Farmers were asked about deaths in the two-month period prior to the survey and illness in the year prior. We used mixed effects models controlling for village clustering, per capita household resources, and herd size to examine if farmers allowing animals to eat ground fruit or actively feeding bitten fruit were more likely to report sick or dead animals. The analysis included 206 villages, 5081 households, 9254 cattle, and 4265 goats. 30% of farmers reported that their animals consumed ground fruit. Compared with farmers who denied their animals ate ground fruit, farmers who reported consumption were more likely to report morbidity in their cattle (42% vs 36%, OR=1.2, CI 1.0-1.5, p=0.02) and in their goats (13% vs 9%, OR=1.8, CI 1.0-2.2, p=0.04). 20% of farmers reported that they actively fed bitten fruit to their herds. Compared with farmers who did not feed bitten fruit, farmers feeding bitten fruit were more likely to report morbidity in their goats (42% vs 31%, OR 1.6, CI 1.2-2.1, p<0.001) as well as mortality (13% vs 9%, OR 1.8, CI 1.2-2.7, p=0.004). The percentage of farmers reporting illness in their goats increased with more frequent feedings: 31% of farmers denying feeding bitten fruit reported illness compared with 42% feeding no more than 2 times/week (OR 1.5, CI 1.2-2.1, p=0.002) and 49% feeding >2 times/week (OR 2.4, CI 1.2-4.9, p=0.015). Feeding ground and bitten fruit is associated with increased mortality and morbidity in goats and cattle. This could be due to the transmission of bat pathogens. Given the close interface between humans and their animals, this represents a potential pathway from bats to human populations. Future serologic studies and pathogen detection aimed at these animals and their human owners may provide an efficient way to observe spillover events.

1117

HUMAN MONKEYPOX DISEASE SURVEILLANCE AND TIME TRENDS IN THE DEMOCRATIC REPUBLIC OF CONGO, 2001-2013

Nicole Hoff[1], Benoît Kebela Ilunga[2], Robert Shongo[3], Jean-Jaques Muyembe[4], Mathias Mossoko[3], Emile Okitolonda[5], Anne W. Rimoin[1]

[1]University of California Los Angeles Fielding School of Public Health, Los Angeles, CA, United States, [2]Minister of Health, Kinshasa, Democratic Republic of the Congo, [3]Ministry of Health, Kinshasa, Democratic Republic of the Congo, [4]National Institute of Biomedical Research, Kinshasa, Democratic Republic of the Congo, [5]University of Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo

Human monkeypox (MPX) is a zoonotic orthopoxvirus that causes a serious smallpox-like illness. The majority of MPX cases are reported in rural villages, located in or near heavily forested areas in the Democratic Republic of Congo (DRC). In DRC, MPX is a reportable disease at the national level (Ministry of Health, Division of Disease Control - Integrated Disease Surveillance and Response (IDSR) system). However, no analyses have been completed to-date looking at the passively collected data on suspected cases of MPX. Assessments of surveillance systems can help shape recommendations and have broad policy implications for disease surveillance systems, especially in low-income settings. We used available data from the IDSR system on MPX-suspected case counts received from every health zone on a weekly basis for an in-depth analysis of yearly and weekly trends, and factors which could influence reporting. A time series analysis will be used to assess the effect of seasonality on the number of suspected cases reported. If detected, additional analyses will be conducted to determine if the seasonality found is actually seasonal variation in MPX occurrence or seasonal variation in disease reporting. In order to examine this effect, other reportable diseases with or without seasonal patterns will be used in place of MPX as the outcome. Between 2001 and 2013, an increase in suspected cases of MPX was reported through the IDSR: 19,437 suspected cases were reported, with 269 of 514 (52.3%) health zones reporting. Additionally, 326 suspected deaths due to MPX were reported (CFR=1.7%). When all years (2001-2013) are collapsed, the average number of suspected cases reported per week is 34 (min=0, max 360). Weeks 9-11 and 42-43 have the highest average number of suspected cases, while weeks 51-52 have the lowest reporting. Apparent increases in reported annual MPX cases, and trends in weekly reporting, may be artifacts of improvements in disease surveillance. Further analyses should examine such trends, providing critical information for prevention and control strategies and suggesting areas of improvement for future data collection efforts.

1118

TUBERCULOSIS INFLUENCES THE PROGRESSION OF NEUROLOGIC DISEASE IN HTLV-1 INFECTED SUBJECTS

Maria de Lourdes S. Bastos, Yuri Neves, José Abraão Neto, Dorival Araújo, Valéria Bittencourt, Sérgio Arruda, Eduardo M. Netto, Edgar M. Carvalho

Federal University of Bahia, Salvador, Brazil

The human T cell lymphotropic virus type 1 (HTLV-1) has a high prevalence in Central and South American. The HTLV-1 associated myelopathy or tropical spastic paraparesis (HAM/TSP) is observed in less than 5% of the cases but a large percentage of infected subjects have neurologic involvement mainly of overactive bladder. HTLV-1 increases from 2 to 4 fold the risk for tuberculosis (TB) but it is not known if TB influences HTLV-1 infection. We determine in a cohort of HTLV-1 infected subjects the prevalence of active or past history of TB, perform tuberculin skin test (TST) and evaluate if TB influences neurologic disease associated to HTLV-1. TB was defined by past or present history of TB or by X-ray showing scar lesions characteristic of TB. Participated of this cross-sectional study 190 HTLV-1 infected individuals presenting in our outpatient clinic for HTLV-1 from April of 2010 to March of 2012. They were evaluated for present or past clinic manifestations of TB and a neurologic examination, chest X-ray and a TST was performed. TB was detected in 39 (20%) of the subjects and latent TB (LTB) in 76 (58,9%). Of the 39 patients with TB 28 had history of the disease. Among them, 28 had pulmonary TB, one had lymph node TB and two pleural TB. In 9 (36%) of the 25 patients with past history of TB the RX was normal at the time of the present study. In patients who had lesions of TB in the X-rays there was no major sequel. The TST was positive in 108 (56,8%) of the 190 patients. There was a predominance of males (51,3%) among TB patients than in LTB (38%) and without TB (20%) groups (P=0.02)). Moreover HAM/TSP was strongly associated with TB but not with LTB or without TB (P=0.02). The chance of findings TB in HAM/TSP patients was 3.5 fold higher than in LTB. The frequency of HAM/TSP was similar in patients with history of TB and in patients who had the diagnosis based on the TST and chest X-ray. While severity of TB does not appear to be influenced by HTLV-1, TB influences progression of neurologic disease to HAM/TSP.

1119

IMPACT OF MATERNAL MALARIA AND HYPERGAMMAGLOBULINEMIA ON TRANSPLACENTAL TRANSFER OF RSV NEUTRALIZING ANTIBODIES IN COASTAL PAPUA NEW GUINEA (PNG)

Jessica E. Atwell[1], Bhagvanji Thumar[1], Roselyn Tobby[2], Danielle I. Stanisic[3], Leanne J. Robinson[4], Holger W. Unger[5], Stephen J. Rogerson[5], Peter M. Siba[6], Christopher L. King[7], Ruth A. Karron[1]

[1]Johns Hopkins School of Public Health, Baltimore, MD, United States, [2]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [3]University of Queensland, Brisbane, Australia, [4]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea; Walter and Eliza Hall Institute, Parkville, Victoria, Australia, [5]University of Melbourne, Melbourne, Australia, [6]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea, [7]Case Western Reserve University, Cleveland, OH, United States

Passively acquired RSV neutralizing antibody (Ab) protects against RSV-associated lower respiratory illness, but placental malaria (PM) and hypergammaglobulinemia can interfere with transplacental transport of

immunoglobulins. To determine efficiency of transport in the context of PM and hypergammaglobulinemia, 313 paired maternal and cord sera from 2 cohorts in *P. falciparum* and *P. vivax*-endemic areas of coastal PNG were tested: the Alexishafen cohort (PM= 59%, 2005-08, 157 pairs) and the FIS cohort (PM=9.6%, 2012-13, 156 pairs). RSV Ab was measured in maternal and cord blood obtained at delivery by 60% complement-enhanced plaque reduction neutralization assay (60% PRN). Cord to maternal titer ratios (CMTR) were calculated for each pair. Maternal IgG was measured by radial immunodiffusion and hypergammaglobulinemia defined as total IgG ≥1700 mg/dL. Impaired and highly impaired transport were defined as CMTR <1.0 and <0.8 respectively. Maternal titers varied substantially (range, 11-7,150 Alexishafen; 19.5 -2,259 FIS). Impaired transport occurred across the spectrum of titers. In the Alexishafen cohort, impaired and highly impaired transport were observed in 34% and 17% of pairs respectively, and in 33% and 18% respectively in the FIS cohort. Rates were nearly identical despite substantial differences in PM prevalence. Analysis of impaired transport by PM status revealed no statistically significant differences in either cohort. Hypergammaglobulinemia was detected in 54% of Alexishafen mothers and was significantly associated with impaired transport (OR=2.2 [95% CI 1.06 - 4.67), p=0.02). However, hypergammaglobulinemia was detected in only 8% of FIS mothers and was not associated with impaired transport. In summary, we observed impaired transplacental transport of RSV PRN Ab in coastal PNG, but these data suggest that PM was not the primary driver. Further work is needed to determine the effect of hypergammaglobulinemia and assess other factors that may impair transport of RSV PRN Ab. These data may have important implications for future maternal RSV immunization programs.

1120

FACTORS ASSOCIATED WITH FAILURE IN SMEAR POSITIVE PULMONARY TUBERCULOSIS: USING SYMPTOMS PLUS SPUTUM SMEAR AND CHEST RADIOGRAPHY

Najeebullah Rahimy[1], **Viravarn Luvira**[2], Prakaykaew Charunwatthana[2], Maleerat Sutherat[2], Weerapong Phumratanaprapin[2], Thavisakdi Bamrungtrakul[3], Sopon Iamsirithaworn[4], Benjaluck Phonrat[2]

[1]Department of Infectious Diseases, Medical Faculty, Kandahar University, Kandahar, Afghanistan, [2]Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [3]Prasarnmit Hospital, Bangkok, Thailand, [4]Office of Disease Prevention and Control 1, Department of Disease Control, Ministry of Public Health, Bangkok, Thailand

Successful outcome of smear positive pulmonary tuberculosis (PTB) is necessary to control the spread of this contagious disease. A retrospective study was conducted to identify outcomes and factors associated with failure in smear positive PTB. The target population was adult, HIV negative, smear positive PTB patients treated with standard category I regimen in new cases or category II regimen in retreated cases at Prasarnmit Hospital, Bangkok, Thailand between 2003 and 2012. Of 297 patients, the outcomes were cure in 229 (77.10%), complete in 5 (1.69%), failure in 16 (5.39%), transfer out in 29 (9.76%), default in 18 (6.05%), and no death. Failure cases were compared with successful ones (cure and complete) and analyzed by Epi Info version 3.4.3. Age of more than 50 years old ,sputum smear 3+ at diagnosis and drug resistance were significantly associated with failure (p-values 0.002, 0.002, and <0.001, respectively). Symptoms such as cough, fever, haemoptysis, chest pain and weight loss were not significantly associated with failure. By using a combination of symptoms with sputum smear in analysis, patients presenting with cough, fever or haemoptysis with a smear of 3+ had a higher risk of failure when compared with those having a smear of 1+/2+ (p-values 0.004, 0.007, and 0.004, respectively). Cavitary lesions in chest radiography (CXR) at diagnosis were not significantly associated with failure (p-value 0.206). When combining symptoms with CXR, only patients complaining of haemoptysis with cavitary lesions were 8.54 times more likely to fail when compared with hemoptysis without cavitary lesions (p-value 0.038). Although we did not reach the target of an 87% success rate of smear positive PTB recommended by the WHO, we were able to identify the risk factors of failure by using symptoms plus simple laboratory tests which might be useful in resource limited areas.

1121

PREVALENCE OF LATENT TUBERCULOSIS INFECTION AND ASSOCIATED RISK FACTORS IN AN URBAN AFRICAN SETTING

Florence N. Kizza[1], Justin List[2], Allan Kizza Nkwata[1], Alphonse Okwera[3], Amara Ezeamama[1], Christopher C. Whalen[1], Juliet Sekandi[1]

[1]University of Georgia, Athens, GA, United States, [2]Yale University, New Haven, CT, United States, [3]Makerere University School of Public Health, Kampala, Uganda

The aim of the present study was to determine the prevalence of Latent Tuberculosis Infection (LTBI) and the associated risk factors in an urban African setting. This is a secondary analysis of data of a house-to-house cross-sectional survey of chronic cough that was conducted from January 2008 to June 2009. The survey included residents aged ≥15 years who were members of households visited. For our study we considered all participants who were tested with Tuberculin skin testing (TST) using the Mantoux method (183 with chronic cough and 100 without chronic cough). The primary outcome was latent TB infection (LTBI), defined as a TST with induration ≥10mm. The potential risk factors considered were; age (15-24, 25-34 and ≥35 years), being employed or a student, sex, marital status, smoking status and chronic cough. Bivariate and multivariable logistic regression analyses were used to assess the risk factors associated with LTBI. The overall prevalence of LTBI was 49% [95% CI 44-55]. Stratifying by age, and using the youngest category as a reference, the risk of LTBI increased with age (25-34, OR (95%CI) =2.03(1.78, 3.52); >=35, OR (95%CI) =3.33(1.77, 6.24). Furthermore, being a student or employed OR (95%CI) =1.88(1.17, 3.04); male OR (95%CI) =1.80(1.08, 2.98) and previously OR (95%CI) =2.22(1.13, 4.35) or currently married OR (95%CI) =2.11(1.18, 3.78) were associated with increased risk. On multivariable logistic regression analysis, age 25- 34, OR (95%CI) = 1.94(1.12, 3.38); ≥35 years OR (95%CI) = 3.12(1.65, 5.88) and being a student or employed OR (95%CI) = 1.72(1.05, 2.81) were found to be associated with LTBI. The prevalence of LTBI was high in this urban African setting. Older age and being a student or employed were factors associated with LTBI suggesting cumulative risk with age and a potential underlying risk related to expansion of one's social network outside the home. Our results provide justification for TB infection control interventions like LTBI screening and preventive treatment programs.

1122

INFLUENZA VIRUS IN SEVERE ACUTE RESPIRATORY INFECTIONS AT INTENSIVE CARE UNITS AND RESPIRATORY SPECIALIZED UNITS IN PERU. CASE CONTROL STUDY

Victor A. Laguna-Torres, V. Ocaña, S. Mendocilla, I. Paz, V. Felices, A. Barrionuevo, J. Gomez de la Torre, N. Bolaños, R. Centeno, J. Perez, JS Ampuero

Naval Medical Reseach Center Unit-6, Lima, Peru

Acute respiratory tract infection, especially due to influenza virus, remains one of the most significant causes of morbidity and mortality worldwide. Two types of syndromes may be cause by influenza A virus: 1) Milder upper respiratory tract disease or "influenza-like illness" (ILI), typically defined as sudden onset of fever (oral temperature ≥38°C or axillary temperature ≥37.5°C) and either cough or sore throat for 7 days, and 2) Severe acute respiratory infection (SARI), typically defined as ILI plus dyspnea and need for hospitalization. We conducted a matched case-control study in three civilian and two military hospitals in Peru to determine risk factors for SARI versus ILI due to influenza virus. Cases were patients of all ages with SARI and controls were ambulatory patients with ILI. Cases and controls were matched by hospital, age group (<5, 5-14, 15-

29, 30-44, 45-59, ≥60), and date of enrollment, with a 7 day maximum between cases and controls. Oropharyngeal swab and/or aspirate bronchoalveolar samples were taken and tested for influenza viruses by RT-PCR. From October 2012 to March 2014, 1374 subjects were enrolled in the study (687 in each arm).The mean age was 13.9, and the median was 2 years old, 67% under 5 years of age, and only 9% age 60 years or older, with 50% males. Influenza A virus was diagnosed in 187 patients 77 in cases and 110 in controls. SARI and ILI were equally as frequent in persons infected with influenza A H1N1 pdm09 virus (OR 1.08, 95%CI 0.62-1.89). However, SARI was significantly less frequent than ILI in persons infected with influenza A H3N2 (OR 0.36, 95%CI 0.21-0.62) and influenza B (OR 0.18, 95%CI 0.08-0.38) viruses. Factoring all sub-types of influenza virus infection, respiratory (OR 1.61, 95%CI 1.26-2.04), cardiovascular (OR 2.54, 95%CI 1.71-3.88), and other premorbid chronic diseases (OR 2.50, 95%CI 1.81-3.45) were risk factors for SARI. We conclude that influenza A H1N1 pdm09 virus is a more frequent cause of SARI than influenza A H3N2 and B viruses. We plan to next extend this analysis to other non-influenza respiratory viruses.

1123

AN EXAMINATION OF POTENTIALLY SUPPRESSIVE INFLUENCES ON INFLUENZA VACCINE EFFECTIVENESS IN THE U.S. MILITARY

Michelle J. Hiser

Armed Forces Health Surveillance Center, Silver Spring, MD, United States

The Armed Forces Health Surveillance Center (AFHSC) assesses influenza vaccine effectiveness (VE) in the US military and select civilian populations annually and semiannually. VE estimations frequently differ between service members and civilians, with higher VE estimates seen among the later. In addition, VE estimates have also varied by type of vaccine administered, with inactivated virus vaccines consistently out performing live attenuated vaccines among non-recruit military members. We propose several possible explanations for these differences. First, an important issue that may impact analysis of military members' VE is their consistently high vaccination rates (for example, in 2013-2014, over 96% were reportedly vaccinated). This could prevent valid comparisons between vaccinated and unvaccinated cases and controls since there are so few unvaccinated individuals in the analyses. Second, military members are required to be vaccinated each year. Thus, extensive vaccine-induced antigenic exposure takes place on a consistent base. Repeated exposure to these vaccines may diminish immunological response and, therefore, may lead to diminished VE. Third, the US military starts vaccinating very early in the season, typically three to four months before the Northern hemisphere usually sees a peak in influenza cases. It is theorized that the lower VE in military members could be due to a waning immunity (i.e., protective effects of the vaccine diminish quickly over a period of 3-6 months), perhaps leaving this population unprotected at the peak of the influenza season. For recent seasons, 40-50% of our military population received vaccines with live, attenuated influenza virus which have been shown to be less effective compared to inactivated virus vaccines. Data will be presented to examine potential vaccination waning within a single influenza season, and to examine the potential impact of high vaccination rates for US military members on statistical models designed to evaluate VE.

1124

EFFECT OF HELMINTH INFECTION ON TUBERCULOSIS DISEASE SEVERITY

Kari R. Meyer[1], Steveen Rios Ribeiro[2], Mary Gaeddert[3], Lisa Ganley-Leal[3], Jerrold J. Ellner[3], Reynaldo Dietze[2], Edward C. Jones López[3], Rodrigo Ribeiro-Rodrigues[2], Natasha S. Hochberg[3]

[1]Boston Medical Center, Boston, MA, United States, [2]Núcleo de Doenças Infecciosas, Universidade Federal do Espírito Santo, Vitória, Brazil, [3]Department of Medicine, Section of Infectious Diseases, Boston Medical Center and Boston University School of Medicine, Boston, MA, United States

The global burden of disease caused by tuberculosis (TB) remains high, with an estimated 12 million prevalent cases in 2012. There is a clear need to identify factors that alter the severity of TB disease and potential infectiousness to others. Recent data suggest an association between helminth infections and mycobacterial disease. This pilot study aims to describe differences in TB severity in persons with TB disease and helminth infection compared to TB disease alone. We evaluated persons with suspected pulmonary TB presenting to local TB clinics in Vitoria, Brazil and analyzed data for those with culture-confirmed pulmonary TB. We collected demographic and symptom information, sputum AFB smear and culture including colony counts, and a postero-anterior xray of the chest; up to three stool ova and parasite examinations were performed for each person. To date, we have data on 14 people. Of these, 11 (79%) were male, the median age was 28 years (range 18-72) and four (29%) had helminths in their stool, including hookworm (n=4), *Schistosoma mansoni* (n=1), and *Strongyloides stercoralis* (n=2). Those with TB and helminth infection had more people living in their house than those with TB alone (7 vs 5.4; p=0.01). Those with TB and helminth infection also tended to have more than 200 mycobacteria on colony counts compared to those with TB alone (100% vs 50%; OR=9.00; p=0.17) and involvement of ≥3 lung zones on chest xray (100% vs 60%; OR=6.23; p=0.26), but the differences were not significant. The small sample size limits our findings, but we have started to identify potential trends in clinical parameters between those with TB and helminth infection compared to TB disease alone, with the former potentially exhibiting more severe clinical disease. Further enrollment is ongoing in order to confirm these results, power the study to achieve statistical significance, and perform multivariate analyses. If the study findings hold true, treatment of helminth infections could serve as a way to reduce the severity and infectiousness of TB disease.

1125

STRENGTHENING DISEASE SURVEILLANCE AND OUTBREAK RESPONSE CAPACITY OF HUMAN AND ANIMAL HEALTH LABORATORY SYSTEMS THROUGH IMPROVED SUPPLY CHAIN, COLD CHAIN AND INFECTIOUS WASTE MANAGEMENT PRACTICES

Claudia Allers, Patrick Msipa

United States Agency for International Development | DELIVER PROJECT, Arlington, VA, United States

Under the One Health approach to detecting, preventing, and coordinating the response to zoonotic disease transmission between the human and animal health sectors, the USAID | DELIVER PROJECT, Task Order 6, supported assessments of the human and animal health laboratory supply chains for influenza surveillance and outbreak response in three high-risk Emerging Pandemic Threats (EPT) countries - Indonesia, Uganda, and Vietnam. The results highlighted the need to standardize and improve logistics, cold chain, and infectious waste management procedures and practices in the laboratories and throughout the specimen collection, storage, and transport systems. In response to the identified needs in these three critical areas, the USAID | DELIVER PROJECT: • designed and implemented a laboratory logistics system for a national influenza-like illness (ILI) and severe acute respiratory infection (SARI) surveillance program to ensure reliable supply and quality of laboratory

reagents and specimen collection supplies for routine surveillance and diagnosis of suspected cases of disease outbreaks; and timely availability of personal protective equipment (PPE) and other infection prevention and control supplies to protect health worker safety in the event of an outbreak • developed and implemented standard operating procedures (SOPs) to improve cold chain monitoring practices and maintenance of cold chain equipment for proper storage and transport of specimens from the field and at the reference laboratories • developed SOPs for effective and safe management of biomedical waste in the laboratory. A consistent supply of the appropriate laboratory reagents and specimen collection supplies is needed to ensure specimen quality and to provide timely and accurate detection of disease outbreaks. In addition, to protect health worker, patient, and field staff safety, it is equally important to ensure the availability of PPE and other infection prevention and control supplies at laboratories, healthcare facilities, and field-based collection sites.

1126

UTILITY OF STRING TEST AND STOOL FOR DIAGNOSING PULMONARY TUBERCULOSIS USING GENE XPERT MTB/RIF

Andrew R. DiNardo[1], Andrew Hahn[1], Jacinta Leyden[2], Charles Stager[1], Ellen Jo Barron[3], Edward A. Graviss[4], Anna M. Mandalakas[1], Elizabeth Guy[1]

[1]Baylor College of Medicine, Houston, TX, United States, [2]Rice University, Houston, TX, United States, [3]Stanford University School of Medicine, Stanford, CA, United States, [4]The Methodist Hospital Research Institute, Houston, TX, United States

Until recently, diagnosis of pulmonary tuberculosis (pTB), has relied on smear microscopy and culture, however microscopy is only 40-80% sensitive and diagnosis via liquid culture requires at best 7-14 days. While Xpert® MTB/RIF has significantly advanced the field of TB diagnostics, it fails to yield a diagnosis in 33-45% of smear-negative cases. Therefore, in cases in which sputum samples are challenging to obtain or in paucibacillary disease, complementary means of diagnosis are needed. We therefore compared sputum to string test and stool in 13 patients with suspected pTB having received <72 hours of anti-tuberculosis therapy (ATT). String test was performed as previously described: the capsule was swallowed and the trailing end taped to the cheek until removal by gentle traction after 4 hours. Stool was processed by two low-technology (not requiring centrifugation) methods, one using sugar flotation and the other using TB MicroSense Beads ®. Of the 13 patients with suspected pTB, 8 had culture-confirmed pTB including 2 with smear-negative pTB. The string test was well tolerated, with a median and mean Wong Baker score of 2. The Xpert from the string test was positive in 100% (8/8) of culture-confirmed pTB including both cases of smear-negative pTB. Stool was collected in 10 of 13 participants before 72 hours of ATT. Using both stool methods, Xpert detected 7 of 7 cases, however 30% of stool specimens were read as invalid. The sugar method detected 3 of 7 cases compared to 5 of 7 by MicroSense beads. Specificity was 100% for both the stool and string test including 2 cases of Non-Tuberculous Mycobacteria. This pilot highlights the potential utility of Xpert on specimens obtained from string test and stool. The string test had 100% concordance with sputum culture-confirmed pTB and does not require electricity or a trained respiratory therapist, making it an attractive means of obtaining respiratory specimens in resource-limited settings. With improved methods to remove the fibrous material, stool has the potential to be a non-invasive means of pTB diagnosis.

1127

RELATIONSHIP BETWEEN NEWS ABOUT INFLUENZA AND ILI OUTBREAKS. A SENTINEL STUDY IN PIURA, PERÚ

Victor R. Ocana Gutierrez

National University of Piura, Piura, Peru

ILI outbreaks reported in the sentinel sites relates to movement of new strains of virus or susceptibility of individuals, among others. Our country has not studied the relationship between the news about influenza and number of cases of ILI sentinel surveillance. A retrospective study (2013) in Piura was performed to determine the association between the publication of news about H1N1 influenza pdm09 and the number of visits for ARI / ILI at sentinel site. In one year the number of cases of ILI, ARI and news published in 6 newspapers is recorded. The correlation between the number of published news per week with the number of cases of ARI / ILI and relationship with two outbreaks of ILI is calculated. A total of 4,172 IRA cases and 1,316 of ILI, 86 cases of Influenza A and 36 influenza B are reported by RT; ILI cases correlate with influenza A virus ($p < 0.05$). Also 373 news on influenza A H1N1 pdm09 are published; the news content is 25.3 % of cases, 23.9 % of deaths, 23.7 % of prevention, 17% of the influenza vaccine, and 9.4 % on other issues related to Influenza A; 85 % of stories about cases and 95 % of deaths were from other cities. Total news published correlates with ILI, but not with the IRA cases. News of most newspapers correlate with ILI cases, but only one with ARI. ILI cases correlate with the news of flu cases, deaths from influenza and prevention issues ($p < 0.05$). Also shows that after a peak news happens peak ILI. At first outbreak of ILI in late summer 11 news (average 2.06 - range 0-6) per week spread over Influenza A virus , at the second in late winter diffuse 331 (average 22.07 , range 0 to 90). In the first outbreak diffuse 1/11 news on cases, no of deaths, 3/11 on vaccines, 3/11 for the prevention and 4/11 others; in the second 87/331 on cases, 85/331 deal deaths, 55/311 on the vaccines, 77/331 on prevention and 27/331 others. There are significant differences in both outbreaks: in the first outbreak the average age is 18.44 (SD 19.39) years, the influenza A H3N2 virus predominates, go elsewhere 40.5 % of ILI cases, detected 61.8% of cases of influenza A with rapid tests; in the second, the average age is 20.99 (SD 19.01), Influenza A H1N1 pdm09 predominates, go elsewhere 55.5 % of ILI cases, 38.0 % of cases of influenza A with rapid test is detected. Therefore there is a relationship between the increase in influenza A H1N1 pdm09 news and features of ILI outbreaks as older age, lower proportion of influenza virus identified and greater attention seeking ILI at sentinel site.

1128

KNOWLEDGE AND PRACTICES REGARDING TB PREVENTION IN MEDICAL STUDENTS FROM A PUBLIC UNIVERSITY OF LIMA, PERU

Percy Soto-Becerra[1], Carlos Diaz[1], Edwin Moreno[1], Danitza Cárdenas[1], Gianina Herrera[1], Paolo A. Wong[2]

[1]Universidad Nacional Mayor de San Marcos, Lima, Peru, [2]Instituto Peruano de Investigación en Ciencias Médicas IPICMED, Lima, Peru

Medical students are in high risk for nosocomial transmission of tuberculosis (TB) during their clinical practices. Adequate knowledge about TB and practices of biosecurity measures, particularly use of respiratory protective masks, are important to prevent nosocomial infection. This is the first study about knowledge and practices in Peruvian medical students. We aimed to describe the knowledge and practices regarding TB among medical students from a public university in Lima, Peru. In December 2012, a self-administered questionnaire-based survey was carried out in third-year medical students from San Marcos National University during the first clinical course of their career. Information was obtained about sociodemographic profile, knowledge about symptoms and ways of TB transmission, and use of masks (N95 or similar). Among 110 respondents, only 50% correctly recognized cough, coughing up blood, fever, night sweeting, fatigue and weight loss, as main symptoms of TB. In addition, 56.36% correctly answered that coughing, sneezing and speaking can transmit TB, and 60% incorrectly considered to share meals, drinks, utensils or cups, as ways of TB transmission. During the 15 weeks that lasted their clinical practice in teaching hospitals, 4% never used any respiratory protective mask, 55% used 1-5 masks, 30% used 6-14 masks, and only 11% used an optimal number of masks (≥15). Those findings suggest that an important proportion of Peruvian medical students are not aware of the main symptoms and routes of TB transmission. Furthermore, many students engage in risky behaviors: 89% used a suboptimal number

of masks during their clinical practice. Moreover, most of them have incorrect knowledge associated to TB stigma. We recommend active learning experience to improve knowledge and promote use of respiratory protective masks.

1129

PREVALENCE AND TRENDS OF RESISTANCE TO FIRST LINE DRUGS IN A HIGH TUBERCULOSIS BURDEN AREA IN LIMA, PERU

Giancarlo A. Valle[1], Renato Quispe[1], Tatiana Cáceres[1], Eduardo Gotuzzo[2], Carlos Zamudio[1], Carlos Seas[2]

[1]Instituto de Medicina Tropical "Alexander von Humboldt", Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Departamento de Enfermedades Infecciosas, Tropicales y Dermatológicas, Hospital Nacional Cayetano Heredia, Lima, Peru

The last Peruvian national surveillance of drug resistance for tuberculosis (TB) included isolates from 2005-2006 and reported high resistance patterns. However, TB prevalence in Peru varies greatly by region, with highest TB concentration in Lima. Few information exist about resistance patterns in these areas. This study reports drug sensitivity prevalence and trends from 2007-2011 in San Juan de Lurigancho (SJL), the most populated district of Lima and one of the highest reported cases of TB. Data from respiratory symptomatic TB suspects enrolled in parallel diagnostic trials during 2007-2011 were included. Isolates from TB patients; confirmed by Löwenstein-Jensen (LJ) cultures and positive Capilia test; were processed for drug susceptibility test (DST) using proportion method in LJ for isoniazid (INH) rifampin (RIF), streptomycin (SM) and ethambutol (EMB). Wayne test was performed for pyrazinamide (PZA). Results were categorized according to previous TB history and divided in two periods (2007-2008, 2009-2011). Annual-trends of resistance prevalence for each drug were evaluated. TB was confirmed in 1027 patients, all with final DST result. Pan-susceptible isolates were 695 (67.7%). Overall resistance isolates were: INH 207 (20.2%), RIF 119 (11.6%), EMB 91 (8.9%), SM 239 (23.4%), PZA 40 (4%), and multidrug resistance (MDR) were 105 (10.2%). In 780 naïve TB patients, resistant patterns were: INH 133 (17.1%), RIF 61 (7.8%), EMB 60 (7.7%), SM 167 (21.4%), PZA 16 (2.1%), and MDR 54 (6.9%). In naïve TB group, comparison of DST patterns during the 2 evaluated periods showed a not significant decrease in RIF, SM, PZA and MDR, and a not significant increase in INH. Increase in resistance patterns was founded (p=0.003) for EMB with 24/453 (5.3%) vs 36/327 (11%) with an increasing annual trend (p=0.0034). We found a significant increasing trend for EMB resistance. Special concern on resistance surveillance should be encouraged especially in TB hot spots like SJL.

1130

MOLECULAR DIFFERENTIATION OF *ENTAMOEBA* SPP. AMONG CAMEROONIAN HIV PATIENTS

Pechangou N. Sylvain[1], Upninder Kaur[2], Kapil Goyal[2], Moundipa F. Paul[3], Rakesh Sehgal[2]

[1]University of Yaounde 1 and Postgraduate Institute of Medical Education and Research, Yaounde and Chandigarh, Cameroon, [2]Postgraduate Institute of Medical Education and Research, Chandigarh, India, [3]University of Yaounde 1, Yaounde, Cameroon

Entamoeba histolytica is an important cause of dysentery. *Entamoeba* spp. has been reported to colonize with increased frequency among HIV positive individuals. Routine microscopic examination of stool sample is most widely used technique but microscopy alone has low sensitivity and it is insufficient for differentiation between *Entamoeba* spp. Molecular techniques are newer methods which are currently used for the identification of *Entamoeba* species. The present study was planned to investigate the *Entamoeba* species and its genotypes by gene sequencing for the confirmation of microscopic findings of stool samples of HIV positive patients of Cameroon. Twenty eight stool samples diagnosed positive for *Entamoeba* spp. by microscopy were collected from Cameroonian HIV patients and studied for the differentiation of *Entamoeba* species. DNA was extracted from infected stool samples and used to amplify a part of the genus *Entamoeba* small-subunit ribosomal RNA gene (16S-like SSUrDNA) as well as the serin riched *Entamoeba histolytica* protein gene and chitinase gene. The 16S-like SSUrDNA was sequenced to identify the other species that could not be done by PCR and for the differentiation of *E. histolytica* from *E. dispar* and *E. moshkovskii*. Sequence analysis identified six different species of *Entamoeba* which were related to *Enamoeba*; *E. histolytica* (27.59%), *E. dispar* (13.79%), *E. moshkovskii* (3.45%), *E. coli* (20.69%), *E. hartmanni* (6.9%), *E. polecki* (10.34%), and *E. struthionis* (10.34%). The phylogenetic analysis within the sequences of *E. histolytica* isolates showed that two distinguishable variants are present among Cameroonian HIV patients. Thus, there is a possibility that specific genotypes are more prevalent among HIV positive patients and molecular diagnosis is of utmost importance in establishing the correct diagnosis of amoebic dysentry.

1131

ESTABLISHING WHETHER *GIARDIA INTESTINALIS* HAS A PROTECTIVE ROLE AGAINST THE INCIDENCE OF DIARRHEA AND ITS ASSOCIATED MORBIDITY, DURING THE FIRST 24 MONTHS OF LIFE IN A BIRTH COHORT IN RURAL TROPICAL ECUADOR

Julie F. McManus

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Giardia intestinalis is a common protozoal infection (approx. 2.8 billion cases worldwide/year).The aim of this research is to establish whether *G. intestinalis* has a protective role against the incidence of diarrhoea and its associated morbidity.The study included sub-cohort of 195 children from the ECUAVIDA study.Stool samples have been collected at 7, 13, 18, and 24 months and samples were collected whenever the child has an episode of diarrhoea.PCR was used to detect *G. intestinalis*-specific DNA in the stool samples.Exposure for stunting/wasting was a positive stool sample collected at 13 and 24 months. Exposure for incidence of diarrhoea between 7 and 13 months was a positive stool sample collected at 7 months of life when child is asymptomatic and for incidence of diarrhoea between 13 and 24 months will be a positive sample collected at 13 months. The outcome observed is the number of acute diarrheal episodes each separated by 7 days free of diarrhoea. Anthropometric measurements of length and weight were taken at 13 and 24 months. Length-for-age, weight-for-age and BMI-for-age Z scores were calculated. Student t tests, Pearson's Chi-Squared analysis and uni- and multivariate regression analysis were carried out for the statistical analysis of results.There was no significant difference in the mean number of ADEs in those with and without Giardia in the two age-groups. Multivariate regression analysis revealed no significant associations between *G. intestinalis* infection and the incidence of acute diarrhoeal episodes between 7-13 months (RR=0.508, CI 0.165-1.562, p=0.237) and 13 to 24 months (RR=1.078, CI 0.694-1.676, p=0.738). Furthermore, no significant relationship could be established between Giardia infection and stunting. However, children who had *Giardia* infection at 7 months were less likely to have stunted growth at 13months, thus infection with Giardia demonstrated a protective effect, which remained when adjusted for age and sex. *G. intestinalis* infection was not associated with the incidence of diarrhoea in this cohort. There were no significant associations demonstrated between *G. intestinalis* infection and stunting/or BMI-for age scores. *Giardia* was significantly predictive of wasting in children at 13 months.This study questions the status of giardiasis as an important parasitic disease and suggests a review may be needed into treatment protocols in the rural tropics.

1132

CLINICAL EVALUATION OF *E. HISTOLYTICA* QUIK CHEK - A RAPID CASSETTE IMMUNOASSAY FOR THE SPECIFIC DETECTION OF *ENTAMOEBA HISTOLYTICA* IN HUMAN FECAL SPECIMENS

Blake R. Hanbury[1], Hans P. Verkerke[2], Abdullah Siddique[3], Rashidul Haque[3], William A. Petri, Jr.[2], Joel F. Herbein[1]

[1]TechLab, Inc., Blacksburg, VA, United States, [2]University of Virginia, Charlottesville, VA, United States, [3]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Entamoeba histolytica is responsible for 100 million cases of amebiasis annually, causing diarrhea, dysentery, and colitis. Microscopic examination of fecal specimens for the presence of *E. histolytica* can approach 10% sensitivity. False positive results occur due to the morphologically-identical and non-pathogenic *E. dispar,* which can be 3-10 times more prevalent. Immunoassays and laboratory PCR assays have improved diagnosis; however, no test has combined a rapid format with specific identification of pathogenic *E. histolytica*. Two commercial microwell ELISAs provide specific detection of *E. histolytica*, but all other immunoassays suffer from poor specificity due to co-detection of *E. dispar*. These data describe results to date for an ongoing clinical evaluation of the *E. HISTOLYTICA QUIK CHEK* (*EHQC* - TECHLAB®, Inc.), the first rapid cassette-based immunoassay for the specific detection of *E. histolytica* in human fecal specimens. The *EHQC* and the ProSpecT *Entamoeba histolytica* microwell ELISA (*ProSpecT*, Remel) are being compared to the *E. HISTOLYTICA II* microwell ELISA (*EHII*, TECHLAB®, Inc.) for specific detection of *E. histolytica* in human fecal specimens from an endemic site in Bangladesh and a US clinical reference laboratory. To date, 160 retrospective specimens tested include *Entamoeba* spp. negatives, *E. histolytica* positives and *E. dispar* positives. Immunoassay-discrepant specimens are further evaluated by *Entamoeba* spp. specific PCR. Compared to *EHII* and PCR, the *EHQC* displays 99% correlation and the *ProSpecT* displays 91% correlation; 13 of the 14 *ProSpecT* discrepants are due to PCR-confirmed *E. dispar* positives. Analytical testing with cultured *E. histolytica* trophozoites determined a limit-of-detection of 586 trophozoites/mL of original specimen for *EHQC* and 1172 trophozoites/mL of original specimen for *ProSpecT*. The *ProSpecT* assay was the only test that reacted with cultured *E. dispar*. The *E. HISTOLYTICA QUIK CHEK* provides a rapid format for sensitive and specific detection of pathogenic *E. histolytica* without non-pathogenic *Entamoeba* cross reactivity.

1133

IN VIVO DISTRIBUTION OF MYELOPEROXIDASE IN A BALB/C MOUSE MODEL OF AMEBIC LIVER ABSCESS

Luz María Cárdenas-Jaramillo[1], Juan Manuel Gutiérrez-Meza[2], Rosa Adriana Jarillo-Luna[1], Víctor Rivera-Aguilar[3], Rafael Campos-Rodríguez[4], **Judith Pacheco-Yepez**[2]

[1]Coordinación de Ciencias Morfológicas, Escuela Superior de Medicina, Mexico City, Mexico, [2]Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina, Mexico City, Mexico, [3]Microbiología, UBIPRO, Fes-Iztacala, Mexico City, Mexico, [4]Departamento de Bioquímica, Escuela Superior de Medicina, Mexico City, Mexico

The invasive protozoan Entamoeba histolytica is the etiologic agent of amebiasis. There is great debate among researchers whether the pathogenic mechanisms of *E. histolytica* cause amebic liver abscess (ALA) or are principally employed by the ameba for survival and proliferation in the host organism. It is known that the majority of individuals are resistant to *E. histolytica*, and that in resistant individuals an appropriate immune response clears pathogens. The BALB/c mouse model is used to explore the mechanisms of resistance to *E. histolytica*. Neutrophils are the most abundant cells of the inflammatory response and the principal killers of amebae. These immune cells have various components with anti-amebic activity, such as myeloperoxidase (MPO). In an earlier work we demonstrated that MPO binds to *E. histolytica* and induces morphological changes. The aim of the present study was to explore to the importance of the participation of MPO in the resistance to hepatic tissue damage during the pathogenesis of amoebiasis in the BALB/c mouse model. BALB/c mice were inoculated with *E. histolytica* and then sacrificed at 3, 6 and 12 h post-inoculation. In order to study the presence of MPO, liver samples were processed for immunohistochemical MPO analysis. The *in situ* expression of MPO was also studied by qRT-PCR in order to determine the expression of this enzyme in the ALA. At 3 h post-inoculation, amoebae were surrounded by neutrophils stained for the MPO enzyme. At 6 h, inflammatory foci composed of neutrophils were positive for the presence of MPO. At 12 h of ALA evolution, acute and chronic inflammatory cells were labeled for MPO. The qRT-PCR of MPO mRNA revealed the expression of the enzyme in the ALA tissue. The results demonstrate the induced expression of mRNA for MPO in immune cells of hepatic lesions, suggesting that this enzyme is synthesized in response to the amoebic infection. Therefore, the resistance of the mice to *E. histolytica* probably lies in the nonspecific immune response, and MPO activity is apparently important in this sense.

1134

NAEGLERIA SP. IN TOURIST PONDS OF LIMA, PERU

Giancarlo Castillo[1], Marcos Chambi[1], Eliza Contreras[1], Luis Crispin[1], Yrma Espinoza[2]

[1]Universidad Nacional Mayor de San Marcos, Lima, Peru, [2]Instituto de Medicina Tropical Daniel A. Carrión, UNMSM, Lima, Peru

Naegleria fowleri causes primary amoebic meningoencephalitis, which affects immunocompromised and immunocompetent persons, and has a mortality rate near 100%. A recognized source of infection is direct contact with contaminated water, such as ponds, lakes, thermal baths or pools. Ponds of Chilca (also known as "La Encantada", "La Mellizera" and "La Milagrosa") are located 68 km south of Lima, Peru. Ponds of Chilca are tourist attraction that receives about 300,000 visitors a year because people believe in the healing properties of their water. The purpose of this study was to isolate *Naegleria* sp. from the water of the three ponds of Chilca and to provide information about the potential risk for the health of residents and tourists. Thirty water samples were collected in one liter containers, ten for each pond: half from surface water and half from one meter deep. After sedimentation, each sample was separated into two tubes with non-nutritive agar 2% with *Escherichia coli* inactivated at 56°C, and was cultured for 14 days at two temperatures, one at environmental temperature (20-25°C) and the other at 37°C. It was observed through the microscope at 10X and 40X. *Naegleria* sp. cysts were found in 40% (12/30) of samples. Forty-two percent of positive samples (5/12) were from "La Milagrosa"; 33% (4/12) were from "La Encantada" and 25% (3/12) were from "La Mellizera". Proportion of positive samples by each pond was 50% (5/10) in "La Milagrosa"; 40% (4/10) in "La Encantada" and 30% (3/10) in "La Mellizera". Approximately 67% (8/12) of positive samples came from deep water. This finding suggests that ponds of Chilca could be a source of transmission of *N. fowleri*, and there is a potential risk of disease in people and tourists who dive into their water. Therefore, we recommend immediate preventive activities.

1135

ENTAMOEBA DIVERSITY IN DISADVANTAGED BANGADESHI CHILDREN

Rashidul Haque[1], Mamun Kabir[1], Karla Pamela Elliott[2], William A. Petri, Jr.[2], **Carol A. Gilchrist**[2]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]University of Virginia Health Science Center, Charlottesville, VA, United States

Entamoeba histolytica is the causal agent of amebic diarrhea and dysentery in young infants in developing countries. The recent identification of a novel *Entamoeba* species *E. bangladeshi* and the discovery that *E. moshkovski* could also cause diarrhea instigated a study

into diversity of *Entamoeba* spp in children living in an urban slum in Dhaka, Bangladesh. In this study a next generation sequencing approach was used to characterize nineteen samples that by microscopy contained ameboid trophozoites or cysts but which could not be assigned to an *Entamoeba* species by conventional qPCR or immunodiagnostic methods. Broad range primers (adapted for next generation sequencing) were used to amplify the 18S rDNA region previously used for taxonomic assessment in the *Entamoeba* genus. However to provide more detailed information, broad range primers targeting regions within the actin gene and heavy subunit of the Gal/GalNac Lectin were used to develop a multilocus typing system. One of the sequenced samples (8170) contained a novel *Entamoeba* variant. While the 18S region of the sequenced DNA was identical to that of the *E. bangladeshi* gene, the actin and Gal/GalNAc transmembrane regions were markedly divergent. The biological effect of these changes is unclear at present (the variations in the novel *Entamoeba* resulted in either no changes in the encoded amino acids or a conserved substitution) and currently we have no evidence that either *E. bangladesi* or *Entamoeba-8170* have the capacity to cause disease. The significance of this finding is that we have proven that an unplumbed diversity exists in the *Entamoeba* species circulating in Bangladesh. Preliminary evidence suggests that structural re-assortment is common in the parasitic *E. histolytica,* and the finding that several closely related species exist in endemic populations suggest that these could provide a reservoir of diversity that the parasite could draw on.

1136

PREVALENCE OF INTESTINAL PARASITES IN SCHOOL-AGED CHILDREN WITHIN THE FRAMEWORK OF THE FIGHT AGAINST NEGLECTED TROPICAL DISEASES (NTDS) IN THE CITY OF LOMÉ, TOGO

Monique A. Dorkenoo[1], Kossi Yakpa[2], Anna N. Chard[3], Emma A. Moore[3], David Plate[3], Gédéon Attiogbe[4], Efoué Soussou[2], Poukpessi Adjeloh[2], Amétépé Kivi[2], Kwame C. Amlaga[2], Harishu Dokoto[5], Mawèkè Tchalim[2], Kossi M. Mensah[6], Rachel Bronzan[7], Koffi S. Sognikin[2]

[1]Ministry of Health and University of Lome, Lomé, Togo, [2]Ministry of Health, Lomé, Togo, [3]Hope Educational Foundation International, Inc., Pompano Beach, FL, United States, [4]Hope Educational Foundation International, Inc., Togo Representation, Lomé, Togo, [5]Faculté des Sciences de la Santé, University of Lome, Lomé, Togo, [6]Ministry of Primary and Secondary Education, Lomé, Togo, [7]Health and Development International, Seattle, WA, United States

In Togo in 2009, a nationwide survey of the prevalence of soil-transmitted helminths (STH), schistosomiasis and trachoma was conducted and, based on the results, nationwide MDA was started in 2010. The region of Lomé, which includes the capital city and its environs and represents around one fifth of the population of Togo, was considered a low-risk zone for these neglected tropical diseases (NTDs) based on available data and was excluded from the national mapping, Yet a high prevalence of NTDs has been found in other countries in urban areas that were assumed to be low-risk,. and school-aged children constitute a risk group for many intestinal parasites. In order to not lose the opportunity to treat exposed children in this region, we conducted a study to determine the prevalence of intestinal parasites in the school-aged population. Lomé region's five districts were each considered as separate ecological entities, based on their specific geographic and climatic characteristics. In each district, 5 primary schools were randomly selected and 30 children from each of the third, fourth, fifth and sixth grades were tested. The Kato-Katz method was performed to identify STH species on fresh stool samples collected from the children on site on the morning of the survey day. Direct examination using Lugol's solution was added to identify protozoa for the children in the 3rd and 6th grades. Across the five districts, 2,944 school-aged children aged 6-15 years, representing 25 schools, were tested. The mean prevalence of STH was relatively low, 5% across all sites, ranging from 1.5% to 8.6% at district level; prevalence also varied with the child's sex. The prevalence increased with age and with grade level. Protozoa were found in 52.2% of the 1,465 children tested and were represented primarily by *Entamoeba histolytica* (51.3% of all children examined). Double and triple infections were noted in 10% of cases. *Ancylostoma duodenale* and *Necator americanus* were the species of STH most frequently identified (3.4%) and infection was light in 80% of cases. *Schistosoma mansoni* was found in 0.3% of cases. The findings of this survey confirm that it is not indicated to extend integrated MDA to Lomé region, but instead highlighted the high prevalence of protozoa, and in particular of *E. histolytica,* in the school-aged population. These results demonstrate the need for a national strategy to address the high prevalence of intestinal protozoa in school-age children.

1137

HISTOPATHOLOGICAL DESCRIPTION OF PROTOZOANS AND HELMINTHS IDENTIFIED IN SMUGGLED TURTLES AND TORTOISES IN BANGKOK

Eric Lombardini[1], Kwanta Chayapum[2], Maethira Lerthiranwong[2], Thanida Haetrakul[2], Nantarika Chansue[2]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]VMARC, Bangkok, Thailand

In January of 2014, upwards of 500 tortoises and turtles were confiscated from the bags of a single smuggler at Bangkok's International Suvarnabhumi Airport. Many of these animals died during the process and complete necropsies were performed. Histopathologic diagnosis of parasites observed in tissue section remains one of the more challenging mechanisms of disease identification, however it also allows for a rapid holistic mechanism of diagnosis of parasitic disease. Histopathological evaluation of all submitted tissues revealed a wide range of nematodiasis, trematodiasis, and protozoal diseases in various organ systems. In each case, the genus of the parasites was identified through descriptive microscopic criteria and through consultation and concurrence with other board certified veterinary pathologists. The identified parasites included renal myxozoanosis, intestinal ascaridiasis, multiorgan spirorchiasis, multiorgan coccidiosis, suspected biliary serpinemiasis as well as other unidentified intestinal nematodes. Microscopic descriptions of helminths hinged on location within the body, concurrent tissue pathology, measurements of size, external ornamentation, lateral chords and alae, type of musculature and hypodermis, presence or absence of a body cavity and gastrointestinal tract, and various features of the gastrointestinal tract and reproductive organs as well as ova or larvae. Protozoal identification similarly was governed by phenotypic characteristics of the parasite in tissue sections, to include location in tissue and concurrent pathology, size, shape, number, shape and location of nuclei as well as a wide range of minute identifying structural features. Without speciating the parasites it is impossible to assess their zoonotic potential, however their introduction into a naïve environment presents the possibility for the emergence of disease in novel hosts or even of epizootic outbreak. While most of the focus remains on the potential spread of viral diseases such as H5N1 influenza virus or monkeypox, parasitic infections can be host adapted, clinically silent and as such present a more subtle and insidious etiology. To our knowledge, this is the first histopathological survey of parasitic disease of smuggled testudines.

1138

DETECTING *CRYPTOSPORIDIUM*: A COMPARISON OF MICROSCOPY, IMMUNOFLUORESCENCE, RAPID TESTS AND RT-PCR

Susan Madison-Antenucci, Noel Espina, Allen Teal

Wadsworth Center, New York State Department of Health, Albany, NY, United States

Cryptosporidium is the cause of numerous outbreaks of intestinal illness due to contaminated water, especially recreational water, in first world countries and world wide is a major cause of diarrheal disease, particularly in children under five years of age. *Cryptosporidium* passes easily from

person to person due to a low infective dose, high numbers of oocysts shed from infected individuals, resistance to common disinfection methods, oocysts that are both immediately infective after passage and are hardy in the environment. There is only one drug that has been FDA-approved for treatment. Nitazoxanide is up to 75% effective in eliminating *Cryptosporidium* and can take up to five days to alleviate the diarrhea associated with infection. There are no vaccines or prophylactic treatments for cryptosporidiosis. Thus detection of the parasite is key to identifying sources of outbreaks and taking special measures to stop the spread of infection. Traditionally detection has been *via* acid fast staining and microscopy. However, microscopic methods are labor intensive and require specially trained personnel. In response to a need for easier means of diagnosis, rapid test that use immunochromatographic methods have been developed to detect parasite antigens. While these are approved for clinical use, in practice the sensitivity and specificity of some rapid tests has been found to be disturbingly low. To evaluate detection methods we compared results from clinical specimens tested by, staining and microscopy, immunofluorescent microscopy and a rapid test. Further, we developed a real time PCR assay and that was used to resolve discrepant results. The RT-PCR assay specifically identifies *C. parvum* and *C. hominis*, the two species that cause the majority of infections in humans, and also detects other species at the genus level. A comparison of the sensitivity and specificity of the methods will be discussed along with identification of unusual species.

1139

CRYPTOSPORIDIUM INFECTION IN RURAL GAMBIAN CHILDREN: EPIDEMIOLOGY AND RISK FACTORS

M. Jahangir Hossain[1], Debasish Saha[1], Martin Antonio[1], Dilruba Nasrin[2], Usman N. Ikumapayi[1], Grant A. Mackenzie[1], Mitchell Adeyemi[1], Muhammad K. Abdul[1], Momodou Jasseh[1], Richard A. Adegbola[1], Stephen Howie[1], Karen L Kotloff[2], Myron M Levine[2]

[1]Medical Research Council Unit, The Gambia, Banjul, Gambia, [2]Centre for Vaccine Development, University of Maryland, Baltimore, MD, United States

The Global Enteric Multicenter Study (GEMS) documented *Cryptosporidium* as the 3rd commonest pathogen of moderate-to-severe diarrhoea (MSD) in children age <5 years. We investigated the epidemiology and risk factors for *Cryptosporidium* diarrhoea among children enrolled in GEMS from an enumerated population in rural Gambia. We recruited MSD cases (Dec. 2007- Dec. 2010), and cases with both MSD and less severe diarrhoea (LSD) (Nov. 2011-Nov. 2012) presenting at health centres, along with 1-3 controls matched for age, sex and community within two weeks of recruitment of cases. A questionnaire generated information on socio-demographic, water, sanitation and presence of animals in the compound. Each subject provided a stool sample to identify enteropathogens, including *Cryptosporidium* by immunoassay. We described the prevalence of *Cryptosporidium* in diarrhoea cases and their controls. Case-control analysis determined the association of risk factors with *Cryptosporidium* positive diarrhoea compared to matched controls negative for *Cryptosporidium.* We enrolled 1938 cases (1381 MSD, 557 LSD) and 2969 matched controls; 231 (11.9%) diarrhoea cases and 141 (4.7%) controls were positive for *Cryptosporidium*. Most *Cryptosporidium* diarrhoea cases (198/231, 86%) were aged 6-23 months and presented during the May-October rainy season (188/231, 81%). *Cryptosporidium* prevalence was similar between MSD and LSD (12.1% vs. 11.5%, p=0.711). Independent risk factors for *Cryptosporidium* diarrhoea were the compound having a cow (aOR 2.9, 95% CI 1.6-5.2), a cat (aOR 2.0, 95% CI 1.1-3.7) or rodents (aOR 2.1 95% CI 1.1-4.0), consumption of stored drinking water (aOR 4.1, 95% CI 1.8-9.7), and rainy season (aOR 25.2, 95% CI 4.4-146.0). In conclusion, continued surveillance is essential to assess the *Cryptosporidium* burden. Sheltering animals outside the compound, improved hygienic practices and treatment of drinking water should reduce *Cryptosporidium*-associated diarrhoea.

1140

DETECTION OF *NAEGLERIA FOWLERI*, *ACANTHAMOEBA* SPP. AND *BALAMUTHIA MANDRILLARIS* IN FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUES BY REAL-TIME MULTIPLEX PCR

Andrew P. Norgan, **Lynne M. Sloan**, Caterina Giannini, Bobbi S. Pritt

Mayo Clinic, Rochester, MN, United States

The free-living amoebae (FLA), *Naegleria fowleri*, *Acanthamoeba* spp. and *Balamuthia mandrillaris*, can cause significant human ocular and/or central nervous system (CNS) infections with high associated morbidity and mortality. There have been several recent efforts to develop molecular methods (e.g., multiplex PCR, isothermal amplification) for the diagnosis of FLA in CSF, fresh tissue and other clinical specimens with excellent sensitivity and specificity (e.g., triplex PCR assay described by Qvarnstrom et al, Centers for Disease Control and Prevention; reported limit of detection of 1 organism per sample). However, the performance of such assays has been less rigorously evaluated using formalin-fixed paraffin-embedded (FFPE) tissue, an important specimen type that presents challenges for DNA recovery. Twenty-eight human corneal or brain FFPE specimens with FLA infection diagnosed by histopathology (gold standard) as well as 11 FFPE human tissues from patients not suspected to have FLA infection were tested in duplicate by multiplex PCR (adapted from Qvarnstrom et al). Tissue sections (50-60 µm) were digested with proteinase K followed by DNA extraction using the Roche MagNA Pure system. Nucleic acid amplification and detection were performed using the Roche LightCycler 480. Twenty-two of the 28 positive FFPE specimens were detected by PCR (sensitivity 78.6%), with all organism-specific positive results matching the corresponding histopathologic diagnoses. One false positive Acanthamoeba sp. result was detected in a section of brain also confirmed to have *Naegleria fowleri* by histopathology and PCR but no positives were detected in the negative FFPE control specimens (specificity 91.7%). Given the ubiquitous environmental distribution of *Acanthamoeba*, this may represent exogenous contamination of the tissue or paraffin block. No inhibition was noted in the PCR reactions based on amplification of an internal control in each reaction. Although sensitivity was reduced using FFPE specimens, the Qvarnstrom et al. triplex FLA PCR assay may serve as a valuable tool for detection and confirmation of FLA infections in ocular and CNS specimens when fresh specimens are not available.

1141

SEROPREVALENCE OF TOXOPLASMOSIS AMONG CHILDBEARING WOMEN IN BAGHDAD DURING 2013

Sumaia B. Hassan[1], K. Muftin Al-Zadawi[1], D. Adnan[1], J. Kamil[1], Yousif Jasim[2], Yousif Jasim[2], K. Yahya Obaid[2]

[1]Zoonoses Diseases Section, Centers for Disease Control and Prevention, Ministry of Health, Baghdad, Iraq, [2]Central Public Health Laboratories, Baghdad, Iraq

Toxoplasmosis is a global parasitic disease caused by the protozoan *Toxoplasma gondii,* with estimates that up to a third of the global population and up to 95% in some subpopulations have been infected. Official assessment in the United Kingdom places the number of new infections at about 350,000 per year in the UK. In Iraq toxoplasmosis is widely endemic but often underreported since not all cases seen in private clinics are reported to the Ministry of Health. During the years 2009 to 2013, the official incidence of toxoplasmosis was reported to have doubled from 692 to 1390. We designed a study to estimate the incidence of toxoplasmosis in women of childbearing ages between 14 and 45 years old and to determine risk factors associated with seroconversion rates. There were 950 subjects recruited for this study and blood samples were collected at four health centers including two in Baghdad, one in Alkarkh and another in Al-Rusafa. Samples were tested for IgG and IgM specific antibodies by ELISA. Demographic data for each subject was collected

and stored in a database, including personal information and a series of questions related to possible risk factors. Our study revealed that the mean age of a patient with positive ELISA response for *T. gondii* was 24 years. There were 72 subjects who were IgM positive and 159 IgG positive. IgG or IgM antibodies indicative of toxoplasmosis were found in 89.9% of house wives. There was no significant difference between observed rates of seroconversion between students and employees (4.5% and 4.8%, respectively), and 0.8% did not complete the questionnaire. There was an association between positive ELISA responses and contact with stray house cats (43.3%), eating unclean vegetables (13.9%), eating outside the house in restaurants (12.6%) and failure to regularly wash hands (19.5%.) In conclusion, our study confirmed the high incidence of toxoplasmosis in Iraq, the public health importance of this disease, and identified some possible risk factors for disease transmission in women of childbearing age. This information will be very useful for new public health education campaigns and control programs throughout Iraq.

1142

DETECTION OF "EARLY WARNING" MONITOR MICE USING PROTEOMIC CLINPROTOOL ALGORITHM ESTABLISHED BY ACUTE SCHISTOSOMIASIS JAPONICA MICE

Yuzheng Huang

Jiangsu Institution of Parasitic Diseases, China, Wuxi, China

The objective of this study was to establish proteomic ClinProTool algorithm with different expressed peptides in sera of acute Schistosomiasis japonicum mice for a new rapid and accurate detection method for "early warning" monitor mice. The acute schistosomiasis japonica infection mice was generated, sera peptides were enriched from the infected and control group by MB-IMAC Cu kit separation(Bruker Daltonics GmBH), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and ClinProTool algorithm were used to generate the proteomic pattern based on the differential expressed peptides. The infected "early warning" guard mice were simulated in our labrotory, sera from different infectiosity(1, 2, 4, 6, 10, 14, 18, 22 cercaria post-infection) and different infection time (every week) were collected, after serum peptides-peaks acquired by mass spectrometry and applied with ClinProTool algorithm, which was used to generate the proteomic pattern. Sera peptides were captured in the mass range from 800 to 20 000Da, seven peaks with a clear difference in amplitude were detected between the 5-weeks post-infection and control groups, 4 peaks with mass charge ratio (m/z) of 3493, 2869, 2024 and 4965 were down-regulated and 3 peaks with a m/z of 9068, 2082 and 4533 were up-regulated in infected mice($P<0.01$). Proteomic pattern was established with the seven difference peaks. The result also showed high sensitivity and specificity of the proteomic pattern. In weeks 1 to 4 post-infection, characteristic proteomic patterns could be detected in 5%(1/20), 37%(7/19), 79% (15/19) and 85%(17/19) of the infected mice, whereas ELISA testing resulted in positive results from week 3 onwards. The infectiosity assay showed 28%(2/7), 50%(4/8), 83%(5/6) positive in 4, 6, 10 cercaria post-infection groups, respectively. And 100% positive in 14, 18, 22 cercaria post-infection groups, All T. gondii control sera were detected *S. japonicum* negative. MALDI-TOF MS coupled with peptide enrichment can be a desired method in detecting the biological markers of schistosomiasis in a mouse model. ClinProTool algorithm could be a good method for the rapid detection of "early warning" guard mice in monitoring Schistosomiasis in China. The result also cast a fundamental research in schistosomiasis in the level of peptide and protein.

1143

RAPID ASSESSMENT OF SCHISTOSOMIASIS RISK FOLLOWING AN EARTHQUAKE IN SICHUAN, CHINA AND IMPLEMENTATION OF PREVENTION MEASURES

Yang Liu

Sichuan Center for Disease Control and Prevention, Chengdu, China

On April 20, 2013 a magnitude 7.0 earthquake struck Ya'ancity, located in Sichuan, China.The earthquake caused a large number of casualties, altered the ecological and social environment and damaged medical treatment health facilities. The impacted areas were historically endemic areas for schistosomiasis transmission, raising concerns about disease reemergence. We conducted a rapid assessment of schistosomiasis risk in the area impacted by the earthquake and used the results of the assessment to rapidly deploy surveillance and control measures.The assessment included a comprehensive literature review, an analysis of schistosomiasis surveillance data in the earthquake-affected counties, and a field investigation. Based on our initial risk assessment,we formulated and carried out schistosomiasis surveillance and control measures including 1) locating displaced person settlements in areas so as to minimize exposure risk, 2) constructing carefulexcrement management systems in the displaced person settlements 3) carrying out snail control (molloscicidingor black plastic)in high-risk environments, 4) conducting health education, 5) increasing patient monitoringand treatment, and 6) expanded chemotherapy.We conducted an evaluation 40 days following the earthquake,including snail surveys,human infection surveys and health education assessments. We found no infected snails or acute Schistosomiascases in humans, but the average density of *Oncomelaniahupensis* snails was higher than observed before the earthquake (4.1snails/m^2 vs. 0.3 snails/ m^2). In the short term, the implementation of effective prevention and control measures may have helped to reduce the risk of schistosomiasis transmission in an area impacted by a natural disaster.However the rise in snail populations and the history of disease transmission in the region suggests health education, snail control and surveillance for schistosomiasis should continue.

1144

HIGH PREVALENCE OF *SCHISTOSOMA JAPONICUM* IN HUMANS AND BOVINES FROM NORTHERN SAMAR, THE PHILIPPINES

Catherine A. Gordon[1], Luz P. Acosta[2], Geoffrey N. Gobert[1], Mario Jis[2], Remigio M. Olveda[1], Allen G. Ross[3], Darren J. Gray[1], Gail Williams[4], Donald Harn[5], Donald P. McManus[1]

[1]QIMR Berghofer Medical Research Institute, Brisbane, Australia, [2]Research Institute of Tropical Medicine, Manila, Philippines, [3]Griffith Health Institute, Griffith University, Gold Coast, Australia, [4]School of Population Health, University of Queensland, Brisbane, Australia, [5]College of Veterinary Medicine, University of Georgia, Athens, GA, United States

Schistosoma japonicum is the causative agent of schistosomiasis in the Philippines with 6.7 million people living in endemic areas and 1.8 million having direct exposure through daily water contact activities. As a zoonosis *S. japonicum* infects over 40 mammalian species, including water buffalo which have been shown to be major reservoir hosts in China. In the Philippines, water buffalo (carabao) have been considered unimportant hosts due to low prevalence and infection intensity found in previous studies. High prevalence of *Fasciola gigantica* has also been reported from bovines in the Philippines. Previous studies on F. hepatica and *S. mansoni* have suggested that cross-protection occurs while anecdotal evidence from the Philippines suggests this might also be the case for *S. japonicum* and *F. gigantica*. To examine the role of bovines in *S. japonicum* transmission human and bovine (cattle and carabao) stool samples from six barangays from Northern Samar, the Philippines, were collected. Bovine samples were examined with an improved microscopy technique, the formalin-ethyl acetate sedimentation (FEA-SD), and qPCR analysis, while human samples were examined by Kato-Katz (KK) in addition to qPCR. High *S.*

japonicum prevalence was found in humans when using qPCR (90.2%), while KK showed a much lower prevalence (22.9%). High prevalence was also found in bovines when using FEA-SD (62.1%) and qPCR (81.7%). Intensity of infection was higher for cattle (geometric eggs per gram 8.3) than carabao (gmepg 4.7). The Bovine Contamination Index was calculated using the combined carabao and cattle arithmetic epg (7.8) and showed that each bovine was excreting an average of 195,000 eggs into the environment daily. Bovines also had a high prevalence of *F. gigantica* infection by both FEA-SD (96.0%) and qPCR (95.3%) techniques. The identification of bovines as a major reservoir host for *S. japonicum* in the Philippines suggests that bovines should be includes in control programs by chemotherapy and/or vaccination to reduce the burden of disease due to schistosomiasis in the Philippines.

1145

URINE MICROALBUMINURIA, PROTEINURIA AND MICROHAEMATURIA ARE MARKERS OF UROGENITAL SCHISTOSOMIASIS-RELATED MORBIDITY IN INFANTS AND PRE-SCHOOL CHILDREN

Welcome M. Wami[1], Norman Nausch[1], Nicholas Midzi[2], Takafira Mduluza[3], Mark Woolhouse[4], Francisca Mutapi[1]

[1]University of Edinburgh, Institute of Immunology and Infection Research, Edinburgh, United Kingdom, [2]National Institute of Health Research, Harare, Zimbabwe, [3]Department of Biochemistry, University of Zimbabwe, Harare, Zimbabwe, [4]University of Edinburgh, Centre for Immunity, Infection and Evolution, Edinburgh, United Kingdom

Urogenital schistosomiasis, caused by *Schistosoma haematobium* is highly prevalent in Africa. Only recently has the burden of disease in preschool children and infants aged 5 years and below began to be quantified and recognized as a significant attribute in childhood health and development. The tools for diagnosing infection in this age group are less sensitive and those for detecting morbidity poor. In addition, there is a paucity of studies assessing the performance of the available diagnostic tools for infection and morbidity in this age group. The objectives of this study were; to determine the reliability of currently available tools for measuring schistosome-related morbidity and to investigate the effect of antihelminthic treatment on these markers. The study was conducted in an endemic area in Zimbabwe. We examined several indicators using urine albumin-creatinine ratio (UACR), dipsticks, questionnaires and clinical examination at baseline in 298 children (1-5years, n=104; 6-10years, n=194) to identify morbidity markers most attributable to urogenital schistosomiasis. Microalbuminuria assessed from UACR and dipstick microhaematuria and proteinuria were significantly associated with infection. A single dose of praziquantel was given to study participants and showed 95.3% cure rate and 96.1% egg reduction rate at 12 weeks. The effect of treatment on these 3 identified markers was assessed in 92/298 children who received successful curative treatment. Prevalence of microalbuminuria at baseline (45.7%; 95%CI: 35.3-56.0%) significantly decreased (χ^2=39.1; P<0.001) to 1.1% (95%CI: 0.0-3.2%) after treatment. Similarly, there was a significant reduction in proteinuria post-treatment, but no change in microhaematuria. In conclusion, our study showed that microalbuminuria, proteinuria and microhaematuria are useful schistosomiasis-related morbidity markers in untreated children. These findings are important for planning, monitoring and evaluation of schistosome control programmes.

1146

CHRONIC SCHISTOSOMIASIS: COMPARISON OF THE EFFECTS OF TWO ROUNDS OF MASS DRUG ADMINISTRATION (MDA) VS ONE ROUND OF MDA ON PHYSICAL FITNESS

Michael O. Ogutu

KEMRI/Centers for Disease Control and Prevention, Kisumu, Kenya

Chronic schistosomiasis has been associated with reduced physical fitness, but additional evidence of the benefits of praziquantel treatment is needed. The aim of this study was to assess the impact of one and two rounds of mass drug administration (MDA) on physical fitness. The study was carried out among school aged children who were grouped into two cohorts as part of a larger investigation of most effective ways to provide MDA. A cohort that had received one round of treatment at school (SBT) was compared to another cohort that had received community wide treatment (CWT) two times in two years. At baseline and after two years, standardized quality controlled methods were used to determine helminth infections (Kato-Katz technique), hemoglobin levels, anthropometric measurements (weight and height) and physical fitness (20m shuttle run test). In this area of high transmission, there were no significant decreases in *S. mansoni* prevalence or intensity of infection in either SBT or CWT groups from baseline to the second year. However, significant increases in both *P. falciparum* infections ($p < 0.001$) and anemia ($p = 0.002$) were observed in both treatment cohorts. At two years after baseline, the proportion of children demonstrating wasting ($p < 0.001$) was decreased but physical fitness as measured by maximal aerobic capacity (VO2 max) was also significantly lower than at baseline in both treatment arms. Thus, we did not see a benefit of schistosomiasis treatment on physical fitness by either treatment approach. However, because of the high transmission levels of *S. mansoni* and *P. falciparum* during the follow up period, any praziquantel treatment benefits may have been masked.

1147

DETECTING MULTI SCHISTOSOME SPECIES DNA IN SINGLE URINE SAMPLE BY LAMP: A NOVEL DIAGNOSTIC TEST FOR SCHISTOSOMIASIS

Nilanjan Lodh[1], Kei Mikita[1], Jean M. Naples[1], Kwabena M. Bosompem[2], Joseph Quartey[2], Clive J. Shiff[1]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]Noguchi Memorial Institute for Medical Research, Legon, Ghana

Schistosomes are easily transmitted and multiply considerably so if control strategies based on targeted mass drug administration (MDA) are to succeed it is essential to have a simple to operate sensitive and accurate test. As the control programs operating become more and more effective in reducing the parasite burden in the individual, the issue of diagnostic sensitivity will become more critical in the assessment of program effectiveness. Over the past several years we have demonstrated that parasite specific DNA can be detected in human urine by PCR when some specimens are apparently negative. Importantly this method does not require stored urine, but is effective in detecting and amplifying DNA from urine residue on coarse filter paper that is dried after filtration and can be stored for several months without freezing and easy to transport. In the current study we assessed the efficacy of *Schistosoma mansoni* and *S. haematobium* specific DNA detection from 86 urine residues both by PCR and loop mediated isothermal amplification (LAMP) collected in Ghana in an area of low to moderate endemicity. We also compared the DNA extraction techniques by standard extraction kit and field usable LAMP PURE kit and have evaluated these procedures on species-specific DNA detection. With *S. haematobium* all three methods showed similar sensitivity and specificity when compared with PCR amplification (100%). For *S. mansoni* sensitivity was highest for LAMP amplification (100%) than PCR and LAMP PURE (99% and 94%). The LAMP PURE extraction produced false negatives which require further investigation for this field usable extraction kit. Overall high positive and negative predictive values (90% - 100%) for both species were also indicative of a highly robust approach. The same pattern was observed when stratified for sex specific analysis. We have demonstrated a robust measure of prevalence of both species when compared with the classical examination of urine or stool. Our approach of using urine sediment for integrated diagnosis of schistosomiasis and a common DNA extraction procedure with LAMP can be an effective means to detect low intensity infection and would enhance the effectiveness of surveillance and MDA control programs of schistosomiasis.

1148

PRAZIQUANTEL 40 MG/KG IN CHILDREN, CLINICAL EFFICACY AND SAFETY, A META-ANALYSIS

Piero L. Olliaro[1], Julien Zwang[2]

[1]World Health Organization, Geneva, Switzerland, [2]Independent, Geneva, Switzerland

Children carry most of schistosomiasis burden: school-aged children are the main target group of preventive chemotherapy (PC); preschool-aged children are treated on confirmation of infection. The objective of this aggregated data meta-analysis is to evaluate the efficacy and safety of PZQ 40mg/kg, the WHO recommended dose, on intestinal and urinary schistosomiasis in children, and to assess if efficacy varies with age. Thirty-five studies were identified through a systematic literature search, enrolling 12562 preschool- and school-aged children and adolescents, of whom 11564 were assessed within 8 weeks post-treatment. The average attrition risk of bias was acceptable (91%). 6186 (54%) were treated with PZQ tablet at 40 mg/kg. Of these, 45% (n=2765) were infected with *Schistosoma mansoni* and 7% were preschool-aged children. Calculated median age was 10 years (range 1 to 19 years). The overall cure rate (CR) and egg reduction rate (ERR) obtained with PZQ 40 mg/kg were respectively: in *S. mansoni* 75.0% (95%CI 70.2-79.6, n=2754), and 92.3% (95%CI 88.4%-95.3%, n=1927); in *S. haematobium* 76.6% (95%CI 67.4%-84.7%, n=2673) and 93.9% (95%CI 88.8%-99.0%, n=1856); in *S. japonicum* 94.7% (95%CI 92.1%-98.0%, n = 406) and 95.0% (95%CI 90.1%-99.9%, n=203); and in *S. haematobium/S. mansoni* 67.6% (95%CI 53.6%-82.4%, n=372) and 98% (95%CI 90.9%-99.9%, n=54). A multivariate mixed-effect model with random effect on study site shows a significant relationship between age and CR for *S. mansoni* (p=0.001) and *S. japonicum* (p=0.001), but not *S. haematobium*. Age has no effect on ERR. PZQ proved safe across ages, with only mild transient reported adverse events. There is no clear evidence that the PZQ dose should be adapted to age, especially if the objective of the intervention is morbidity reduction in the context of PC (efficacy assessed by ERR rather than CR). However, data for PZQ tablet on preschool-aged children are limited to one country and *S. mansoni*, and studies reported on broad age-categories, making it difficult to derive conclusive estimates for age effect in small children.

1149

STRATIFIED WORM BURDEN APPROACH TO MODELING SCHISTOSOME TRANSMISSION IN AT-RISK POPULATIONS

Nara Yoon, David Gurarie, **Charles H. King**

Case Western Reserve University, Cleveland, OH, United States

Schistosoma infection is widespread in many countries of the world, and WHO has made control of schistosomiasis a priority among tropical diseases. The infection is transmitted between human and snail hosts and multiple biological and ecological factors contribute to its spread and persistence. Mathematical modeling could help to design more efficient and cost-effective transmission control strategies. Conventional approaches to modeling macroparasite transmission have not sufficiently accounted for its complexity. We are developing new approaches that accommodate many important features of infection, such as highly aggregated worm distribution in host populations and model/data uncertainties. An earlier work has successfully applied these methods for prediction of schistosomiasis control in coastal Kenya. The current project extends the applicability of this earlier work - particularly in reference to heterogeneous host demographics and density dependent effects on worm fertility and mortality. We have also addressed diagnostic data uncertainty. New calibration procedures have now been developed and tested with data collected from several endemic areas. This has allowed us to estimate the essential biological and environment parameters of Schistosoma infection in structured population groups, and apply our model to analyze probable outcomes of control interventions in different settings. The new transmission model will next be implemented by the ongoing Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) project for comparison trials of several proposed 'cost-effective' integrated control strategies.

1150

LONG-TERM EFFECT OF MASS CHEMOTHERAPY, TRANSMISSION AND RISK FACTORS FOR *SCHISTOSOMA MANSONI* INFECTION IN VERY LOW ENDEMIC COMMUNITIES OF VENEZUELA

Adriana Tami[1], Stefanie Hofstede[2], Genevieve van Liere[2], Diana Ballén[3], Renzo N. Incani[4]

[1]Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands, [2]Faculty of Earth and Life Sciences, Free University of Amsterdam, Amsterdam, Netherlands, [3]Unidad de Trematodiasis, Centro de Microbiologia y Biologia Celular, Instituto Venezolano de Investigaciones Científicas, Altos de Pipe, Edo. Miranda, Bolivarian Republic of Venezuela, [4]Departamento de Parasitología, Facultad de Ciencias de la Salud, Universidad de Carabobo, Valencia, Bolivarian Republic of Venezuela

The prevalence of *Schistosoma mansoni* infection in Venezuela has changed from high to low mostly due to successful control activities including mass chemotherapy and molluscicide applications. This study examined the impact of mass chemotherapy on *Schistosoma mansoni* transmission and risk factors for infection 12 years after administration of praziquantel in Venezuela. Two relatively isolated rural communities where studied, one with snail control (Manuare) and the second without (Los Naranjos). A cross-sectional survey of randomly selected households included 226 (Manuare) and 192 (Los Naranjos) consenting participants. *Schistosoma mansoni* prevalence was determined using a combination of coprological (Kato-Katz) and serological (circumoval precipitin test, alkaline phosphatase immunoassay and Western blot) tests. Data on epidemiological and socioeconomic risk factors were obtained through individual structured interviews. Univariate analysis and multivariate logistic regression models identified independent risk factors for infection. Water sites were examined for the presence of *Biomphalaria glabrata* snails. Only one participant was positive by coprology. The overall prevalence according to the combined tests was 32.7% in Manuare and 26.6% in Los Naranjos. A lower prevalence (12.7% in Manuare and 13.2% in Los Naranjos) was found in children <12 years of age representing those born after mass chemotherapy. Variables associated with infection in both communities were older age (>25 years), contact with specific water sites, and being a farmer/non-specialized worker. In conclusion, mass treatment with praziquantel applied once to endemic communities led to an important and long-lasting sustained reduction of *S. mansoni* infections independent of the application of snail control. A degree of low active transmission of *S. mansoni* persisted in the treated areas which was associated with similar factors in both communities.

1151

SCHISTOSOMAISIS PREVALENCE IN RELATION TO THE PROXIMITY OF LAKE MALAWI

Jane M. Whitton[1], Samuel Jemu[2], Fiona Fleming[1], Sarah Knowles[1]

[1]Schistosomiasis Control Initiative, Imperial College London, London, United Kingdom, [2]Malawi Ministry of Health, Lilongwe, Malawi

Large freshwater bodies such as the great lakes of East Africa have long been considered key areas of transmission for schistosomiasis. Lake Malawi in particular is used as an example to highlight the risks of schistosomiasis and hot spot areas for transmission. To date there has been no study which evaluates the relationship between living in close proximity to Lake Malawi and risk of infection, or how the lake contributes to transmission within the country. Here we present findings from the re-assessment of five lakeshore districts which have benefitted from four rounds of preventive chemotherapy with praziquantel from the Ministry

of Health. These districts were targeted initially due to the presumption they are at greater risk of transmission. This survey was conducted firstly to identify the areas at greatest risk of infection, secondly to establish the relationship between prevalence of schistosomiasis and living in proximity to the lakes and finally the future frequency of treatment in line with World Health Organisation recommendations. A total of 2440 children from 75 schools were sampled. Fifteen schools from each of the five lakeshore districts were selected by stratifying distance to lake 0-5 kilometres (km), 5-15km and >15km. Thirty children from each school, 15 boys and 15 girls, aged 10-14 years were randomly selected and tested for *Schistosoma haematobium* using urine filtration and *S. mansoni* by Kato Katz. Probability of infection with either *S. haematobium* or *S. mansoni*, in relation to both school level characteristics, including proximity to the lake, as well as pupil characteristics such as age, sex and treatment with praziquantel in the last year were assessed. Overall 9.0% were infected with *S. haematobium* and 5.2% with *S. mansoni* varying dramatically by district; 4.85%(95% CI = 0-9.77) in Nkhata Bay to 22.46%(95% CI = 9.48-35.44) in Zomba despite previous rounds of treatment. Contrary to previous findings, *S. mansoni* was found to have a significantly higher prevalence as you move away from the lake (OR=0.33, 95% CI 0.11-0.97). This relationship was not consistent across species or between the districts accentuating the hazards of predicting at-risk areas based on the presence of large water bodies. This study has highlighted the need for comprehensive mapping during decision making for treatment strategies, in this case within Malawi, due to the heterogeneous nature of schistosomiasis infection.

1152

ASSESSMENT OF MASS TREATMENT FOR SCHISTOSOMIASIS IN KWAZULU - NATAL PROVINCE, SOUTH AFRICA

Ana Randjelovic[1], Synne Gronvold Fronas[1], Mavis Munsami[2], Jane Dene Kvalsvig[3], Siphosenkofi Gift Zulu[3], Andile Maphumulo[3], Eyrun Floerecke Kjetland[4], Leiv Sandvik[5], Myra Taylor[3], Svein Gunnar Gundersen[6]

[1]Faculty of Medicine, University of Oslo, Oslo, Norway, [2]Department of Health, Ugu District, Port Shepstone, South Africa, [3]Discipline of Public Health Medicine, Nelson R. Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa, [4]Centre for Imported and Tropical Diseases, Department of Infectious Diseases Ullevaal, Oslo University Hospital, Oslo, Norway, [5]Department of Biostatistics, Epidemiology and Health Economics, Oslo University Hospital, Oslo, Norway, [6]Research Department, Sorlandet Hospital HF, Kristiansand, Norway

It is estimated that 700 million people worldwide and 5.2 million people in South Africa are in need of annual treatment for schistosomiasis. In accordance with current policy, a Department of Health (DoH) in KwaZulu-Natal Province, South Africa, aimed to reach 75 percent treatment coverage in a mass treatment campaign (MTC) of schools in a schistosomiasis-endemic area. A cross-sectional study was designed to explore the implementation, coverage, challenges and limitations of a DoH MTC in a middle income country. The study was conducted by exploring nurses' and research team records, school enrolment lists and parental consent forms. Slightly more than 10 000 learners in 43 primary and high schools were treated, achieving treatment coverage of 44.3%. A median of two schools per day were visited over the course of 39 days. We found that older learners, being male and being in a large school were independent significant predictors for low treatment coverage. Our results indicate that coverage would likely increase through improved consent procedures and repeated schools visits. Further information is needed on how to increase compliance in teenagers, males and pupils in large schools.

1153

PERFORMANCE OF MICROHEMATURIA IN THE DETECTION OF *SCHISTOSOMA HAEMATOBIUM* IN CHILDREN FROM BENGO, ANGOLA

Miguel Brito, Clara Mirante, Sofia Moura, Isabel Clemente, Teresa Ganga, Catarina Alexandre, Graciete Zumbua

CISA - Health Research Centre in Angola, Caxito, Angola

Urinary schistosomiasis is endemic in Angola and for effective control measures, an efficient, quick and cheap means of diagnosis should be incorporated. The use of reagent strips for detecting microhematuria has long been recommended as a cheap and accurate proxy for schistosomiasis diagnosis. However, their performance levels do vary according to the underlying population prevalence. Moreover, whether or not these methodologies still turn in good performances after mass treatment with Praziquantel also needs determining. A cross-sectional study of children was completed to evaluate the performance of microhematuria in detecting *Schistosoma haematobium* infections in Bengo, Angola, both before treatment with praziquantel and then again in the follow-up (1 month and six months after treatment). Urine samples from 504 children were tested for microhaematuria with Combur-Test (Roche) reagent strips followed up by egg microscopy analysis. A total of 277 boys and 227 girls were analyzed at the baseline with 400 from this sample reanalyzed one month after treatment and 419 six months after. The prevalence of the infection was 64.5% in the sample area, classifying this area as of high prevalence. One month after treatment the prevalence was 29.5% while six months later the rate stood at 45.1%. The application of reagent strips was found to vary both in terms of sensitivity (90.8% at the baseline, 66.1% one month after and 81.5% six months after) and in specificity (69.3% at the baseline, 76.6% one month after and 79.1% six months after). There were no significant differences in the gender based performance. A significant correlation was found between the intensity of infection and the concentration of hematuria in all three samples ($r=0.620$; $r=0.434$; $r=0.419$, $p<0.001$, respectively). Recourse to urine strips in community studies, in endemic areas, where resources and specialized diagnostic methodologies are scarce, thus presents itself as an easy and economic technique with good sensitivity and specificity. Moreover, this method makes possible highly precise estimates of the degree of intensity of *S. haematobium* infection.

1154

SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHES REMAPPING SURVEY IN THE REPUBLIC OF YEMEN

Dhekra A. Annuzaili[1], Michael D. French[1], Abdulhakim Ali Al-Kohlani[2]

[1]Imperial College, London, United Kingdom, [2]Ministry of Public Health and Population, Sanaa, Yemen

Schistosomiasis or Bilharzia (both uro-genital and intestinal) and soil-transmitted helminths (STHs) are widespread in the Republic of Yemen with an estimated 3 million people (of the total population of 24 million) infected with schistosomiasis and a similar number with STH The Yemen Ministry of Public Health and Population have completed three years of the current six year programme (2010-2015) to control schistosomiasis and STHs nationwide Following two/three treatment rounds, a nationwide remapping survey have been conducted and led by local researchers at the Parasitology Department at the University of Sana'a The aims of the remapping are to update the map of the distribution of SCH and STH in the country, to set the treatment approach for the remainder of the current programme, and to inform the need for future control activities, including those that may help aim for elimination of infection. As part of the remapping surveys approximately 90,000 school-aged children from 2,600 schools from 333 districts in the country were surveyed between March and May 2014 Hemoglobin levels have also been recorded from all these individuals in order to provide the richest dataset on anemia ever

collected in the country The results of these surveys will be presented and discussed, against the backdrop of the significant operating challenges experienced in Yemen.

1155

FEMALE GENITAL SCHISTOSOMIASIS: MORPHOLOGIC CHARACTERISTICS OF ABNORMAL BLOOD VESSELS

Sigve D. Holmen[1], Elisabeth Kleppa[1], Hashini N. Galappaththi[2], Kristine Lillebø[1], Fritz Albregtsen[1], Mathias Onsrud[1], Eyrun F. Kjetland[2]

[1]University of Oslo, Oslo, Norway, [2]Oslo University Hospital, Oslo, Norway

Female genital schistosomiasis (FGS) manifests with deposition of *Schistosoma haematobium* eggs in the genital mucosa. This results in the appearance of sandy patches and abnormal blood vessels. The former manifestation is considered pathognomonic to the disease whereas the significance of the latter is still unknown. In the literature, the blood vessels have been described as reticular, branched, circular, convoluted (cork-screw shaped) and having uneven calibre. In this study we wanted to analyse these features objectively using computer image analyses on morphology. In a study on young women with FGS in South Africa, we selected colposcopic images in which the clinicians had indicated the presence of abnormal blood vessels (n = 29) characteristic of FGS. An equal number of negative endemic controls was selected based on clinical findings. Data on blood vessel morphology was extracted using computer image analysis. The morphologic features were analysed by fractal dimensions (a measure of complexity), identification of closed loops (circularity), clusteredness (distance between vessels), number of vessels and size of vessels. We found that vessel size, clusteredness and circularity were significantly associated with the clinician's identification of abnormal blood vessels ($p = 0.036$, $p = 0.046$ and 0.049, respectively). However, we found no association with fractal dimension ($p = 0.957$) or number of vessels ($p = 0.889$). Using clusteredness alone, it was possible to classify the images with a precision of 72.4 %. Adding vessel size or circularity to the classification model did not improve the precision. Automated computer analysis of blood vessels can help decipher the specific features of abnormal blood vessels in FGS. Furthermore, this could possibly be used as part of a diagnostic tool based on image analysis. Further studies are required to look at other morphologic features of abnormal blood vessels in FGS, such as open loops (semi-circular structures) and uneven calibre.

1156

THE ROAD TOWARDS SUSTAINABLE CONTROL OF SCHISTOSOMIASIS IN THE DEMOCRATIC REPUBLIC OF CONGO: PRE-ASSESSMENT OF STAFF PERFORMANCE AND MATERIAL RESOURCES IN ENDEMIC REGIONS.

Liliane Mpabanzi[1], Sylvie Linsuke[2], Joule Madinga[1], Sylvain Nundu[2], Pascal Lutumba[2], Katja Polman[1]

[1]Institute of Tropical Medicine, Antwerp, Belgium, [2]Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of Congo, Kinshasa, Democratic Republic of the Congo

Schistosomiasis is a poverty-related disease affecting more than 200 million people in developing countries, 85% of them in Sub-Saharan Africa. Since a long time, schistosomiasis has been known to be endemic in certain provinces of the Democratic Republic of Congo. However, recent or hard figures to support these observations are not available, and the most recent national prevalence data available were generated over 30 years ago. Recently, the Ministry of Health adopted a national plan against schistosomiasis. For this program to be implemented in an efficient and sustainable way, data on schistosomiasis in the DRC urgently need to be updated. The present study assessed the knowledge and practice of health staff on schistosomiasis as well as the availability of material resources for diagnosis and management of schistosomiasis in two endemic provinces in the DRC. We performed structured interviews with staff from 35 health care facilities in 9 health zones (HZ) of Kinshasa and 2 HZ in Bas-Congo. Schistosomiasis was reported to be present in all of the included HZ. Health workers could name the most important symptoms of schistosomiasis such as bloody diarrhea and hematuria. Interestingly, health workers in rural Bas-Congo were more accurate than those in urban Kinshasa in citing more advanced/long term symptoms of schistosome infection such as ascites and hematemesis. Kato-Katz and urine filtration were not available in any of the health facilities. Diagnosis and treatment mainly relied on reported symptoms. Knowledge on schistosomiasis did not differ between rural Bas-Congo and urban Kinshasa. Fees for consultation, diagnosis and treatment however, were three times higher in Kinshasa than in Bas-Congo. Diagnosis of schistosomiasis in health care facilities in Bas-Congo and Kinshasa is mainly symptom-based. Though knowledge on schistosomiasis among health staff appears sufficient, substantial efforts still have to be made to improve the availability of diagnostic tools and treatment. Reinforcement of the wavering health system would be the first step on the challenging road towards sustainable control of schistosomiasis in a country fighting a heavy burden of schistosomiasis and many other neglected tropical diseases.

1157

HIGH PREVALENCE OF S*CHISTOSOMA MANSONI* IN THE HEALTH ZONE OF KASANSA, DEMOCRATIC REPUBLIC OF THE CONGO

Sylvie Linsuke[1], Sabin Nundu[1], Sylvain Mupoyi[2], Rodin Mukele[3], Faustin Mukunda[2], Madeleine Kabongo[4], Raquel Inocêncio da Luz[5], Jean-Pierre Van Geertruyden[5], Marc Van Sprundel[6], Marleen Boelaert[7], Katja Polman[7], Pascal Lutumba[8]

[1]Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo, [2]Programme National de Lutte contre la Bilharziose et Parasitoses Intestinales, Kinshasa, Democratic Republic of the Congo, [3]University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [4]Georgia State University, Atlanta, GA, United States, [5]University of Antwerp, Antwerp, Belgium, [6]University of Kinshasa, Antwerp, Belgium, [7]Institute of Tropical Medicine, Antwerp, Belgium, [8]Institut National de Recherche Biomédicale/UNIKIN, Kinshasa, Democratic Republic of the Congo

School-aged children suffer the most from Schistosomiasis infections in Sub Saharan Africa due to poverty and limited sanitary conditions. Surveillance of disease burden is recommended and 20-year-old prevalence data needed urgent updating in the Democratic Republic of Congo. Epidemiological and parasitological study was carried out in 2011 in Health zone of Kasansa in Democratic Republic of Congo. Six health areas were included in the study. In each health area, one primary school was selected. Kato-Katz and direct microscopy examinations were performed in school-aged children. High *Schistosoma* prevalence levels (82.7%) were found in the Health Zone of Kasansa and certain study areas presented prevalence levels reaching nearly 100%. These results demonstrate that *S. mansoni* infection is a bigger problem than anticipated and there is an urgent need to implement effective control measures.

1158

COMPARATIVE STUDY OF THE ACCURACY OF DIFFERENT TECHNIQUES FOR THE LABORATORY DIAGNOSIS OF SCHISTOSOMIASIS MANSONI IN LOW ENDEMICITY AREAS

Maria Cristina Carvalho Espírito-Santo

Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

Schistosomiasis constitutes a major public health problem, and estimates suggest that 200 million people are infected worldwide. Barra Mansa, Rio de Janeiro State, Brazil, has an estimated prevalence of 1%. Areas of low endemicity (ALE) represent a new challenge for the helminth control because infections occur with low parasite load (<100 eggs per gram of feces), causing a decrease in sensitivity of parasitological techniques. To compare the performance of the techniques of Kato-Katz (KK), Hoffman, Pons and Janer (HH), ELISA-IgG and ELISA-IgM, Indirect

Immunofluorescence Technique (IFT) and qPCR technique in samples of serum and feces (qPCR in feces and serum) using the *Circumoval Precipitin Test (COPT)* as reference. An epidemiological survey, in a randomized sample of residents in five neighborhoods of Barra Mansa/RJ was undertaken to obtain stool and sera samples. A cross-sectional study conducted from April to December 2011, using a probabilistic sampling that collected 610 fecal samples and 612 serum samples. The laboratory diagnostic techniques used were: KK and HH, ELISA-IgG and ELISA-IgM, IFA-IgM, COPT, qPCR-feces and qPCR-serum. We obtained the following results from different diagnostic techniques: KK and HH, 0.8% (n=5); ELISA-IgG, 11.6% (n=71); ELISA-IgM, 21.4% (n=131); IFA-IgM 15.8 (n=97); RPO 5.4% (n=33); qPCR-feces, 9.8% (n=60) and qPCR-serum, 1.5% (n=9). ELISA-IgM (21.4%) presented the highest positivity while the techniques of HH and KK were the least sensitive to indicate the presence of infection (0.8%). In comparison with COPT, except for qPCR-serum, all other techniques showed a statistically significant difference in positivity ($p<0.05$) and high accuracy (from 82% to 95.5%). The lack of adequate surveillance in areas of low endemicity of schistosomiasis may turn them into areas of medium and high endemicity. This study presents a control perspective, pointing to the possibility of using these combined laboratory tools in the diagnosis of schistosomiasis in low endemicity areas.

1159

SCHISTOSOMIASIS IN DEMOCRATIC REPUBLIC OF CONGO: A LITERATURE REVIEW OF THE LAST SIXTY YEARS DATA

Joule Madinga[1], Sylvie Linsuke[2], Liliane Mpabanzi[3], Lynn Meurs[3], Kirezi Kanokabana[3], Niko Speybroeck[1], Pascal Lutumba[2], Katja Polman[3]

[1]Institute of Health and Society, Université Catholique de Louvain, Brussels, Belgium, [2]Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo, [3]Institute of Tropical Medicine, Kronenburgstraat, Belgium

Since a long time, schistosomiasis has been known to be endemic in the Democratic Republic of Congo (DRC). However, hard figures or detailed epidemiological data are scarce, seriously hampering control. A literature search was conducted in PubMed to identify relevant original articles related to schistosomiasis in the DRC, published between January 1955 and January 2014. This was completed by non-peer-reviewed publications and unpublished data from experts and researchers. Key indicators, including the prevalence and intensity of infection, schistosomiasis-related morbidity and the distribution of schistosome species were retrieved. The search yielded 38 records, of which only 5 (13.1%) were published in the last twenty years. Endemicity of schistosomiasis was described in regions within 10 of the 11 provinces of DRC. Three species of *Schistosoma* were reported: *S. mansoni*, the most widespread species, followed by *S. haematobium* and S. intercalatum (Zaïre or Congo strain), which has a restricted distribution. They are co-endemic in many endemic areas, and hybridization of *S. haematobium* and S. intercalatum has been reported in Kinshasa. The prevalence of schistosomiasis varied greatly between regions and, within these regions, between different villages ranging from 0.6% up to 95%. In Kinshasa, the capital of the country, the level of endemicity has declined to under 10% over the years and is currently low. However, in rural areas, the endemicity is still either moderate or high, with great intra-regional variability. Schoolchildren and mine workers were the two most infected groups with, in some areas, prevalences exceeding 90%. Hepatosplenomegaly, urinary tract lesions, anemia and stunting were commonly reported in schistosomiasis endemic areas of the DRC such as Bas-Congo, Kinshasa and Kasai. Still, the epidemiology of schistosomiasis and its distribution have not been sufficiently explored in the DRC. Data summarized in the present review are limited to certain endemic areas only, most of them are simply outdated, and some present methodological limitations. This review discusses the knowledge gaps regarding schistosomiasis in DR Congo. It also highlights the need for effective control strategies, as well as for updated epidemiological data through well designed studies.

1160

COMMUNITY PERCEPTIONS, ATTITUDE, PRACTICES AND TREATMENT SEEKING BEHAVIOR FOR SCHISTOSOMIASIS IN LAKE VICTORIA ISLANDS IN UGANDA

Narcis Kabatereine[1], Fiona Fleming[2], Wangechi Thuo[3], Tinkitina Benjamin[4], Edridah M. Tukahebwa[4], Alan Fenwick[2]

[1]Schistosomiasis Control Initiative of Imperial College London, Kampala, Uganda, [2]Imperial College London, London, United Kingdom, [3]The Global Network for Neglected Tropical Diseases, Washington, DC, United States, [4]Vector Control Division, Kampala, Uganda

Over 200,000 people, most of them infected with *Schistosoma mansoni* inhabit 150 islands in Lake Victoria in Uganda. Although a programme to control the disease has been ongoing since 2003, its implementation in islands is inadequate due to high transport costs on water. In 2011 and 2012, the Global Network for Neglected Tropical Diseases (GNNTD) through Schistosomiasis Control Initiative (SCI) provided financial support to ease treatment delivery on the islands and over that period, therapeutic coverage has been increasing. We conducted a study with an objective to assess community awareness of existence of the disease, its signs, symptoms, causes and transmission, as well as attitude, practice and health seeking behavior. This was a cross-sectional descriptive study which used pre-tested interviewer administered questionnaire among purposively selected individuals in schools, health facilities and communities. Frequency distribution tables, graphs and cross-tabulations were the main forms of data presentation. Our results showed that there are numerous challenges that must be overcome to achieve effective control of schistosomiasis in the islands. Many people especially young men are constantly on the move from island to island in search for richer fishing grounds and such groups are commonly known to miss treatment by mass chemotherapy. Unfortunately case management in health facilities is very poor; health facilities are few and understaffed mainly with unskilled personnel who are overburdened by other illnesses such as malaria and HIV and the supply of praziquantel in health facilities is inadequate. Furthermore, sanitation is appalling, with no clean water and community knowledge about schistosomiasis is low even among biomedical staff. Rather than elimination, our results indicate that the programme should continue to target morbidity control beyond the 2020s until preventive measures have been instituted. The government should provide adequate trained health workers and stock praziquantel in all island health facilities.

1161

SURVIVAL AND GROWTH OF *VIBRIO CHOLERAE, ESCHERICHIA COLI* AND *SALMONELLA* SPP. IN WELL WATER MICROCOSMS

Moussa Djaouda[1], Bouba Gaké[2], Daniel Ebang Menye[1], Serge Hubert Zébazé Togouet[3], Song Liang[4], Moïse Nola[3], Thomas Njiné[3], Thomas Njiné[3]

[1]University of Maroua, Maroua, Cameroon, [2]Centre Pasteur du Cameroun, Annexe de Garoua, Garoua, Cameroon, [3]University of Yaoundé 1, Yaoundé, Cameroon, [4]University of Florida, Gainesville, FL, United States

Faecal contamination of drinking water is believed to be responsible for recurrent cholera outbreaks and many other waterborne diseases in north-Cameroon. However, very few studies report the isolation of faecal bacteria from drinking water in this region. Little is also known about the survival and growth of bacterial pathogens in well water, largely consumed by local populations. The ability of strains of faecal bacteria (Vibrio cholerae, Escherichia coli ATCC 25922 and four strains of Salmonella isolated respectively from well water, pig, poultry, and human urine in Garoua) to survive or grow in well water microcosms was compared. Water samples were obtained from two wells in Garoua (north-Cameroun). Autoclaving at 121°C for 15 min and filtration through 0.2 µm filter were used to make microcosms. Microcosms were constituted of unfiltered-autoclaved-; filtered-non-autoclaved- and filtered-autoclaved well waters. Bacterial strains were inoculated at initial cell

concentration of 3 Log10CFU/ml. All strains were able to survive/grow in used microcosms, and a maximal concentration of 5.61 Log10CFU/ml was observed. Survival abilities were strain-and-microcosm-dependent. The declines were more pronounced in filtered-non-autoclaved water than in the other microcosms. *E. coli* and *Salmonella* sp (Poultry strain) lowered to undetectable levels (<1Log10CFU/ml) after two days of water storage. *Vibrio cholerae* decreased over time but surviving cells persisted for longer in filtered-non autoclaved water from well W1 (1.91 Log10CFU/ml) and well W2 (2.09 Log10CFU/ml). Competition for nutrients and/or thermolabile anti-microbial substances synthesized by "ultramicrocells" or by the autochthonous bacteria retained by the filter might affect the bacterial survival.

1162

HOUSEHOLD AIR POLLUTION AND ITS RESULTANT HEALTH EFFECTS IN RURAL KENYAN WOMEN

Diana Menya

Moi University, Eldoret, Kenya

Household air pollution caused by the burning solid fuels for cooking and heating, is a major cause of respiratory morbidity for nearly a third of the world's population. 84% of Kenyan households use solid fuels for cooking. Studies have suggested that reducing HAP may improve respiratory health in women and children. We conducted a stove intervention trial to evaluate the respiratory benefits of reducing HAP by replacing traditional stoves with fuel-efficient, low emission local stoves. We conducted a pre-post intervention trial in 50 randomly selected households in Western Kenya. Fifty women aged over 18 years, willing to allow a home assessment for HAP, accept a new improved stove, and spending at least 4 hours per day in the cooking area of the house were enrolled. One month before stove replacement demographic, smoke exposure, respiratory morbidity and cooking practices data were collected also direct observations and measurements of cooking spaces. Spirometry with bronchodilator challenge, blood pressure, oximetry, hemoglobin and anthropometry were conducted also 24-hr mean PM2.5 levels using the pDR1000 passive sampler; Relative Humidity using HOBO data logger and 24-hr mean CO using Easylog USB CO Monitor. We designed, fabricated and replaced traditional stoves with the Eldoboma stove. It is inexpensive, durable dependable and locally acceptable. It was found to have very low emissions of PM 2.5 and CO in laboratory testing. Mean age of the women is 34 years, 94% are married and 74% are farmers. Mean years of education is 9. They started cooking at the mean age of 13.6 and had spent an average of 20 years cooking. They cooked an average of 3 meals daily. Thatch (56%) and tin (44%) were the most common type of roofing material. Mud and thatch was the most common type of wall (52%). The kitchens had one permanently opened door and window. None of the women were currently smoking. Exhaled carbon monoxide was 4.3 ppm. Percentage of carboxyhemoglobin was 0.82% . Arterial carboxyhemoglobin saturation; 5.76%; Arterial Oxygen saturation 96.12%, Perfusion Index 4.2%, systolic blood pressure 126.43 mmHg, and diastolic 80.8 mmHg. Kitchen PM2.5 was 8145.73 µg/m3 and Carbon Monoxide level was 55.76 ppm. At baseline, indoor levels of PM2.5 and CO were much higher than in many prior studies of HAP. Women spending ≥4 hours in poorly ventilated kitchens using a traditional cookstove had abnormally high levels of SpCO, likely due to HAP exposure.

1163

INTEGRATING WATER, SANITATION AND HYGIENE WITH COMMUNITY-BASED NUTRITIONAL COUNSELING IN FOND DES BLANCS, HAITI, 2013-2014

Janell Routh[1], W. Roodly Archer[1], Rachel Bedenbaugh[2], Taryn Silver[2], Mirielle M. Henry[3], Joseline P. Marhone[3], Efua Gyan[2], Eric Mintz[1], Barbara J. Marston[1], Daniel Lauture[4], Samuel Bernard[2], Miliane Clermont[2]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]St. Boniface Haiti Foundation, Fond des Blancs, Haiti, [3]Ministry of Public Health and Population, Port au Prince, Haiti, [4]UGP, Port au Prince, Haiti

Reducing the burden of malnutrition through community-based nutritional counseling programs has shown promising impact in Haiti and worldwide. In Haiti, the prevalence of acute malnutrition is low (4.1%), but chronic malnutrition, based on rates of stunting, remains high (23.4%). Poor water, sanitation, and hygiene (WASH) conditions worsen malnutrition: 9% of the population has piped water, 36% of the population travels ≥ 30 minutes to retrieve drinking water and 63% of households in rural areas have an unimproved or no toilet. Insufficient water and sanitation infrastructure contributed to the rapid spread of epidemic cholera in Haiti in 2010 and to its subsequent persistence. To reduce the risk of cholera and other diarrheal diseases and to educate mothers about locally available nutrition, we integrated a 2-week community-based nutritional counseling program for undernourished children <5 with distribution of WASH education and kits (safe water container, a household water chlorination product [*Aquatabs*], and soap). During three monthly home visits, community health workers emphasized educational messages, collected anthropometric data, and distributed refills of soap and *Aquatabs*. We surveyed 103 families before program implementation. At baseline, 86 (84%) families used an improved water source for drinking, 96 (93%) had ever tried a water chlorination product, and 60 (58%) had no toilet facilities. All 103 (100%) families demonstrated correct handwashing procedures. While 95 (92%) reported using chlorine product that day, only 7 (7%) of baseline water samples contained residual free chlorine. In 288 monthly follow-up visits completed in the first half of the 6-month follow-up period, 177 (60%) of water samples contained residual chlorine. This evaluation suggests that the integration of community-based nutritional counseling and WASH incentives is feasible and acceptable, and may help motivate improved WASH behaviors.

1164

DETERMINANTS OF PESTICIDE EXPOSURE AND NEUROBEHAVIORAL IMPACT OF SUBSISTENCE FARMERS IN THE GAMBIA

Jaime Butler-Dawson, Peter S. Thorne

University of Iowa, College of Public Health, Iowa City, IA, United States

Pesticide use greatly impacts the health of agricultural communities, including both beneficial and detrimental impacts. There is increasing concern regarding the widespread use of pesticides and their potential impacts on public health. Acute high-level exposures to certain insecticides have well-known adverse neurobehavioral (NB) effects. Chronic exposures have more subtle effects which are harder to measure and evidence is limited on the NB aspects of low-level exposures to insecticides. We assessed levels of chronic pesticide exposure and effects on NB performance of subsistence farmers. NB tests were administered to rural residents in the Upper River Region of The Gambia. Participants (N=158, ages 18 - 40 years) completed eight NB tests to assess attention, memory, response speed, and coordination. Questionnaires were administered to participants on sociodemographic characteristics and agricultural and home pesticide use. Among participants who had ever directly used agricultural pesticides (N=77, 58%), practices that were potentially main determinants of pesticide exposure were duration and frequency of use, lack of personal protective equipment use (58%), mixing techniques (bare hands/leaves, 27%, and jerry can, 22%), application methods (hands,

40%, and hand-held sprayer, 23%), and hygiene practices (not bathing or changing clothes after use, 42%, and not washing hands before eating, 42%). These exposure determinants were weighted individually by six subject matter experts to create exposure scores that included frequency and duration of use to estimate exposure levels of participants. The average age of the studied population was 28 years and 48% of males and 63% of females had never been to school. Females had statistically significant higher exposure scores than males and certain ethnicity groups had statistically significant higher exposure scores. The results of such studies are critically important especially in developing countries where adverse health effects could be the greatest due to lack of protective measures and regulations.

1165

RAINWATER CATCHMENT AND PURIFICATION SYSTEM FOR IMPOVERISHED COUNTRIES

Katelyn Boone[1], Lucas Potter[1], Kristina Kelly[1], Viktoria L. Pretzman[1], Christopher Hagerty-Hoff[1], Anathea Pepperl[1], James T. McLeskey[1], Michael P. Stevens[2]

[1]Virginia Commonwealth University, Richmond, VA, United States, [2]Virginia Commonwealth University Medical Center, Richmond, VA, United States

Access to clean drinking water is one of the principle health needs faced by people living in rural Honduran communities. In a survey done by Virginia Commonwealth University's Global Health and Health Disparities Program (GH2DP), in one rural community (Lomitas) only 22% of respondents (11/50) had access to private water faucets. The majority of respondents obtained their water directly from the river (62% or 31/50). According to GH2DP's microbial testing, the river does not meet the drinking water standards set forth by the World Health Organization (WHO). VCU's chapter of Engineers Without Borders (EWB) has created a rainwater catchment system to allow individual households in Lomitas to collect clean drinking water that meets or exceeds the drinking water standards set forth by the WHO. The goal is to deploy a system that can be set up and maintained by local inhabitants without extensive training. This novel, clean-drinking water apparatus is sustainable and comprised of inexpensive and readily available materials such as tarps, water hoses, polyvinyl chloride (PVC) pipes, and polyethylene terephthalate (PET) bottles. We are currently studying the rigor of our rainwater catchment system through exposure to several extreme environmental conditions, through assessment of possible microbiological hazards, and through the determination of potential effectiveness for rural communities with sufficient rainfall.

1166

POST-EARTHQUAKE DRINKING WATER SURVEILLANCE IN THE OUEST DEPARTMENT OF HAITI DURING THE GREAT HAITIAN CHOLERA EPIDEMIC: THE MODERN DAY JOHN SNOW

Thomas A. Weppelmann

Emerging Pathogens Institute, Gainesville, FL, United States

The 2010 earthquake in Haiti led to thousands of deaths, destruction of drinking and waste-water infrastructure and displacement of millions into temporary camps with little sanitation and hygiene. Even worse, the introduction of *Vibrio cholera* in October, 2010 resulted in a massive cholera outbreak that spread rapidly throughout Haiti. Once it was recognized that many displaced persons lacked access to water and the outbreak likely resulted from consumption of contaminated surface water, thousands of wells were installed by Non-Government Organizations. However, despite an ongoing epidemic, no water quality data was collected from these wells to verify their safety. To determine if the wells were a source of exposure, 359 sources of drinking water in the Leogane flood basin, located at ground zero of the earthquake, were screened for *V. cholerae* and fecal coliform bacteria. While no toxigenic strains of *V. cholerae* were identified, non-toxigenic *V. cholerae* was isolated from six water sources. Of these, all contained fecal coliforms and displayed significant clustering near the major highway, which was hypothesized as a major route of dissemination the cholera outbreak. Over 80% of unimproved water sources (WHO classification) had the presence of fecal coliforms along with 25% of the recently installed improved water sources, which showed increases in contamination after hurricane Sandy. This study provides evidence that the source of transmission was most likely from person to person and from consumption of surface water sources, which our research group has recently identified to be contaminated with toxigenic *V. cholerae* O1. The results also suggest that while drilling wells in emergency situations is a necessity, the long-term sustainability of those wells will likely require the support from the national government in the form of monitoring maintenance, and not by a fragmented patchwork of NGOs. If *V. cholerae* has become endemic, improvements in water and sanitation infrastructure might be the only way to eliminate the transmission of cholera in Haiti.

1167

INTRODUCTION OF INNOVATIVE LOW-COST FOOD BANKING SCHEME AS A MEANS OF PREVENTING MALNUTRITION AMONG UNDER FIVE CHILDREN AMONG FARMERS IN RURAL COMMUNITIES

Irene R. Moshi, Emmanuel Kaindoa, Elihaika Minja, Fredros Okumu

Environmental Health and Ecological Sciences Thematic Group, Ifakara Health Institute, Ifakara, United Republic of Tanzania

Malnutrition among other health problems in under-five children, contributes about 50% of all child mortality in Sub-Saharan Africa. Malnourished child during the first five years of his/her life leads to slowed physical and mental development. A child's physical growth, cognitive development and overall performance depend much on feeding of the child during the first five years. This is a preventable condition, among other solutions food security within the household is among them. So we intend to introduce the use of low-cost food banking schemes as a means of reducing and preventing malnutrition among children during their first five years. We intend to conduct cross sectional study in five villages with a sample 50 households among farming communities. Mothers and care givers will be recruited from the houses that have under five children. Malnutrition diagnostic test will be performed to all children understudy to get their status. Mothers and care givers will identify all food products which are locally available then will be trained on nutritional foods for children as well as proper feeding practices and food storage techniques. Monitoring of food types given to the children, feeding patterns and food availability within the household will be conducted. All this is to ensure children get the required nutrients from the local available food products within the areaFrom the provided training and knowledge sharing on nutritious foods, feeding patterns and storage techniques, we expect the following: 1) knowledgeable mothers and care givers on nutritional foods for their children, 2) improved feeding pattern of under five children and 3) improved storage facilities from the locally available means to ensure availability of at least 3 meals per day and food security within these households. Actual results will be presented during the conference. In conclusion. mothers and care givers knowledge on nutritional foods and feeding pattern for children are among the important entities in reducing child malnutrition without forgetting the role played by food security.

1168

THE IMPACT OF HYDRATION ON COGNITION AMONG SCHOOL CHILDREN: RESULTS FROM A RANDOMIZED CONTROL TRIAL IN ZAMBIA

Victoria Trinies, Matthew Freeman

Emory University, Atlanta, GA, United States

Access to drinking water during the school day may improve children's ability to learn through the positive effect of hydration on attention,

concentration, and short-term memory. The link between dehydration and lower cognitive performance has been well-established among adult populations and among school children in high-income settings. However, until recently this relationship has not been evaluated among children in low-resource settings. We conducted a randomized control trial to investigate the relationship between cognition, hydration, and water consumption among children residing in a water-scarce setting. The protocol for the study was adapted from a pilot conducted in Mali. We visited five schools in Chipata province, Zambia, for one day each. Pupils were randomly assigned within each school to receive either a bottle of drinking water that they could refill throughout the day (water group, n=149) or were not provided with supplemental water and could only access drinking water that was normally available at the school (control group, n=143). We assessed hydration in the morning and afternoon using urine specific gravity (USG) measured with a portable refractometer. Children were considered dehydrated if their USG exceeded 1.015. In the afternoon, we administered six cognitive tests to assess short-term memory, concentration, visual attention, and visual motor skills. Independent samples t-tests were used to compare cognitive test scores between the water and control groups, and linear regression was used to compare hydration level and cognitive test score. Mean morning USG was 1.018 for both water and control groups. Afternoon USG increased among the control group (1.022) and decreased among the water group (1.006). Mean scores for one of the cognitive tests were significantly higher among the water group. There was no significant difference in mean scores between the water and control groups for any other test, and there was no significant correlation between afternoon USG and any test scores. Results show that moderate dehydration among school children is prevalent and increases throughout the day in the absence of supplemental water, and increased access to water decreases dehydration prevalence. There is some evidence that hydration improves cognitive test performance, but we found no clear association.

1169

THE IMPACT OF SCHOOL-BASED WATER, SANITATION AND HYGIENE IMPROVEMENTS ON THE PRESENCE OF BLOOD ANTIBODIES FOR ENTERIC AND NEGLECTED TROPICAL DISEASES AMONG SCHOOL CHILDREN IN MALI

Anna Chard, Victoria Trinies, Howard Chang, Matthew Freeman

Emory University, Atlanta, GA, United States

The role of improvements in water, sanitation, and hygiene (WASH) on the reduction of enteric and neglected tropical diseases among children and adolescents is well supported. However, health impact evaluations of WASH interventions in low-resource settings are limited by the existing, and often biased, expensive, or laborious methods and tools used to measure diarrheal and NTD incidence. We piloted a novel, objective method for evaluating the impact of school WASH improvements on enteric and neglected tropical diseases incidence among pupils in Mali. Capillary blood in the form of dried blood spots (DBS) was collected from 400 students attending beneficiary schools of comprehensive school-based WASH program (intervention), and 400 students attending schools that did not receive any WASH improvements (control). Using a Luminex multiplexing assay, we will analyze the DBS for blood antibodies for a range of enteric and neglected tropical diseases. Levels of antibodies will be compared between pupils in intervention and control schools to provide biological evidence of the school WASH program impact on pupil health. Data collection will conclude in May, and DBS antibody analysis will be complete by June. Results from this pilot study will be used to assess the feasibility of using the Luminex multiplexing assay to detect disease incidence among school-aged children (SAC) - a novel age range for this approach - to identify the leading pathogenic causes of enteric and neglected tropical diseases among SAC, and to quantify the impact of a comprehensive school WASH program on blood antibody levels among SAC. Additionally, results may inform power and sample size calculations for future research.

1170

EFFECT OF SANITATION CONDITIONS AND HYGIENE ON THE PREVALENCE OF ENTERIC PATHOGENS IN AN IMPOVERISHED COMMUNITY IN THE PERUVIAN AMAZON

Jennifer L. Rice[1], Nikolai S. Tolstoy[1], Hannah Ross-Suits[1], Drake Tilley[2], Erik B. Lehman[1], David W. Craft[1]

[1]The Pennsylvania State University, Hershey, PA, United States, [2]U.S. Naval Medical Research Unit - 6, Lima, Peru

Diarrheal diseases are the fourth most common cause of death worldwide and the second most important factor in global disease burden (WHO, 2011) and represent an important target for global health interventions. Many enteric parasites are transmitted by the fecal-oral route via waterborne sources and can lead to diarrhea or nutritional deficiencies. We hypothesized the prevalence of the roundworms *Ascaris lumbricoides* and *Trichuris trichiura*, and the protozoans *Entamoeba coli*, *Giardia species*, and *Cryptosporidium parvum* is higher in households without quality sewage treatment, treated water, proper water storage, and good hand washing practices than in those households that have access to and utilize these services and hygiene practices. We conducted this prospective observational study in Belen, an urban, riverside slum in the Amazon Basin in Iquitos, Peru. Interviews were used to assess source of drinking water, water treatment, and sanitation practices and environmental sanitation concerns as well as qualitative drinking water satisfaction. Ova and parasite analysis of stool and total coliform determination of drinking water was performed for each household surveyed. 64% of stools sampled had 1 or more parasites, with *A. lumbricoides*, *Giardia spp.*, *E. coli*, *T. trichiura*, and *C. parvum* at 34%, 18%, 14%, 8%, and 0% prevalence, respectively. Water source, water storage, and sanitation methods were not associated with parasite infection or water coliform presence. Water treatment method was significantly associated with *E. coli* prevalence, as was the presence of cats near the household. Seeking repeated treatment for diarrhea was also linked to *E. coli* infection. Coliform contamination of drinking water was significantly correlated with the number of children under 5 living in the household. These results suggest that parasite infection is common in this community. Future studies are needed to define the roles of drinking water, environmental contamination, and microorganism carriage in the etiology of gastrointestinal illness in this population.

1171

THE VALUE OF FRAGMENTED FORESTS FOR MITIGATING PATHOGENS IN SURFACE WATER IN RURAL UGANDA

Gail Emilia Rosen

University of Wisconsin Madison, Madison, WI, United States

While the value to human health of ecosystem services is well established, questions remain regarding the scales at which these services operate. In particular, it is not clear whether forest fragments provide the same benefits to human health that are expected of undisturbed forests. One crucial ecosystem service provided by intact forests is water filtration. This process is highly relevant to human health, especially in the developing world, where water treatment is often nonexistent and forest fragmentation is common. This pilot study takes place in the region bordering Kibale National Park in western Uganda, which was once continuous forest; now, large swatches have been cleared for smallholder agriculture, and only small fragments of forest remain. In the villages surrounding these fragments, as in much of the developing world, lack of clean drinkable water is a major public-health problem. These forest fragments thus represent an ideal model system in which to examine the potential of forest fragments to improve the quality of surface water with regard to pathogens. To examine the effect of forest fragments on water-borne pathogens, I sampled streams and wells inside, upstream and downstream of representative fragments. Control samples were taken from intact forest in the park. Along with basic water-quality data, three complementary indicator systems of pathogen contamination (coliform

bacteria counts; adeno- and polyomaviruses; and complete bacterial metagenome analysis) provide a standard, well-established benchmark of fecal contamination, a sensitive, source-species-specific indicator system, and a snapshot of local bacterial communities. Preliminary findings indicate a clear mitigating effect of forest fragments on loads of fecal coliforms in surface water, while the effects on other quality indicators are more complex. Such combinations of water-quality data with respect to land use are uncommon and provide novel insights into connections between human health and the altered environment.

1172

THE BUMPED KINASE INHIBITOR, 1561, IS EFFECTIVE AGAINST EXPERIMENTAL TOXOPLASMOSIS

Joseph S. Doggett[1], Rama S. Vidadala[2], Kayode K. Ojo[2], Kasey L. Rivas[2], Dustin J. Maly[2], Wes C. Van Voorhis[2]

[1]Portland Veterans Affairs Medical Center, Portland, OR, United States, [2]University of Washington, Seattle, WA, United States

Toxoplasma gondii causes severe brain and eye disease. Current drugs for *T. gondii* are limited by toxicity. Bumped kinase inhibitors (BKI) selectively inhibit calcium-dependent protein kinases of the apicomplexan pathogens *T. gondii*, cryptosporidia and plasmodia. We have recently shown that the BKI, 1294, is metabolically stable, orally bioavailable and effective in a mouse model of acute toxoplasmosis at 100 mg/kg and 30 mg/kg given for 5 days. At the 63rd annual meeting of the American Society for Tropical Medicine and Hygiene, we will describe a BKI, 1561, that has greater efficacy than 1294. In 2 independent experiments (n=8,) a one time oral dose of 10 mg/kg given 48 hours after intraperitoneal *T. gondii* inoculation reduced the burden of infection of the Type 1 RH strain of *T. gondii* in the brain and spleen by 72% and 99%, respectively, compared to a vehicle only control. 1561 administered as a loading dose of 20 mg/kg followed by 4 daily doses of 5 mg/kg reduced the level of *T. gondii* infection in mice below the limits of detection (n=4.) Burden of infection in the brain and spleen was evaluated with quantitative real time PCR. The burden of infection was also evaluated in the peritoneal fluid with fluorescent microscopy of RH strain *T. gondii* expressing yellow fluorescent protein and was found to be reduced by 98% with a single oral dose of 10 mg/kg and was below the limits of detection in the group of mice given 20 mg/kg once followed by 5 mg/kg for 4 days (n=4.) The efficacy of 1561 is in part due to its favorable pharmacokinetics. The serum concentration of a 10 mg/kg dose in mice is 4.7 μM after 24 hours and 3.61 μM after 48 hours. The serum concentration of a 20 mg/kg dose at 24 hours was 11.05 μM. These findings show that 1561 is highly effective against experimental toxoplasmosis and is an outstanding candidate for further investigation as an anti-*Toxoplasma* drug.

1173

MULTIPLEXED RECOMBINASE POLYMERASE AMPLIFICATION DETECTION OF INTESTINAL PROTOZOA USING LATERAL FLOW STRIPS

Zachary Crannell[1], Miguel M. Cabada[2], Alejandro Castellanos-Gonzalez[3], Ayesha Irani[3], Mauricio Rebolledo[3], Clinton White[3], Rebecca Richards-Kortum[1]

[1]Rice University, Houston, TX, United States, [2]University of Texas Medical Branch, Cuzco, Peru, [3]University of Texas Medical Branch, Galveston, TX, United States

Protozoan infections of *Cryptosporidium*, *Giardia*, and *Entamoeba* are increasingly being recognized as important causes of diarrheal episodes and are associated with growth and cognitive impairment. While each is treatable, the treatments differ. However, the clinical presentations are similar, underlying the importance of accurate diagnostics. Current diagnosis relies heavily on stool smear, which even in the best hands is neither sensitive nor specific. We have developed isothermal nucleic acid amplification assays that can detect low-level infections as well as PCR, but does not require expensive equipment that is often unavailable in low-resource settings. The recombinase polymerase amplification (RPA)- *Cryptosporidium* assay demonstrates a limit-of-detection (LOD) comparable to or better than PCR (100 parasites/ml stool). Similarly the RPA- *Giardia* assay has a LOD that compares well with established PCR assays (1,000 parasites/ml stool). The RPA- *Giardia* assay was field tested in the highlands of Peru using 111 stool specimens collected from rural communities. For specimens containing low and medium concentrations of DNA (90/111 specimens), the sensitivity was 93% and the specificity was 94%. The RPA- *Entamoeba* assay is currently undergoing bench-top testing and, like the other RPA assays, demonstrated a LOD equivalent to the gold standard PCR. We are currently working on integrating the three assays with our low-resource DNA extraction protocols to build a multiplex assay that can detect all three parasites on a single lateral flow strip.

1174

CRYPTOSPORIDIOSIS: EPIDEMIOLOGY AND CORRELATES OF IMMUNITY IN BANGLADESHI CHILDREN

Poonum Korpe[1], Rashidul Haque[2], A. S. Faruque[2], Feiyang Nu[1], Miao Lu[1], Abdullah Siddique[2], Mamun Kabir[2], Jennie Ma[1], William A. Petri, Jr.[1]

[1]University of Virginia, Charlottesville, VA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

.Cryptosporidiosis has been identified as a leading cause of moderate-to-severe diarrhea in infants worldwide. Human immunity to *Cryptosporidium spp*. is thought to be T-cell mediated, however acquired immunity to this parasite has not been defined. The role of humoral immunity has not been established, though our group has demonstrated the protective effect of maternal breast milk IgA on breastfeeding infants in Bangladesh. In this community-based prospective cohort study, we aim to describe the natural history of *Cryptosporidium spp.* infection and identify correlates of mucosal and humoral immunity in slum-dwelling Bangladeshi children. Children were enrolled at birth and followed for two years, with active surveillance for diarrheal illness. Stool samples were tested for *Cryptosporidium spp*. using real time qPCR. Enzyme-linked immunosorbent assay was used to test for serum anti- *Cryptosporidium* IgG and fecal anti- *Cryptosporidium* IgA. Anthropometric measurements were taken every 3 months. We followed 392 children from birth to age two. Almost 80% of children had been infected with *Cryptosporidium spp*. Asymptomatic infection (75% of children) was more common than diarrheal infection (25% of children). Higher parasite burden, as measured by quantitative real time PCR, was associated with diarrhea rather than asymptomatic infection (T-test, $p < 0.0001$). Using multivariable regression analysis, we found that children with asymptomatic *Cryptosporidium spp.* infection during the first two years of life were significantly more likely to have growth stunting at age two, compared to children who were never infected ($p = 0.035$). Positive anti- *Cryptosporidium* serum IgG at 12 months of age was associated with lower risk of infection during the second year of life (log-rank test, $p = 0.033$). No protective effect was seen with positive fecal anti- *Cryptosporidium* IgA. We also found that over 90% of samples tested were of *C. hominis* subtype, which is consistent with previous reports. In summary, the burden of *Cryptosporidium spp.* infection in Bangladeshi children is largely subclinical, but is associated with significant growth faltering. This is the first study to demonstrate that *Cryptosporidium spp.* infection associated with diarrhea is related to higher parasite burden. Our findings suggest that human immunity to *Cryptosporidium spp*. may be acquired, which has important implications for the potential for vaccine development.

1175

RNA-SEQ-BASED STRUCTURAL ANNOTATION AND REGULATORY MOTIF DISCOVERY IN *THEILERIA PARVA*, AN APICOMPLEXAN PARASITE OF CATTLE IN SUB-SAHARAN AFRICA

Kyle Tretina[1], Hanzel T. Gotia[1], Joshua Orvis[1], Olukemi O. Abolude[1], Priti Kumari[1], Vishvaneth M. Nene[2], Claudia Daubenberger[3], Richard P. Bishop[2], Joana C. Silva[1]

[1]University of Maryland, Baltimore, Baltimore, MD, United States, [2]International Livestock Research Institute, Nairobi, Kenya, [3]Swiss Tropical and Public Health Institute, Basel, Switzerland

Understanding the biology of transmission, colonization and pathogenesis is essential to the rational development of efficacious therapeutics and vaccines against Apicomplexa parasites. Two critical steps that greatly bolster this process are the full structural annotation of their genomes and the identification of the full complement of transcription factor binding sites. Many apicomplexans have high AT nucleotide content and gene-dense genomes, which often thwart the accurate characterization of gene structures and gene regulation. Transcriptional regulation remains poorly understood in these pathogens, which are remarkable in their lack of canonical transcription factors and regulatory motifs. With ever more apicomplexan genomes being sequenced and annotated, including those of human and livestock parasites, there is a need for novel approaches to these issues. Theileria species are distinctive for their very high gene density and apparent lack of enrichment for the binding site of AP2 transcription factors, believed to be principal transcription factors in Apicomplexa. Therefore, *Theileria parva* is ideal as a model system for the development of novel approaches to gene structure annotation and of techniques that can give insight into transcriptional gene regulation in Apicomplexa. RNA-seq technology is particularly well suited to address this problem, providing the ability to identify the precise genomic coordinates of the start and end of transcripts and of introns, as well as relative expression levels. Leveraging RNA-seq data extensively, in standard and novel ways, we re-annotated the *T. parva* genome, identifying 121 new genes, altering 48% of the existing gene structures, and discovering three regulatory motifs. Experiments are ongoing to identify additional regulatory motifs and to determine the applicability of these approaches to other Apicomplexa, such as Babesia and *Cryptosporidium* species.

1176

PROFILING OF AGENTS AGAINST *BABESIA DIVERGENS*

Alexis Dziedziech Dziedziech[1], Bernina Naissant[1], Elizabeth A. Lawrence[1], Louis M. Weiss[2], **Laura Kirkman**[1]

[1]Weill Cornell Medical College, New York, NY, United States, [2]Albert Einstein College of Medicine, New York, NY, United States

Babesiosis is a tickborne zoonotic disease found worldwide. There has been a recent increase in both the total number of human cases as well as the number of patients presenting with severe disease. The limitations of established treatment regimens, especially in immunosuppressed patients, are well recognized with prolonged treatment and frequent recurrences. In addition, parasitemia can be slow to respond to treatment in these patients, thus complicating management. B divergens in particular is noted to be difficult to treat with medications alone and red blood cell exchange transfusions are frequently required. To date, alternative treatment regimens have primarily been chosen empirically, based on activity against other apicomplexan parasites. We have been using an *in vitro* SYBR Green-based assay originally developed for malaria parasites to evaluate the anti-parasitic activity of various compounds against *B. divergens*. Compared to parallel assays in *P. falciparum*, the majority of agents were much less potent when tested against B divergens, with notable exception of agents previously shown to be effective against various species of Babesia; imidocarb, diminazene and atovaquone. We are currently exploring both cytostatic and cytocidal profiles of various agents as well as characterizing interactions between different drug combinations.

1177

INVASION BY ORGANELLAR DNA GENERATES STRAIN-SPECIFIC DIFFERENCES IN THE NUCLEAR GENOME OF *TOXOPLASMA GONDII*

Sivaranjani Namasivayam[1], Cheng Sun[2], Assiatu Barrie[2], Wenyuan Xiao[1], Cedric Feschotte[3], Ellen Pritham[3], Jessica C. Kissinger[1]

[1]University of Georgia, Athens, GA, United States, [2]University of Texas Arlington, Arlington, TX, United States, [3]University of Utah, Salt Lake City, UT, United States

Toxoplasma gondii is one of the most successful zoonotic parasites, essentially capable of infecting any warm-blooded animal. We have determined the extent of mitochondrial (NUMT) and plastid (NUPT) DNA fragment assimilation by the *T. gondii* nuclear genome. While NUMTs have been reported in numerous eukaryotic species they typically constitute a very small fraction of the genome. *T. gondii* has ~13,000 organellar DNA insertions. This is the highest number of insertions and NUMT density (~1.66%) ever reported; ~10 times more than the honeybee genome and almost 100 X greater than is observed in the human genome. The NUMT fragments originate from all regions of the mitochondrial DNA and are distributed across all of the *T. gondii* chromosomes. Careful examination of the regions flanking insertion sites suggests these NUMTs are independent insertions and are not post-integration amplifications. Age estimation of the nucleotide insertion sequences reveals integration events spanning the last 20 million years suggesting that acquisition of organellar DNA by the *T. gondii* nuclear genome is a continual process. Comparison to the closely related species (28 million years separation), Neospora caninum revealed a much lower NUMTs density (~0.71%) in N. caninum and revealed very few conserved NUMTs between the two species. Comparisons of NUMTs between sequences of several *T. gondii* strains and the N. caninum genome sequence reveals that most insertions decay rapidly and that the insertion rate is high. Most interestingly, we have identified strain-specific NUMTs in every strain examined thus far suggesting a possible role for NUMTs in strain-specific biology and diversification. We are currently expanding this analysis to the 62 available strains to obtain a comprehensive picture of the scope, rate and potential implications for this aspect of genome evolution. We have preliminary evidence suggesting NUMTs upstream of genes could foster occasional functionalization and regulate gene expression.

1178

GIARDIA-BACTERIAL INTERACTIONS: DEPLETION OF MICROBIOTA FACILITATES GROWTH IN A MURINE MALNUTRITION MODEL OF GIARDIASIS

Luther A. Bartelt, David T. Bolick, Glynis L. Kolling, Cirle A. Warren, Richard L. Guerrant

University of Virginia, Charlottesville, VA, United States

Giardiasis affects up to 90% of children within the first year of life in resource-limited settings. The clinical manifestations of endemic pediatric Giardiasis range from protection against acute diarrhea to associations with persistent diarrhea and development shortfalls. While pathogen and host variables are known to affect *Giardia* pathogenesis, complex interactions between nutritional factors and microbiota may be highly influential in determining Giardiasis outcomes. Using a C57Bl/6 mouse model, protein energy malnutrition was recently shown to accentuate *G. lamblia*-induced growth faltering through 15 days post-infection. Concurrently, there was altered villus architecture and mucosal inflammatory response in malnourished compared with nourished *Giardia*-infected animals. In the current study, 10^6 *G. lamblia* H3 cysts were used to infect 5-week-old male C57Bl/6 mice sustained on a 2% low-protein diet. Mice were given an antibiotic cocktail containing ampicillin, neomycin, and vancomycin administered in drinking water beginning 7 days prior to and continuing throughout infection. Animal weights were compared to infected mice given water without antibiotics. RT-PCR was

used to confirm infection. In a separate experiment, mice on antibiotics were challenged with *G. lamblia* H3 cysts 35 days prior to challenge with 10^10 enteroaggregative Escherichia coli strain 042. The ampicillin, neomycin, vancomycin cocktail depleted stool microflora that remained suppressed with continued administration through 21 days. *G. lamblia* H3 cyst infection caused growth faltering in non-antibiotic treated mice compared with uninfected controls ($p<0.01$ d13-d14), and compared with antibiotic-treated *G. lamblia* infection ($p<0.05$ d5, $p<0.01$ d6, $p<0.001$ d7, and $p<0.001$ d8-14). Duodenal burden at 15 days post-infection by RT-PCR revealed similar burden of parasites irrespective of antibiotic treatment. In *G. lamblia*-enteroaggregative E. coli 042 co-infection models, growth faltering was worse in the co-enteropathogen infection ($p<0.05$ d8, d10) than with either single infection when compared with uninfected controls. Elucidating the mechanisms accounting for growth faltering in Giardiasis as related to microbiota and malnutrition may help to improve our understanding of this enigmatic parasite and its influence on co-pathogen infections, enteropathy, and childhood development.

1179

DETERMINATION OF LOCAL FECAL CONTAMINATION OF SURFACE WATER BY MICROBIAL SOURCE TRACKING AND ASSOCIATION WITH SCHISTOSOMIASIS RISK IN BRAZIL

Rafael Ponce-Terashima[1], Amber M. Koskey[2], Lúcio M. Barbosa[3], Luciano K. Silva[3], Eliana A. Reis[3], Jackson M. Costa[3], Sandra L. McLellan[2], Mitermayer G. Reis[3], Ronald E. Blanton[1]

[1]Center for Global Health and Diseases, Cleveland, OH, United States, [2]School of Freshwater Sciences, Great Lakes Water Institute, Milwaukee, WI, United States, [3]Laboratory of Pathology and Molecular Biology, Gonçalo Moniz Research Center, Salvador, Bahia, Brazil

Schistosomiasis is to a degree a disease of contact with fecally contaminated surface waters, rather than ingestion. Since it is a chronic infection, schistosomiasis may be amenable to analysis of the quantitative relationship between fecal contamination and risk of infection. In order to examine this relationship, we surveyed an endemic community in rural Bahia, Brazil that straddles a shallow river. All residents were examined for eggs and samples taken from the major water contact sites. Nearly all of the population (98.3%) uses an indoor toilet, but only 44% flush to a septic tank. The remainder discharge to the river. We validated two widely used markers of human fecal contamination, human *Bacteroides* and *Lachnospiraceae* by pyrosequencing and qPCR of local stool samples. We observed that the concentration of these human-associated stains dramatically increased in the downstream portion of the village. Using map algebra in ArcGIS, we developed a model for individual infection risk based on the distance of a person's home to the nearest contact site and the concentration of human fecal contamination at this point. This model explained ~50% of the risk of infection. Following treatment of those infected in 2009, 89% were confirmed egg-negative. At follow-up in 2012, prevalence had declined by 48% and intensity by 44%. Those infected were again treated in 2012 and reexamined in 2013 when prevalence fell by 32% and intensity by 30%. Reinfection rates were 33.7 and 18.4%, in 2012 and 2013 respectively. Incidence was 22.0 and 12.9%. In both 2012 and 2013, all new infections were confined to the most downstream section. Genetic differentiation analysis showed that the reinfecting populations in individuals were moderately differentiated from their pretreatment populations (mean $D = 0.055$ and 0.077, respectively). This confirmed that these were new infections rather than localized absence or failure of treatment. Models of schistosome transmission as well as control planning might be improved by considering local concentrations of fecal contamination of surface water.

1180

SURPRISING INTERACTIONS BETWEEN SCHISTOSOMES AND AMPHISTOMES IN KENYAN *BIOMPHALARIA PFEIFFERI*: AMPHISTOME DEPENDENCE ON AND DOMINANCE OF SCHISTOSOME INFECTIONS, WITH SOME IMPLICATIONS FOR SCHISTOSOME CONTROL

Martina R. Laidemitt[1], Martin W. Mutuku[2], Celestine K. Dweni[2], Gerald M. Mkoji[2], Eric S. Loker[1]

[1]University of New Mexico, Albuquerque, NM, United States, [2]Kenya Medical Research Institute, Nairobi, Kenya

Schistosoma mansoni is commonly transmitted by *Biomphalaria pfeifferi* in streams in western Kenya. Also transmitted by *B. pfeifferi* in these streams are amphistomes, trematodes that mature as adults in domestic ruminants. Because amphistomes are common (some of our collections retrieve 25% or more snails shedding amphistomes), and because their intramolluscan stages may have predatory effects on schistosome sporocysts, amphistomes may have an unappreciated detrimental effect on schistosome transmission in such streams. We have examined amphistome-schistosome interactions to learn if amphistomes are dominant when present in co-infections, and if amphistomes can be manipulated to achieve an even greater effect on schistosome transmission. Thus far we have identified 19 distinct lineages of amphistomes in Kenya, at least four of which rely on *B. pfeifferi* as their snail host. Field-collected *B. pfeifferi* with patent amphistome infections can rarely be superinfected with *S. mansoni*. Attempts to establish experimental infections with amphistomes in *B. pfeifferi* have been unsuccessful, but further study has revealed that if snails are first exposed to amphistomes, and later exposed to *S. mansoni* infection, that the snails subsequently shed amphistome cercariae, or in some cases, amphistomes and schistosomes. We have since confirmed that field-derived snails not shedding any kind of cercariae when exposed to *S. mansoni* surprisingly often shed amphistome cercariae. We interpret the results to mean that amphistome miracidia may frequently penetrate *B. pfeifferi*, but then require a later facilitating effect from *S. mansoni* to achieve a patent infection. Once facilitated though, they largely dominate the schistosome infection. Widespread exposure of snails to amphistomes, at least with the isolates we are working with now, may have two distinct effects: 1) preventing subsequent production of *S. mansoni* cercariae by these snails; and 2) only snails exposed to *S. mansoni* (or possibly other trematodes) would shed amphistome cercariae.

1181

DETECTION OF MIXED SCHISTOSOME INFECTIONS BY AMPLIFYING SPECIES-SPECIFIC DNA FROM URINE SEDIMENT OBTAINED BY FILTRATION

Nilanjan Lodh[1], Jean M. Naples[1], Kwabena M. Bosompem[2], Joseph Quartey[2], Clive J. Shiff[1]

[1]The Johns Hopkins University, Baltimore, MD, United States, [2]Noguchi Memorial Institute for Medical Research, Accra, Ghana

Differential diagnosis of *Schistosoma mansoni* and *S. haematobium,* which often occur sympatrically in Africa, requires both urine and stool and the procedures are low in sensitivity. Frequently used diagnostic tests, such as Kato-Katz (KK) for *S. mansoni* eggs and presence of haematuria for *S. haematobium* both lack sensitivity, produce false-negative results and show reduced accuracy with decreasing intensity of infection. The need for a single diagnostic procedure with high sensitivity, specificity and ease of operation for both parasites is important as many African countries are implementing Mass Drug Administration (MDA) following recommendations of the World Health Organization (WHO). Our approach simplifies the collection and performance of DNA based diagnostic test and is more sensitive and specific than KK and haematuria. Importantly transport costs are considerably reduced as dried filter papers are easy to store, light weight and stable. Eighty-six samples of urine sediment obtained by filtration were collected from a group of 5 - 23 years old

people from an endemic area of southern Ghana where both parasites live sympatrically. DNA was extracted from urine sediment on filter paper from which a species-specific repeat fragment was amplified by polymerase chain reaction (PCR) with specific primers for *S. mansoni* and for *S. haematobium*. Additionally, all participants were tested by KK (stool) and dipstick for haematuria. Diagnostic parameters for all three tests were analyzed statistically. Amplification of species-specific DNA by PCR showed much higher sensitivity (99% - 100%) and specificity (100%) compared to KK and haematuria (sensitivity: 76% and 30% respectively) for both schistosome species. The same pattern was observed when the data were stratified for age group and sex specific analysis. In addition PCR amplification detected DNA from 11 individuals infected with both parasites who were negative by KK and haematuria. This approach of detecting parasite specific DNA from either or both species in a single urine specimen has practical advantages of simplicity of collection avoids the need for two specimens and is more effective than standard tests including those based on serology. This will be important for long term success of parasite control by Mass Drug Administration (MDA) intervention because of the need to detect low intensity infections that persist even after treatment has occurred.

1182

DIAGNOSTIC PERFORMANCE OF KATO-KATZ, MINI-PARASEP AND MINI-FLOTAC TECHNIQUES IN DETECTION OF HELMINTH INFECTIONS IN MBITA, WESTERN KENYA

Annette A. Imali[1], Kimberly Y. Won[2], Fredrick R. Ouma[1], Pauline N. Mwinzi[1], Henry S. Bishop[2], **Maurice R. Odiere**[1]

[1]Kenya Medical Research Institute, Neglected Tropical Diseases Branch, Kisumu, Kenya, [2]Centers for Disease Control and Prevention, Division of Parasitic Diseases and Malaria, Atlanta, GA, United States

This study evaluated the diagnostic performance of Kato-Katz (the WHO recommended diagnostic technique for detecting helminth infections), Mini-Parasep (a concentration method; DiaSys, England) and Mini-FLOTAC (a floatation method based on two different floatation solutions, FS2 and FS7; University of Naples, Italy) for detection of *Schistosoma mansoni* and soil-transmitted helminths (Hookworm, *Ascaris lumbricoides* and *Trichuris trichiura*) ova. Single stool samples were collected from 282 inhabitants from four villages along the shores of Lake Victoria, in Mbita, western Kenya. Aliquots for the Mini-Parasep and Mini-FLOTAC techniques were preserved in 10% and 5% formalin, respectively, before processing. Prevalence of *S. mansoni* was 47.2% (n = 282), 59.3% (n = 209), 3.2% (n =127) and 12.6% (n = 127) by Kato-Katz, Mini-Parasep, Mini-FLOTAC FS2 and Mini-FLOTAC FS7, respectively. Sensitivities for detection of *S. mansoni* were 76.0%, 70.9%, 2.3% and 9.1% for Kato-Katz, Mini-Parasep, Mini-FLOTAC FS2 and Mini-FLOTAC FS7, respectively, and their specificities were 73.0%, 41.7%, 60.3% and 54.4%, respectively. The prevalence of STH was too low for meaningful comparisons. Kato-Katz and Mini-Parasep had a fairly good agreement for *S. mansoni* detection (k=0.49), and a substantial agreement for *A. lumbricoides* (k=0.66). Kato-Katz and Mini FLOTAC FS7 had a fair agreement both for *S. mansoni* (k=0.28) and *T. trichiura* (k=0.35) detection. Kato-Katz diagnosed a higher number of eggs compared to Mini-Parasep (204 vs 105, p= 0.0059) and Mini-FLOTAC FS7 (204 vs 22, p= 0.0211). Kato-Katz also detected higher proportion of heavy intensity *S. mansoni* infections compared to Mini-Parasep which detected higher proportion of light intensity infections. Consistent with other studies, the saturated sodium chloride (FS2) detected more hookworm and less *S. mansoni* compared to the zinc sulphate (FS7) solution. Mini-Parasep performed better than Mini-FLOTAC and is a promising technique with comparable sensitivity and potential logistical advantages compared to the standard Kato-Katz technique.

1183

PHARMACOKINETICS/PHARMACODYNAMICS (PK/PD) OF 40 MG/KG VS. 60 MG/KG DOSING OF PRAZIQUANTEL IN UGANDAN CHILDREN 3-8 YEARS OLD WITH INTESTINAL SCHISTOSOMIASIS

Amaya Bustinduy

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Whilst praziquantel (PZQ) is widely used for control of schistosomiasis by mass drug administration, there has been no pharmacokinetic/pharmacodynamic (PK/PD) study in children; treatment at either 40 mg/kg or 60 mg/kg is a direct extrapolation of common practice dosing in adults. In a time where millions of children receive PZQ in sub-Saharan Africa, there is a pressing need to optimize dosing for more effective treatment of schistosomiasis. To address this deficit, we conducted the first PZQ PK/PD study in children aged 3-8 living in a highly endemic area for *Schistosoma mansoni* around Lake Albert, Uganda. Sixty children with patent intestinal schistosomiasis, ascertained by eggs in stool and/or urine antigen test, were randomized to receive PZQ at either 40 mg/kg or 60 mg/kg dosing. Parasitological data were collected alongside and included soil-transmitted helminths eggs, a malaria rapid test as well as HIV test. Subsequent blood samples were taken at different time points (0, 1, 2, 4, 6, 12 & 24h) for later analysis of venous concentrations of PZQ. Quantification of the enantiomers of PZQ was carried out using chiral chromatography via LC-MS/MS analysis. A population methodology using the program Pmetrics was used with two structural models constructed and fitted to the observed PK data. Models were distinguished on the basis of the linear regression of the observed-predicted values before and after the Bayesian step, log-likelihood values and various measures of bias and precision. As a PD estimate, egg reduction rates at 24 days were significantly greater in older children (> 5 yo) and those with medium and heavy intensity *S. mansoni* infection receiving 60 mg/kg doses of PZQ. Although our results favour the use of higher dosing in school-age children, especially in those with moderate and heavy egg-intensity infections, ongoing PK population modelling will be used to identify optimal paediatric dosages associated with maximal anti-parasitic activity and comparable drug exposures in adults.

1184

HIGH INTENSITIES OF *SCHISTOSOMA MANSONI* IN UGANDAN PRIMARY SCHOOL CHILDREN AFTER TEN YEARS OF MASS DRUG ADMINISTRATION

Poppy H. Lamberton[1], Thomas Crellen[1], Charlotte Gower[1], Narcis B. Kabatereine[2], Edridah Muheki Tukahebwa[3], David W. Oguttu[3], Joanne P. Webster[1]

[1]Imperial College London, London, United Kingdom, [2]Schistosomiasis Control Initiative at Vector Control Division, Kampala, Uganda, [3]Vector Control Division, Kampala, Uganda

The Schistosomiasis Control Initiative (SCI) began mass drug administration (MDA) with praziquantel (PZQ) in Uganda in 2003. Knowledge on how parasite infections change as a result of PZQ-treatment may have important implications for the success of this and other control programmes. Baseline prevalence, intensity of infection, morbidity, host behavioural data and side-effects post-PZQ-treatment were recorded for *Schistosoma mansoni* infections, the causative agent of intestinal schistosomiasis, in children from three primary schools on the shores of Lake Victoria, in Mayuge district, Uganda. Schools were visited at twelve time points, over three years from 2004 to 2006. Children at these schools were resampled in February and March 2013, and will be revisited in April and May 2014, now over 10 years after the MDA programme began. Follow-up epidemiological and parasitological data collection pre- and post-PZQ-treatment will be repeated. Baseline field data revealed that 80.6% of children were still excreting eggs one-week-post-treatment with 40 mg/kg PZQ, and 39.9% of these had counts >100 eggs per gram (epg). Four-weeks-post-treatment 23.9% of children were still excreting

eggs with 2.9% having counts >100 epg. Hatching tests indicated that the eggs being released at both one- and four-weeks-post-treatment were viable. From 2004 to 2006, although infection intensities were lower each year, prevalence returned to baseline levels. Ten years later, infection prevalence and intensities, in 2013, were higher than at baseline, raising concerns over lower than expected treatment success and questions about the potential reasons for this. Epidemiological and parasitological data collection from 2014 will be compared with 2013 and baseline data to establish if these maintained high intensities are due to potential reduced susceptibility, poor adherence to treatment and/or a consequence of high transmission.

1185

MATHEMATICAL MODELLING OF INTEGRATED CONTROL OF SCHISTOSOMIASIS: TOWARDS ELIMINATION

Gail M. Williams[1], Darren Gray[2], Allen Ross[3], Don P. McManus[4], Yuesheng Li[4]

[1]University of Queensland, Brisbane, Australia, [2]Australian National University, Canberra, Australia, [3]Griffith University, Brisbane, Australia, [4]Queensland Institute of Medical Research, Brisbane, Australia

While the prevalence of schistosomiasis has been markedly reduced in many areas of PR China where it was previously endemic it is resistant to elimination in some areas. In other countries, such as the Philippines it remains a serious public health problem. Combination of individual control measures such as bovine and human chemotherapy, improved sanitation, and molluscicide appears to offer the best approach to further decreasing prevalence and achieving elimination. Bovine vaccination appears to offer an additional element to an integrated control strategy. While estimates of the efficacy of individual strategies are available from field trials, the overall effectiveness of integrated control can be estimated by mathematical modelling. This must incorporate realistic assumptions about such parameters as coverage and frequency of intervention and duration of efficacy, as well as knowledge of the local epidemiology and transmission of the infection. We have developed a mathematical model which incorporates a simulation of the transmission of schistosomiasis in a situation which involves multiple and heterogeneous mammalian hosts (e.g humans, water buffalos). It takes into account births, deaths and aging of such hosts, as well as the infection characteristics of each. The model is parameterised using epidemiological data collected in both PR China and the Philippines from extensive field surveillance over many years. We compare the results (in terms of infections, infection-years prevented, and probability of elimination) of various five-year programs within various epidemiological settings. In particular we estimate the minimum bovine vaccine efficacy needed to block transmission of schistosomiasis in these settings, in combination with different levels of uptake of other interventions such as human chemotherapy.

1186

TRANSGENIC TOOLS ALLOW THE STUDY OF P450 MIS-EXPRESSION ON INSECTICIDE RESISTANCE PHENOTYPE IN *ANOPHELES GAMBIAE*

Amy Lynd[1], Dolphine Amenya[1], Adriana Adolfi[1], Vasilia Balabanidou[2], John Vontas[2], Hilary Ranson[1], Gareth J. Lycett[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]University of Crete, Heraklion, Greece

The recent development of molecular tools for use in transgenic mosquitoes has provided the means to enable advanced function genetic analysis to be carried out in *Anopheles gambiae.* These tools include the Gal4/UAS system, PhiC31 recombination mediated cassette exchange (RCME) and enhancer trapping. Together they allow tissue specific expression in a conditional manner and permit the direct effects of gene mis-expression to be compared *in-vivo* by negating the position effects seen using transposon based integration systems. These tools are now being applied to examine metabolic and cuticular insecticide resistance regulated through P450 expression. Resistance to insecticides in the form of target site insensitivity mutations, cuticular and metabolic resistance, is threatening the success of insecticide based vector control programmes. Whilst target site insensitivity is well studied, the mechanisms underlying metabolic and cuticular resistance are poorly understood. Genome wide expression studies have revealed a number of candidate p450 genes whose overexpression is associated with insecticide resistance in *Anopheles* species. Although *in vitro* expression has indicated that several p450 enzymes metabolize insecticides, the function of other p450s, highly over expressed in resistant mosquitoes, have remained elusive, suggesting they may have other roles in resistance. A number of p450's are highly expressed in the oenocytes, which may suggest a role in lipid and hydrocarbon formation that has been linked to cuticular resistance. To investigate the *in vivo* role of these p450 genes, we utilized the Gal4/UAS system to examine the effects of both tissue specific overexpression and stable RNAi knockdown in transgenic *An. gambiae.* Transgenic UAS responder lines were created by RCME. Crossing of these p450 responder lines to tissue specific Gal4 driver lines allowed the phenotypic effects of p450 overexpression and stable knockdown to be studied *in vivo*. The results of these crosses and the phenotypic effects on both insecticide resistance and mosquito fitness are discussed. To our knowledge, this is the first time that transgenic *Anopheles* have been used to study insecticide resistance and the work demonstrates that combining RCME with the Gal4/UAS system provides a useful tool for gene expression studies in the mosquito.

1187

EFFECTS OF THE KDR RESISTANCE MUTATION ON THE SUSCEPTIBILITY OF WILD *ANOPHELES GAMBIAE* POPULATIONS TO *PLASMODIUM FALCIPARUM*: A HINDRANCE FOR VECTOR CONTROL

Mamadou Ousmane Ndiath[1], Jean-François Trape[2]

[1]Institut Pasteur of Bangui, Bangui, Central African Republic, [2]Institut de Recherche pour le Développement, Dakar, Senegal

In the context of generalization of insecticide resistance, we hypothesized that insecticide resistance has a positive impact on the capacity of mosquitoes to transmit malaria. We therefore investigated populations of *Anopheles coluzzii* and *An. gambiae* S molecular form to assess whether different genotypes at the kdr locus are responsible for different susceptibilities. F3 progeny of *An. gambiae* s.l. collected in Dielmo were infected by direct membrane feeding with *Plasmodium falciparum* gametocyte-containing blood sampled from volunteer patients. The presence of oocysts was determined by light microscopy after 7 days, and the presence of sporozoites by ELISA after 14 days. Mosquito species and molecular forms were identified by PCR. Generalized linear models were performed using the R software to test the effect of explanatory variables on infection rate and density. The oocyst rate was 12.6 times greater in RS than in SS mosquitoes, and all RR individuals were infected. The prevalence of sporozoites was 38.6 greater in the RS group and 420 times greater in the RR group than in SS genotypes. In the model of oocyst number and sporozoite density, genotype interactions were not significant, but the effects of strain and genotype were significant. The number of oocysts infecting a mosquito when infection occurred was 9.3 times greater in the S form strain than in the RS group and 42.7 times greater in the RR group than in the SS genotype. In *An. coluzzii*, the number of oocysts was greater in the RS group and 32 times greater in the RR group than in SS. The sporozoite absorbance density was higher in RR and RS than in the SS group and higher in the S molecular form. The presence of the resistance allele at the kdr locus increases susceptibility to *Plasmodium* not only at the oocyst stage but also at the sporozoite stage in non-genetically modified wild mosquitoes. These results have significant implications and should be taken into account in the development of strategies for malaria control.

1188

GENETIC DIVERSITY PATTERNS IN THE MAJOR MALARIA VECTOR, *ANOPHELES FUNESTUS*, REVEAL A GENOMIC FOOTPRINT OF SELECTION ASSOCIATED WITH CONTROL INTERVENTIONS IN SOUTHERN AFRICA

Kayla G. Barnes[1], Themba Mzilahowa[2], Janet Hemingway[1], Charles S. Wondji[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Malaria Alert Center, Blantyre, Malawi

Pyrethroid resistance observed in *Anopheles funestus* is hindering efforts to control this major malaria vector throughout Southern Africa. Despite some similarities, significant differences in the resistance and gene expression profiles have recently been observed between populations of *An. funestus* in this region. The existence of barriers to gene flow and their potential impact on the spread of insecticide resistance genes between populations remains uncharacterized. Here, we resolved the population structure of *An. funestus* in Southern Africa by genotyping 17 microsatellites in 40-48 samples from ten collection sites. This genome-wide analysis revealed the presence of three population clusters across Southern Africa (pairwise Fst score p≤0.05) with northern Malawi populations genetically closer to Zambia than that of southern Malawi and Mozambique. These results parallel previous gene expression and resistance profiles data. Additionally, we observed a temporal shift in population structure associated with LLINs and IRS intervention in Southern Malawi. Fine-scale analysis of polymorphism of the 120kb rp1pyrethroid resistance QTL including the two main resistance cytochrome P450 genes, CYP6P9a and CYP6P9b, revealed a significant loss of genetic diversity in samples collected post intervention while pre-intervention mosquitoes were highly diverse. This reduced diversity spans a region of 70kb around the two genes, supporting the presence of a selective sweep in a genetic area linked to pyrethroid resistance. This study suggests that gene flow is not uniformed across Southern African populations of *An. funestus* and could impact the spread of pyrethroid resistance. More importantly, by detecting a genomic selection footprint associated with control interventions, this work reveals that control interventions may be the main driving force of pyrethroid resistance in this region.

1189

A SIGNIFICANT ASSOCIATION BETWEEN THE *VGSC*-L1014S KDR MUTATION AND INFECTION WITH *PLASMODIUM FALCIPARUM* IN *ANOPHELES GAMBIAE* FROM EASTERN TANZANIA

Bilali I. Kabula[1], Patrick Tungu[1], Emily J. Rippon[2], Keith Steen[2], Stephen Magesa[1], William Kisinza[1], Martin J. Donnelly[2], Franklin Mosha[3]

[1]National Institute for Medical Research, Muheza, United Republic of Tanzania, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]Kilimanjaro Christian Medical University College, Moshi, United Republic of Tanzania

The success of malaria vector control is threatened by widespread pyrethroid resistance. However, the extent to which insecticide resistance mechanisms impact upon the development of the malaria parasite in its vector remains unknown. The objective of this study was therefore, to investigate the association between the presence of the pyrethroid resistance *vgsc*-L1014S allele and infection with *Plasmodium falciparum* sporozoites in *Anopheles gambiae*. WHO standard methods were used to characterise susceptibility of wild female anopheles mosquitoes to 0.05% deltamethrin. PCR based molecular diagnostics were used to identify mosquitoes by species and to detect *vgsc*-L1014S alleles. The presence of *P. falciparum* sporozoites was detected using a CSP-ELISA. *Anopheles gambiae s.l.* were resistant to deltamethrin with mortality rates of 78.6% [95% CI: 74.9-81.9%]) in the dry season and 81.2% [95% CI: 76.8-84.9%]) in the rainy season. Of 545 mosquitoes genotyped, 96.5% were *An. gambiae s.s.* and 3.5% were *An. arabiensis*. The *vgsc*-L1014S mutation was detected in both *An. gambiae s.s.* and *An. arabiensis* at the allelic frequency of 0.45 (95% CI: 0.41-0.48) and 0.32 (95% CI: 0.19-0.47) respectively. In *An. gambiae* s.s., *vgsc*-L1014S was significantly associated with deltamethrin resistance (χ^2= 11.19; p=0.0008). The *P. falciparum* sporozoite infection rate in *An. gambiae s.s.* was 4.11%. There was a significant association between the presence of sporozoites and *vgsc*-L1014S mutation in *An. gambiae s.s.* (χ^2 = 6.89; p=0.009). The presence of this association suggests that *vgsc*-L1014S carrying mosquitoes are more likely to survive sufficiently long to transmit malaria infection. These findings are of importance for the epidemiology of malaria considering the widespread nature of this resistance mutation in Africa.

1190

EFFECTS OF AN EPIGENETIC DRUG ON MALARIA MOSQUITOES

Atashi Sharma, Troy D. Anderson, Igor V. Sharakhov

University of Virginia Tech, Blacksburg, VA, United States

Malarial mosquitoes adapt to a wide range of changes in environment quickly, making malaria control an omnipresent problem in tropical countries. Epigenetic regulation of gene expression may be an important factor for mosquito development and survival at various conditions. Pharmacological studies of drug effects on cell lines and animal models have established the use of epigenetic drugs as a useful tool for modulating the genetics and physiology of cells and organisms. Epigenetic drugs are well established in cancer research, however not much is known about their effects on insects. We designed a study for examining the biochemical effects of 3-Deazaneplanocin A (DZNep), an experimental epigenetic drug for cancer therapy, on the malaria vector, *Anopheles gambiae*. Our aim was to test if DZNep may be used as an effective tool to study effect of epigenetic changes in malaria mosquitoes. A concentration-dependent increase in mortality and decrease in size was observed in larval mosquitoes exposed to DZNep whereas the drug reduced the fecundity of adult female mosquitoes relative to the control treatments. In addition, there was a concentration-dependent decrease in *S*-adenosylhomocysteine (SAH) hydrolase activity in mosquitoes following exposure to DZNep relative to control treatments. Effect of DZNep on the chromatin structure of polytene chromosomes obtained from ovarian nurse cells of exposed females was evaluated. Our results reveal that epigenetic changes in mosquitoes affect the life span and fecundity of mosquitoes. We were able to demonstrate a unique multi-prong approach for exploring the toxicological effects of water-soluble, epigenetic drugs against vector mosquitoes and other insects. The emergence of insecticide resistance warrants the exploration of novel targets for vector mosquito control. Therefore, future directions would involve identifying potential leads for targeting epigenetics of vectors.

1191

INSENSITIVITY TO SPATIAL REPELLENTS: A HERITABLE TRAIT?

Joseph Wagman[1], John P. Grieco[1], Nicole L. Achee[2]

[1]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]University of Notre Dame, Notre Dame, IN, United States

Novel, broadly applicable vector control strategies are needed to augment the currently available options of indoor residual spray and insecticide treated nets. Evidence has shown that the spatial repellent actions of some insecticides can reduce pathogen transmission. For this reason, efforts are underway to outline requirements and challenges for an expanded public health role for spatial repellent products. It is clear much remains to be learned about the underlying mechanisms of action that result in repellency behaviors and the impact of these active ingredients on vector populations over time. The current study was designed to investigate the heritability of spatial repellent responses in the dengue virus vector *Aedes aegypti* using an *in vitro* repellency bioassay and the active ingredient transfluthrin. Specifically, selective breeding of behavioral responder and non-responder cohorts was conducted for nine generations. Results

show the responder cohorts exhibited consistent repellent responses - no positive selection was observed. The selective breeding of non-responders resulted in a mosquito cohort that was not repelled after four generations. It is important to note that the selective breeding scheme used here is likely to produce greater selective pressures than the real world application of spatial repellents. Ongoing studies include back-cross breeding experiments to assess whether the non-responder phenotype is recessive or dominant and evaluations of transfluthrin toxicity susceptibility in both the responder and non-responder cohorts. Data collection from these additional studies will be completed by July, 2014.

1192

PHENOTYPIC EFFECTS OF CONCOMITANT INSENSITIVE ACETYLCHOLINESTERASE (ACE-1R) AND KNOCKDOWN RESISTANCE (KDRR) IN *ANOPHELES GAMBIAE*: A HINDRANCE FOR INSECTICIDE RESISTANCE MANAGEMENT FOR MALARIA VECTOR CONTROL

Benoît Sessinou Assogba[1], Luc Salako Djogbenou[1], Jacques Saizonou[1], Pascal Milesi[2], Laurette Djossou[1], Innocent Djegbe[3], Welbeck Achille Oumbouke[4], Fabrice Chandre[5], Lamine Baba-Moussa[6], Mylène Weill[2], Michel Makoutodé[1]

[1]Institut Régional de Santé Publique/World Health Organization/UAC, Cotonou, Benin, [2]Université Montpellier 2, Institut des Sciences de l'Evolution de Montpellier (UMR 5554, CNRS-UM2-IRD), Montpellier, France, [3]Institut de Recherche pour le Developpement, MIVEGEC (UMR 224- CNRS 5290-UM1-UM2), Centre de Recherche Entomologique de Cotonou, Cotonou, Benin, [4]Centre de Recherche Entomologique de Cotonou, Health Ministry, Cotonou, Benin, [5]Institut de recherche pour le développement, Unité Mixte de Recherche MIVEGEC (IRD 224-CNRS 5290-UM1-UM2), Montpellier, France, [6]Université d'Abomey Calavi, Faculté des Sciences et Techniques, Laboratoire de Biologie et de Typage Moléculaire en Microbiologie, Cotonou, Benin

Malaria is endemic in sub-Saharan Africa with considerable burden for human health. Major insecticide resistance mechanisms such as kdrR and ace-1R alleles constitute a hindrance to malaria vector control programs. *Anopheles gambiae* bearing both kdr and ace-1 resistant alleles become increasingly recorded in wild populations. In order to maintain the efficacy of vector control strategies, the characterization of concomitant kdr and ace-1 resistance, and their pleiotropic effects on malaria vector phenotype toward insecticide are important. Larval and adult bioassays were performed with different insecticide classes used in public health following WHO standard guidelines on four laboratory *Anopheles gambiae* strains, sharing the same genetic background but harboring distinct resistance status: KISUMU without any resistance allele; ACERKIS with ace-1R allele; KISKDR with kdrR allele and ACERKDRKIS with both resistance alleles ace-1R and kdrR. In parallel, acetylcholinesterase (AChE1) activity levels were recorded for the 4 strains. Larval bioassays indicate that the homozygote resistant strain harboring both alleles (ACERKDRKIS) displayed slightly higher but significant resistance level to various insecticides tested: carbamates (bendiocarb, $p<0.001$; propoxur, $p=0.02$) and organophosphates (chlorpyriphos-methyl, $p=0.002$; fenitrothion, $p<0.001$) when compared to ACERKIS strain. However, no differences were recorded between ACERKDRKIS and KISKDR resistance level against permethrin (Pyrethroid, $p=0.7$) and DDT (Organochlorine, $p=0.24$). For adult bioassays, the percentages of mosquitoes knocked down were significantly lower for ACERKDRKIS than for KISKDR with permethrin ($p = 0.003$) but not with deltamethrin. The percentage of mortality from adult bioassays was similar between ACERKDRKIS and ACERKIS with carbamates and organophosphates, or between ACERKDRKIS and KISKDR with pyrethroid and DDT. Concerning acetylcholinesterase enzyme, ACERKDRKIS strain showed similar AChE1 activity than ACERKIS. I conclusion, the presence of both kdrR and ace-1R alleles increase the resistance level to carbamate and organophosphate insecticides and may represent an important threat to malaria vector control in West Africa.

1193

INFLAMMATORY GENES ASSOCIATED WITH SEIZURES OF NEUROCYSTICERCOSIS

Prabhakaran Vasudevan[1], Ramajayam Govindan[1], Josephin Justin Babu[1], Michael P. Anderson[2], Douglas A. Drevets[3], Helene Carabin[2], Jay S. Hanas[4], Vedantam Rajshekhar[5], Anna Oommen[1]

[1]Department of Neurological Sciences, Neurochemistry Laboratory, Christian Medical College and Hospital, Vellore, TamilNadu, India, [2]Department of Biostatistics and Epidemiology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK, United States, [3]Department of Medicine, Oklahoma University Health Sciences Center, Oklahoma City, OK, United States, [4]Department of Biochemistry and Molecular Biology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK, United States, [5]Department of Neurological Sciences, Neurosurgery Unit-II, Christian Medical College and Hospital, Vellore, TamilNadu, India

Seizures are a common and early symptom of a wide spectrum of brain pathologies from infections to malignancies. They are often not treated in developing countries due to stigmatization, resulting in a high burden to the people affected and their families. In countries where pigs are raised traditionally and sanitation is poor, almost one third of epilepsies are associated with neurocysticercosis (NCC), which occurs when the larval form of *Taenia solium* infects the human brain. A reliable diagnostic test for NCC-associated seizures not requiring expensive brain imaging is not available. Hence, people with epilepsy in developing countries who seek treatment are prescribed "traditional" anti-epileptic drugs that aim at reducing brain hyperactivity rather than targeting its cause. Identifying blood markers specific to NCC-associated seizures could contribute to developing diagnostics and more targeted treatments. This study examines inflammatory genes associated with NCC in peripheral blood monocytes. In a study at the Christian Medical College Hospital, Vellore, India, inflammatory genes associated with NCC-associated seizures were determined by comparison of microarray data (Agilent platform) of peripheral blood monocyte RNA of 12 NCC patients with seizures, 10 patients with idiopathic seizures and 10 patients without seizures and normal brain images. Data analysis and normalization by percentile shift were done using GeneSpring GX software 12.0. Overall there was greater similarity in gene expression between the two seizure groups than either seizure group compared to controls. The two seizure groups shared 452 upregulated and 378 downregulated genes with innate immune responses being a significant functional annotation. Importantly, NCC patients had 151 upregulated and 27 downregulated genes that were not changed in patients with idiopathic seizures. These data suggest NCC seizures display a unique mRNA signature and share a common inflammatory background with idiopathic seizures.

1194

BIO-GUIDED IDENTIFICATION OF PROTEINS FOR THE DIAGNOSTIC OF CYSTICERCOSIS IN SWINE

Priscilla Nativel[1], Anjanirina Rahantamalala[1], Sitraka Ramiandrisoa[1], Virginie Rasoamampianina[1], Magalie Duchateau[2], Julia Chamot-Rooke[2], Remy Guebey[1], Harentsoaniaina Rasamoelina-Andriamanivo[3], **Ronan Jambou**[2]

[1]Institut Pasteur de Madagascar, Antananarivo, Madagascar, [2]Institut Pasteur, Paris, France, [3]FOFIFA-Département de Recherches Zootechniques et Vétérinaires, Antananarivo, Madagascar

Cysticercosis is caused by *Taenia solium* and affects both humans and pigs. Human is the definitive host of the adult tapeworm. Pig is infected by eggs emitted in human feces and develops cysticercosis due to larval stage, in the muscles, brain, and eyes. Human develop disease either through self-contamination by eggs emitted from his tapeworm or by ingestion of contaminated food by human feces. Neurocysticercosis (NCC) is one of the most prevalent parasitic infection of the brain and the most common cause of seizures in adults in tropical countries. In Madagascar, a high prevalence of cysticercosis in swine was reported

especially in rural area (up to 30%) and consumption of pig infected meat by villagers is very common propagating taeniasis. The economic value of cysticercosis-infected meat are degraded from 20% to 50%, which is a major cause of income loss for poor farmers. Therefore, villagers are aware of cysticercosis and ask for rapid diagnostic associated with a treatment of pigs. However currently available diagnostic tests need laboratory facilities. The production of a diagnostic test usable at the farm-gate could thus be sustained by the pork-meat market itself. To design an immunochromatographic test usable at the farm gate, we started a bio-guided identification of new target proteins in the liquid of the cyst. This liquid contains water-soluble parasite proteins and is released in the host during the lysis of the dying cyst. Identification of the proteins of interest was done using ion exchange chromatography plus 2D separation of the proteins followed by western blot analysis using serums from infected pigs. Spots from the coomassie-stained gel corresponding to these proteins were then analyzed by mass spectroscopy and identified using a bank of ESTs of *T. solium*. Eighteen new proteins of interest were identified, expressed in E.coli and tested against serums

1195

COMPARISON OF SERUM MASS PROFILING OF PATIENTS WITH NEUROCYSTICERCOSIS EPILEPSY, IDIOPATHIC EPILEPSY AND NO NEUROLOGICAL DISEASE

Jay Hanas[1], James Hocker[1], Ramajayam G[2], Anna Oommen[2], Josephin Justin Babu[2], Michael Anderson[1], Douglas Drevets[1], Prabhakaran Vasudevan[2], Rajshekhar Vedantam[2], Helene Carabin[1]

[1]University of Oklahoma Health Sciences Center, Oklahoma City, OK, United States, [2]Christian Medical College, Vellore, India

Seizures are symptomatic of brain pathologies like infections or malignancies, but also can be idiopathic. In areas where pigs are a food source and sanitation is poor, about one third of seizures are from neurocysticercosis (NCC), a *Taenia solium* larval infection of the brain. Diagnostic tests for NCC-associated seizures, other than imaging, are not readily available. A serological test to identify NCC-related seizures among individuals with seizures of unknown origin is needed to improve treatment and understanding of seizures induced by NCC. In this study, we used serum mass profiling (SMP) to distinguish patients with seizures due to NCC states from idiopathic seizures and from seizure-free control subjects. SMP is based on the hypothesis that a body's serum profile will reflect specific disease phenotypes and physiologies. Sera from patients with solitary cysticercus NCC (SNCC, N=11), multiple cysts NCC (MNCC, N=10), idiopathic seizures (S, N=5), and controls (C, N=9) were analyzed by electrospray ionization ion-trap mass spectrometry. Leave one out cross validation was used to identify m/Z (mass/charge) peaks which differed significantly ($p<0.05$) when comparing two groups at a time (e.g., multiple NCC vs control or solitary cysticercus NCC vs control). Results showed that each patient group was distinguishable from controls (SNCC v C, MNCC v C, S v C) with p-values < 4x10-8. In addition, the idiopathic seizure group was distinguished from the SNCC group or the MNCC group (S v SNCC, S v MNCC) with p-values < 4x10-8. When patient groups were randomized with their respective controls, p-values for each comparison increased to >0.2 suggesting that physiologic processes accounted for the observed differences. Moreover, the sera samples from idiopathic seizures identified with neither control nor NCC patient groups,suggesting that NCC-associated seizures and idiopathic seizures represent different serum physiological states. These data suggest serum mass profiling is useful for development of a sero-diagnostic tool for identifying NCC-induced seizures.

1196

SPATIAL CLUSTERING OF PORCINE AND HUMAN CYSTICERCOSIS AT VILLAGE LEVEL OF NAYALA, BOULKIEMDE, AND SANGUIE PROVINCES IN BURKINA FASO

Ram P. Poudel[1], Helene Carabin[1], Sheryl Magzamen[2], Ramané Ganaba[3], Pierre Dorny[3], Sarah Gabriel[3], Zékiba Tarnagda[3], Pierre-Marie Preux[3], Jean-Bosco Ouédraogo[3], Athanase Millogo[3], Farid Boumediene[3]

[1]University of Oklahoma Health Sciences Center, Oklahoma City, OK, United States, [2]Colorado State University, Fort Collins, CO, United States, [3]University of Ouagadougou, Ouagadougou, Burkina Faso

Taenia solium is a parasitic zoonosis transmitted between humans and pigs. In the intermediate (pigs) and accidental (humans) hosts, the larval form of the parasite causes cysticercosis when establishing in tissues. In humans, larvae may migrate to the brain, causing severe neurological disorders such as epilepsy, severe chronic headaches, and sometimes death. To interrupt the life cycle of *T. solium*, it is essential to identify clusters of porcine and human infection. This study aims at identifying village-level spatial clusters of porcine and human cysticercosis. A total of 60 randomly-selected eligible pig-raising villages located in 30 departments were sampled in three provinces of Burkina Faso. In each village, concessions (a group of households) raising sows and piglets were identified, 10 sow-raising and 30 piglet-raising concessions were randomly selected, and one sow and one piglet was randomly sampled in each selected concession. Another 40 concessions were randomly selected, and the first 60 humans living in one of the 80 selected concessions consenting to a 4 year serological follow-up provided a blood sample. Longitude and latitude coordinates of each concession were measured using PDAs. An ELISA was used to detect the presence of current cysticercosis infection in sera. Spatial analyses were conducted with ArcGIS 10.1 The GI* statistic was used to identify clusters of villages with high prevalence (Hot Spots) and low prevalence (Cold Spots). Twelve spatial clusters of porcine and seven of human cysticercosis were found when using a threshold of 11 and 12 kilometers for porcine and human cysticercosis respectively between neighboring villages. More analyses are required to assess if some of this clustering may be partly explained by socio-demographic factors in humans or management factors in pigs as well as environmental factors such as village-level sanitation indicators, or factors that may affect the survival of the eggs such as temperature, humidity, and land cover.

1197

EFFECT OF ALBENDAZOLE ON SEIZURES IN PATIENTS WITH SYMPTOMATIC NEUROCYSTICERCOSIS

Matthew L. Romo[1], Arturo Carpio[2], Katarzyna Wyka[1], Denise Leslie[3], Howard Andrews[4], Emilia Bagiella[4], W. Allen Hauser[5], Elizabeth A. Kelvin[1]

[1]CUNY School of Public Health, New York, NY, United States, [2]School of Medicine, Cuenca, Ecuador, [3]MRI Diagnostics of Westchester, Valhalla, NY, United States, [4]Department of Biostatistics, Mailman School of Public Health, New York, NY, United States, [5]Gertrude H. Sergievsky Center, College of Physicians and Surgeons, New York, NY, United States

Neurocysticercosis is a major cause of neurological morbidity and mortality in endemic countries; however, it is unclear if antihelminthic treatment reduces the burden of neurological symptoms, particularly seizures. No trial has reported a benefit of albendazole in improving seizure outcomes, with the exception of one trial that reported that treatment with albendazole was associated with fewer "seizures with generalization." Our objective was to examine the effect of albendazole on seizures in more detail, including by seizure type, post-treatment. Data come from a trial conducted in Ecuador in which patients with symptomatic neurocysticercosis were randomized to receive albendazole 400 mg or placebo twice daily for 8 days, both with prednisone. 178 patients were randomized, with 88 in the albendazole group and 90 in the placebo group. 88% of patients in the albendazole group and 86% of patients

in the placebo group completed 12-months of follow-up. Overall, fewer patients in the albendazole group had at least one seizure during follow-up (24% in albendazole group vs. 34% in placebo group), but this difference was not statistically significant (*P*=0.14). A similar proportion of patients had at least one generalized seizure (11% in albendazole group vs. 12% in placebo group; *P*=0.77), and at least one focal seizure (22% in albendazole group vs. 25% in placebo group; *P*=0.65), during follow-up. On average, there was a lower total number of seizures, generalized seizures, and focal seizures in the albendazole group compared with the placebo group, but this was not statistically significant in unadjusted negative binomial models. After adjusting for potential confounders (age and history of generalized seizures at baseline), the difference in the mean number of generalized seizures was significant (rate ratio 0.24; 95% confidence interval: -2.68, -0.15; *P*=0.03). However, the difference in the mean number of total seizures and focal seizures was not statistically significant in adjusted models. In conclusion, albendazole may reduce the frequency of generalized seizures, but more research is needed to determine how albendazole modifies long-term disease course.

1198

RETROSPECTIVE REVIEW OF CYSTICERCOSIS IN RETURNED UNITED STATES TRAVELERS

David Goodman-Meza[1], JeanAnne Ware[2], Andrew Anthony[3], Christina M. Coyle[3], Ted E. Nash[2]

[1]Jacobi Medical Center, Bronx, NY, United States, [2]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [3]Albert Einstein College of Medicine, Bronx, NY, United States

Neurocysticercosis (NCC) has become increasingly recognized in non-endemic countries during the past ten years due to immigration from endemic countries, but disease in travelers is rarely reported in the United States (US). A retrospective review of all travel-related cysticercosis (CC) cases seen at National Institute of Health, Bethesda (NIH) and Jacobi Medical Center's Tropical Medicine Clinic (JMC), Bronx between the years 1985 and 2013 was undertaken. Thirteen cases were identified, 10 at NIH and 3 at JMC. Age was 35.5 (range 22-61), 8 female and 3 foreign born. Most had history of travel to Latin America (69.2%), followed by the Caribbean (38.5%), India (23.1%) and Africa (23.1%). Reason for travel was most commonly tourism (38.5%) and duration of travel had a mean of 2.1 years (days-9 years) with 46.1% traveling less than 3 months. Seven (53.8%) had a history of Taeniasis during or after travel. Most common symptoms were seizures (61.5%), headaches (30.8%), visual changes (30.8%) and subcutaneous nodules (30.8%). Of the 4 with subcutaneous disease, 2 had neurologic symptoms due to NCC, 1 had neuroimaging evidence of NCC and 1 had only subcutaneous involvement. On imaging, 10 had parenchymal lesions, 2 subarachnoid disease (both had the most remote travel history, 14-20 years since last travel) and one had no lesions. Of the parenchymal cases, 3 had single cysts, 3 multiple cysts, 2 multiple enhancing lesions and 2 with multiple calcifications. Eight (61.5%) had positive enzyme-linked immunoblot transfer assay (EITB) serology. All 3 single cystic lesions were EITB negative, whereas all but 1 case with multiple lesions were EITB positive. This is the largest series of returned travelers with NCC in the US. In contrast to the literature, these patients had large burden of disease as evidenced by multiple parenchymal lesions and SANCC. Almost half of the patients had short term travel and greater than half had a history of *Taeniasis*. A high-index of suspicion should be maintained in returned travelers from endemic regions presenting with neurologic or subcutaneous symptoms.

1199

ASSESSING THE ECONOMIC BURDEN OF NEUROCYSTICERCOSIS HOSPITALIZATIONS IN THE UNITED STATES, 2003-2012

Robert Flecker

Oregon Health and Sciences University, Portland, OR, United States

Neurocysticercosis (NCC), caused by brain infection with *Taenia solium* larval cysts, is a leading cause of acquired epilepsy. This disease is of emerging public health concern in the United States, especially among immigrants from and travelers to cysticercosis-endemic regions. The complicated and chronic nature of NCC management results in significant cost; consequently, the economic burden associated with NCC in the United States could be considerable. To assess the economic burden of NCC in the United States, we reviewed the Nationwide Inpatient Sample dataset from 2003-2011 for hospitalizations with ICD-9 diagnostic code of 123.x and at least one additional supporting diagnostic code. We also evaluated hospitalizations for other major tropical diseases for comparison. Based on this stratified sample, we estimated there were 16,103 NCC hospitalizations with hospital charges between $650 - $915 million USD over the 9 years studied. The majority of hospitalized cases were Hispanic (74%), male (57%) and under 44 years of age (70%). Almost 60% of NCC hospitalizations presented with a coexisting diagnosis of seizure or epilepsy. Over 38% of hospitalizations were government pay, either Medicare or Medicaid, and 75% were admitted from the emergency department. California was most affected with 1/3 of all hospitalizations and almost half of total U.S. hospital charges (est. $442,000,000 USD), followed by Texas, New York, Florida and Illinois. There were almost twice as many hospitalizations and three times the total charges for NCC than malaria. NCC results in a greater number of hospitalizations and higher hospitalization charges in the U.S than any of the other tropical diseases we studied. NCC is an increasing public health concern in the United States, especially among Hispanics, and represents a significant economic burden to the U.S. healthcare system. As Hispanic immigration from NCC-endemic continues to increase, we expect to experience a greater number of U.S. cases resulting in increase disease burden.

1200

INSULIN-LIKE GROWTH FACTOR-I EXPRESSION IN THE PATHOGENESIS OF PANCYTOPAENIA IN CANINE AND HAMSTER VISCERAL LEISHMANIASIS

Hiro Goto, Flaviane A. Pinho, Amanda R. Torres, Ive M. Carvalho-Dantas, Samantha I. Miyashiro, Mitika K. Hagiwara

Universidade de São Paulo, São Paulo, Brazil

Pancytopenia is an important alteration in visceral leishmaniasis (VL) which pathogenesis is poorly known. Some cytokines and growth factors are implicated in haematopoiesis in human VL among them the insulin-like growth factor (IGF)-I that likely has stimulatory effect on erythroid precursors. In the present study, we studied haematological parameters, bone marrow morphological alterations and IGF-I expression in *Leishmania (Leishmania) infantum*-naturally infected dogs and experimental VL in hamster. We examined 13 infected and 10 non-infected control dogs, 5 infected dogs presented pancytopaenia and 8 bicytopaenia. All infected dogs had normocytic normochromic anaemia, leukopenia and/or thrombocytopaenia. In myelogram, we observed dysgranulopoiesis (100%), dyserythropoiesis (100%) and dysmegakaryopoiesis (53.8%). VL dogs presented an increase in the myeloid:erythroid ratio compared with non-infected dogs and infected pancytopaenic dogs had a greater erythroid precursor:mature erythroid ratio when compared with infected bicytopaenic dogs. IGF-I expression by qPCR was lower in infected than in non-infected dogs. When we extended our study to *L. infantum* amastigote-infected hamsters, we observed significant haematological alteration such as anemia and leukopenia from 90 days post-infection. In the bone marrow biopsy we observed hipercellularity, increased macrophage proliferation area, reticulin proliferation and increased

myeloid:eritroid ratio. In the mielogram at 90 and 120 days of infection, we observed progressive alterations. IGF-I expression in bone marrow of hamster was higher at 90 days that decreased at 120 days of infection when compared with non-infected controls, coinciding respectively with normal or diminished hemoglobin concentrations. Anaemia was the main haematological alteration in dogs and hamster. Low IGF-I expression in infected dog or hamster with patent anaemia suggests possible involvement of this factor in the pathogenesis of anaemia during VL.

1201

DISCOVERY OF NOVEL SEROLOGICAL BIOMARKERS ASSOCIATED TO CHAGAS DISEASE USING A HIGHLY-MULTIPLEXED DISCOVERY PLATFORM BASED ON HIGH-DENSITY PEPTIDE MICROARRAYS

Santiago J. Carmona[1], Claus Schäfer-Nielsen[2], Carlos A. Buscaglia[1], Juan S. Mucci[1], Jaime Altcheh[3], Oscar Campetella[1], Alberto C. Frasch[1], Morten Nielsen[1], **Fernán Agüero**[1]

[1]Universidad de San Martin, San Martin, Argentina, [2]Schafer-N, Copenhague, Denmark, [3]Hospital de Niños Ricardo Gutierrez, Ciudad Autonoma de Buenos Aires, Argentina

The full set of antibody (B-cell) specificities associated with the response to a natural infection remains largely unexplored. We developed a highly-multiplexed discovery platform based on next-generation high-density peptide microarrays and demonstrate for the first time its potential to simultaneously identify and finely map hundreds of B-cell epitopes from a complex natural human infection. The platform consists of a HD tiling peptide array containing ~200K unique 15mer peptides synthesized *in situ* on a microarray slide, using a maskless photolithographic method. The peptides completely cover the full length of 468 *T. cruzi* proteins, scanning the protein sequence at maximal resolution (1 residue shift). These proteins include 59 previously described antigens, 50 proteins randomly selected from the proteome, 100 proteins selected using a recently published bioinformatics method (Carmona SJ et al 2012); and 232 surface proteins from a number large gene families. The array also contains 24K 15mers corresponding to ~50 neo-proteins of random sequence to estimate the array background baseline (negative control). Using this platform we have analyzed the B-cell immune response in humans with Chagas Disease, assaying 8 independent HD-arrays with 4 pools of purified IgGs from Chagas Disease patients and negative controls. After defining a very conservative cutoff we identified 549 positive 15mers in the set of query proteins (serologically uncharacterized), which define 80 new antigens. We also identified the location of linear epitopes for a number of known antigens, as well as a number of protein regions which are the target of non-specific antibody binding (putative cross-reactive responses). This high-density peptide array platform allows high-throughput identification and mapping of B-cell epitopes, opening the door to large scale studies of immune responses against human infectious diseases.

1202

VECTOR-TRANSMITTED *LEISHMANIA DONOVANI* IN BALB/C MICE DISPLAY DISTINCT FEATURES IN THE HOST IMMUNE RESPONSE AT THE BITE SITE AND SYSTEMICALLY COMPARED TO NEEDLE-INITIATED INFECTIONS

Ranadhir Dey[1], Hamide Aslan[2], Amritanshu Joshi[1], Claudio Meneses[2], Pradeep K. Dagur[3], John. Philip McCoy[3], Robert Duncan[1], Jesus Valenzuela[2], Hira L. Nakhasi[1], Shaden Kamhawi[2]

[1]CBER/Food and Drug Administration, Bethesda, MD, United States, [2]National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States, [3] National Heart, Lung and Blood Institute/National Institutes of Health, Bethesda, MD, United States

Visceral leishmaniasis caused by *Leishmania donovani* is transmitted by sand flies with no available vaccine. The virulence of a sand fly-initiated infection abrogates the protection observed in vaccinated animals following needle challenge. This highlights the need to develop VL models initiated by vector-transmission. We developed such models in BALB/c mice using *L. donovani*-infected *Lutzomyia longipalpis* sand flies and compared to needle-initiated (i.d./i.v.) infections. Sand fly infection in BALB/c mice, developed *Leishmania*-specific antibodies 5-10 weeks post-infection that correlated with early dissemination of parasites into the spleen and liver; by weeks 20-30 post-infection, most mice displayed antibodies to *Leishmania*, a slight increase in spleen parasite burden and loss of liver parasites. Dissemination of parasites following intravenous (i.v.) needle injection was also seen in both spleen and liver similar to sand fly mediated transmission. In contrast, intradermal (i.d.) needle injection of parasites failed to disseminate to visceral organs. Splenic CD4+ and CD8 T+ cells displayed a silent immune response after sand fly-transmitted infections producing less IFNγ and TNFα at 5 and 30 weeks post-infection but similar levels of IL-10 when compared to i.v. infection. Further, vector-initiated infection induced persistent recruitment of leukocytes (neutrophils and monocytes) to the bite site compared to a weaker and transitory recruitment following i.d. inoculation of *L. donovani*. Infected sand fly bites also initiate an acute pro-inflammatory (IFNγ, IL1β, TNFα and IL12) response 3h post infection that subsided at 6h, followed by a sustained induction of IL-10 and MCP1(monocyte chemoattractant protein 1) up to 18h post-infection. Altogether, these results demonstrate that in contrast to needle-initiated infections, vector-transmission induces a distinct acute inflammatory response and a strong cellular infiltration at the bite site that may facilitate dissemination of *L. donovani* in an immunologically silent environment that favors parasite survival.

1203

IMMUNE RESPONSE TO *LUTZOMYIA INTERMEDIA* SALIVA IN INDIVIDUALS FROM A CUTANEOUS LEISHMANIASIS ENDEMIC AREA

Augusto M. Carvalho[1], Juqueline Cristal[1], Regis Gomes[1], Lucas P. Carvalho[2], José C. Miranda[1], Aldina Barral[1], Edgar M. Carvalho[2], Camila I. De Oliveira[1]

[1]Centro de Pesquisas Goncalo Moniz, FIOCRUZ, Salvador, Brazil, [2]Immunology Service, Federal University of Bahia, Salvador, Brazil

Sand fly saliva contains a variety of molecules that modulate the host's hemostatic and immune responses. Immunization with *Lu. intermedia* saliva, one of the main vectors of *Leishmania braziliensis* in Brazil, does not confer protection against *L. braziliensis* infection in mice. In addition, Cutaneous Leishmaniasis (CL) patients display higher levels of anti- *Lu. intermedia* saliva antibodies when compared with sera individuals with sub-clinical *L. braziliensis* infection. In the present work, we conducted a prospective study and characterized the immune response against *Lu. intermedia* saliva in residents of a CL endemic area (Corte de Pedra, BA), where *L. braziliensis* is prevalent. To this end, 264 participants were enrolled and were tested for their humoral immune response against *Lu. intermedia* saliva: antibodies were found in 150 (56.8%) subjects and a positive serology was associated with home arrival after 4:00 pm ($p=0.01$). There was a predominance of IgG1 and IgG4 subclasses and sera from naturally exposed individuals preferentially recognized *Lu. intermedia* proteins of 31, 38, 52, 76 kDa. In a subset of individuals displaying positive serology to salivary proteins, we evaluated cytokine and chemokine production following stimulation of Peripheral Blood Mononuclear Cells (PBMCs) with *Lu. intermedia* saliva. In exposed individuals, we observed higher ($p<0.01$) concentrations of IL-10, IL-13, IFN-γ, CXCL9 and CCL2 compared to non-exposed controls. The main sources of IL-10-secreting cells were $CD4^+CD25^+Foxp3^+$ expressing-cells. The co-culture of *L. braziliensis*-infected macrophages with saliva-stimulated autologous lymphocytes increased the number of intracellular amastigotes. This effect was reversed in the presence of anti-IL-10. Lastly, we observed an association between presence of an immune response to *Lu. intermedia* saliva and CL development, after a 3 year follow-up. We conclude that natural exposure to *Lu. intermedia* saliva polarizes the immune response to a regulatory phenotype, predisposing development of CL caused by *L. braziliensis*.

1204

THE INTERFACE OF THE ANTIVIRAL CELL RESPONSE IN THE INFECTION BY *LEISHMANIA AMAZONENSIS*: ROLE OF THE RNA SENSORS MOLECULES TLR3 AND PKR

Carolina Rath

Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

The parasite *Leishmania amazonensis* infects and replicates inside host macrophages leading to persisting cutaneous infections in humans. Although devoid of viral particles, it has been demonstrated that the dsRNA induced kinase, PKR, is activated and plays an important role in the infection by this Leishmania species. The present study was designed to test the role of TLR3, an endosomal dsRNA receptor, classically engaged in cellular antiviral response, in the infection. We demonstrated that TLR3 and the adaptor molecule TRIF (Toll/IL-1R domain-containing adaptor inducing IFN) are colocalized to the parasitophorous vacuole (PV). We also showed the TLR3 was proteolytic processed, a step required for TLR3 signaling, during the infection. Supporting the notion that TLR3 is engaged in the infection, we also demonstrated that the inhibition of TLR3 cleavage impaired the intracellular parasite growth and reduced the expression of the cytokines Interferon beta (IFN1β) and IL-10, while it induced high levels of IL-12. Moreover, the nuclear translocation of the transcription factor IRF3 was impaired when TLR3 cleavage was inhibited in infected macrophages. Accordingly, TLR3-/- macrophages infected by *L. amazonensis* restricted the intracellular parasite infection and expressed reduced levels of IFN1β, IL-10 and exhibited increased levels of IL-12. The *in vivo* infection of TLR3-/- mice by *L. amazonensis* revealed a significant reduction of lesions in the foot pad. Furthermore, we showed that the RNA sensor PKR (dsRNA activated protein kinase) cooperates with TLR3 signaling to potentiate the expression of IL-10, IFN1β and enhanced the parasite survival. Altogether, our results showed that *L. amazonensis* although devoid of viral particles does engage TLR3 signaling during the infection and utilizes this component of the innate immunity to evade the host cell response in conjunction with the PKR pathway.

1205

ROLE OF PD1/PDL1 IN THE INDUCTION OF REGULATORY T CELLS DURING *LEISHMANIA AMAZONENSIS* INFECTION

Herbert L. de Matos Guedes[1], Eric D. Carlsen[2], Yuejin Liang[2], Christie Hay[2], Calvin Henard[2], Iryna V. Pinchuk IV[2], Lynn Soong[2]

[1]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [2]University of Texas Medical Branch, Galveston, TX, United States

Leishmania amazonensis is the etiological agent of diffuse cutaneous leishmaniasis in South America. In murine models of this infection, dysregulated expansion of effector T cells is related to pathogenesis, while the induction of regulatory T cells (Treg) promotes lesion resolution. The most important co-stimulator/receptor pairs for Treg induction are PD1/PDL1, ICOS/ICOSL, OX40/OX40L and GITR/GITR. In this study, we examined the roles of these molecules in *L. amazonensis*-infected C57BL/6 mice. We found that infected foot tissues had a 10- and 5-fold increase in PD1 and PDL1 expression levels, respectively, with minimal changes for other receptor/ligand pairs. In skin-draining lymph nodes of infected mice, there were an increase in the percentage of CD11c+PDL1+ dendritic cells (DC) and PD1+CD4+ T cells. To evaluate PDL1 expression on DC, we performed *in vitro* infection with promastigotes and amastigotes. *L. amazonensis* infection resulted in an increased PDL1, but decreased PD-L2, expression on DC surface. This induction-triggered PDL1 expression was partially dependent on STAT3, PI3K, mTOR and MYD88 with minor participation of MAPK/ERK, but not on JKI, JKII, JKIII and STAT5. Infected DCs were more competent in inducing CD25+FoxP3+ Treg *in vitro* than the control cells, and this Treg-promoting effect was dependent on PDL1 expression but not on TGF-beta production. Together, these data suggest a role for PD1/PDL1 in the regulation of local immune responses during *L. amazonensis* infection. This study provides new insights on immune regulation of cutaneous leishmaniasis.

1206

IL-33 DECREASES INFLAMMATORY RESPONSE IN CUTANEOUS LEISHMANIASIS PATIENTS

Lucas P. Carvalho, Sara Passos, Daniela Celestino, Paulo Machado, Edgar M. Carvalho

Universidade Federal da Bahia, Salvador, Brazil

Inflammatory response in cutaneous leishmaniasis (CL) patients leads to tissue damage and skin ulcer development. Lesion infiltrate is composed mostly by lymphocytes and monocytes, and few parasites are observed. Monocytes from these patients infiltrate lesion and produce high amounts of TNF, contributing to parasite destruction and tissue damage. Conversely, subjects with L. braziliensis subclinical infection do not develop disease and produce lower levels of TNF. We believe that the less inflammatory environment in these individuals controls parasitemia and prevent tissue damage. In recent studies, it was reported a protective role of IL-33 in atherosclerosis, type 2 diabetes, cardiac remodeling and toxoplasmosis through the Th1/Th2 balance. The aim of this study was to determine the role of IL-33 in immune response from CL patients. IL-33 was not detected in supernatants from biopsy cultures or peripheral blood mononuclear cells (PBMC) stimulated or not with soluble Leishmania antigen (SLA), assessed by ELISA. To evaluate the effect of IL-33 on PBMC and cells from biopsies from CL patients we stimulated these cells with SLA in presence or absence of recombinant IL-33 and after 72 hours the levels of IL-5, IL-13, IFN-γ, TNF, IL-6 and IL-1β were determined by ELISA. As expected, SLA induced high levels of IFN-γ, TNF, IL-6 and IL-1β. The addition of exogenous IL-33 to cultures of PBMC stimulated with SLA and biopsies decreased the levels of IL-1β and IL-6 and increased the production of IL-5 and IL-13. IL-1β is produced upon inflammasome activation. To test the pathway by which L. braziliensis triggers IL-1β production we infected C57BL/6 mouse macrophages lacking NLRP3, AIM2, Caspase1, ASC and IL-1R. We found that L. braziliensis-induced IL-1β production is dependent on NLRP3, Caspase1 and ASC. Altogether our data show that IL-33 decrease inflammatory responses in CL patients and may have a protective role in these individuals.

1207

DOXYCYCLINE TREATMENT SHOWS A STRONG EMBRYOSTATIC EFFECT AND CLEARANCE OF PERSISTENT MICROFILARIAE IN THE SKIN OF ONCHOCERCIASIS PATIENTS IN WHOM REPEATED IVERMECTIN TREATMENT HAD FAILED TO CLEAR MICROFILARIDERMIA

Alexander Y. Debrah[1], Sabine Specht[2], Linda Batsa[3], Ute Klarmann[2], Sabine Mand[2], Alexander Kwarteng[3], Marcelle Buettner[4], Mike Osei-Atweneboana[5], Daniel Boakye[6], Charles D. Mackenzie[7], Ohene Adjei[8], Achim Hoerauf[2]

[1]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]Institute for Medical Microbiology, Immunology and Parasitology, University of Bonn, Bonn, Germany, [3]Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana, [4]Ahrensburger Stieg 17, Hamburg, Germany, [5]Centre for Scientific and Industrial Research, Accra, Ghana, [6]Noguchi Memorial Institute, Accra, Ghana, [7]Department of Pathobiology and Diagnostic Investigation, Michigan State University, Lansing, MI, United States, [8]Komfo Anokye Teaching Hospital, Kumasi, Ghana

Ivermectin (IVM) has been the drug of choice for the treatment of onchocerciasis. However, there have been reports of persistent microfilariae (Mf) in the skin of some people after many rounds of IVM treatment in Ghana, consistent with the emergence of drug resistance or sub-optimal response (SOR) to IVM. To assess the effect of targeting Wolbachia endosymbionts in *Onchocerca volvulus* on onchocerciasis patients in whom repeated IVM treatment had failed to clear Mf, 167 patients were recruited in 2 districts in Ghana where IVM SOR has been reported. They were treated with either 100mg/d doxycycline or matching placebo for 6 weeks. Three and 12 months after doxycycline treatment,

the patients took part in the IVM mass treatment. Patients were snipped before, 12 and 20 months after doxycycline treatment to assess the levels of Mf. Onchocercomata were extirpated from the patients after 20 months for assessment of embryostatic as well as macrofilaricidal effects. 20 months after treatment, 76% of living female worms from the placebo group were Wolbachia-positive, whereas only 4% in the doxycycline-treated group had a few remaining bacteria. At the same time point, 49% of living females in the placebo group showed normal embryogenesis compared to only 4% in the doxycycline group. More importantly, at 20 months post therapy, none of the nodules removed from doxycycline treated patients contained Mf. This is reflected by the absence of microfilaridermia in the same patients (97% compared to 21% of the placebo patients (P<0.001)). Due to the low dose doxycycline treatment, 52% (consistent with earlier reports) of 136 worms had died compared to 38% in the placebo group. In conclusion, targeting Wolbachia in *O. volvulus* is effective in clearing Mf in the skin of onchocerciasis patients in whom repeated standard treatment has failed to clear. Thus strategies may be developed including anti-wolbachial treatment to control the re-emergence of onchocerciasis in areas where infections persist despite the frequent use of IVM.

1208

ESTABLISHING AND TESTING THE TOOLS TO SUPPORT THE TEST AND (NOT) TREAT (TNT) STRATEGY: THE KEY TO IMPLEMENT ONCHOCERCIASIS AND LYMPHATIC FILARIASIS ELIMINATION PROGRAMS IN LOIASIS ENDEMIC AREA

Sébastien D. Pion[1], Michael D'Ambrosio[2], Matthew Bakalar[2], Céline Montavon[1], Jean Bopda[3], Steve Mbickmen[3], Cédric J. Chesnais[1], Amy D. Klion[4], Joseph Kamgno[3], Jules B. Tchatchueng[3], Charles D. Mackenzie[5], Wilma Stolk[6], Samuel Wanji[7], Michel Boussinesq[1], Daniel A. Fletcher[2], Thomas B. Nutman[8]

[1]Institut de recherche pour le Développement, Montpellier, France, [2]Department of Bioengineering, Berkeley, CA, United States, [3]Center for Research on Filariasis and Other Tropical Diseases, Yaoundé, Cameroon, [4]Eosinophil Pathology Unit, Laboratory of Parasitic Diseases, Bethesda, MD, United States, [5]Department of Pathobiology and Diagnostic Investigation, East Lansing, MI, United States, [6]Erasmus Medical Center, Rotterdam, Netherlands, [7]Research Foundation in Tropical Diseases and the Environment, Buea, Cameroon, [8]Helminth Immunology Section and Clinical Parasitology Section, Laboratory of Parasitic Diseases, Bethesda, MD, United States

Implementation of elimination programs for lymphatic filariasis and onchocerciasis in areas where loiasis is co-endemic is problematic because individuals heavily infected with *Loa loa* may develop severe adverse events (SAEs) when treated with ivermectin. A test and treat approach aims to identify individuals with high microfilarial densities at the point-of-care (POC) prior to treatment. Individuals with microfilarial densities >30,000 microfilariae (mf)/mL are at risk for potentially fatal neurologic SAEs and should be excluded from treatment; those with >8,000 mf/mL present a risk for non-neurological side effects. We tested a POC smartphone-enabled portable video microscope, CellScope, that provides automated counting of *Loa* mf in peripheral blood. During a pilot community study conducted in two villages of Cameroon, CellScope results were compared to a calibrated blood smear (CBS) method and quantitative PCR (qPCR). Out of 205 participants, using either qPCR (from DNA extracted from dried blood spots) or CBS, 66 (32.2%), 16 (7.8%) and 1 (0.5%) individuals were found with mf density >0, >8,000 and >30,000 mf/mL, respectively. CellScope outcomes were missing from 37 participants because of initial technical problems related to movement artifact or failure to manual focus. Among the remaining 168, there were strong correlations between automated counts and counts from CBS (r= 0.96, p<0.0001) and qPCR results (r=0.88, p<0.0001). The results from the CellScope were available within 3 minutes of blood draw, while the results of qPCR and CBS required transport to a centralized laboratory and were not available for at least a week. Although the proportion of individuals with >30,000 mf/ml precludes a formal evaluation of the CellScope, the sensitivity and specificity for levels between 0, 1-8,000 mf/mL and >8,000 mf/mL were excellent. Thus, CellScope screening appears to provide a POC tool to identify individuals at risk of SAEs in community-based MDA programs where *L. loa* is endemic. Validation of the CellScope V2.0 in ~30,000 individuals is planned for late 2014.

1209

DRUG DISCOVERY AND DEVELOPMENT FOR THE TREATMENT AND CONTROL OF FILARIASIS: REPURPOSING EMODEPSIDE

Sabine Specht[1], Charles D. MacKenzie[2], Simon Townson[3], Robert Don[4], Achim Hoerauf[1], Ivan Scandale[4]

[1]University Hospital, Bonn, Germany, [2]Michigan State University, East Lansing, MI, United States, [3]Tropical Parasitic Diseases Unit, London, United Kingdom, [4]Drugs for Neglected Diseases Initiative, Geneva, Switzerland

Lymphatic filariasis (lymphoedema and hydrocoele) and onchocerciasis (dermatitis and ocular inflammation) caused by the parasitic filarial nematodes *Wuchereria bancrofti*, *Brugia malayi* and *Onchocerca volvulus* lead to severe morbidity in developing tropical countries. Mass drug administration (MDA) programmes use ivermectin or diethylcarbamazine, combined with albendazole, with the aim to eliminate filarial diseases. However, these drugs primarily only kill the first stage larvae. Co-endemicity of these diseases with loiasis impedes MDA due to the high risk of encephalopathy. Additionally, resistance to the standard MDA drugs is also a concern. Therefore, new drugs and regimes need to be in the pipeline. Here we describe research on old drug candidates such as emodepside into new treatment options. Emodepside is a registered drug for animal health, commercialized by Bayer under the name of Profender® (in combination with praziquantel) or Procox® (in combination with toltrazuril). Emodepside is extremely potent *in vitro* against various filarial nematodes (*Achatocheilonema vitae*, *Litomosoides sigmodontis*, *Brugia malayi*, *Onchocerca gutturosa, Onchocerca lienalis*). Jirds infected with *L. sigmodontis* were administered 0, 12.5, 25, 50 and 100 mg/kg orally once a day for 5 consecutive days. Tissue pathology was scored, identifiable worms from the peritoneal and pleural cavities isolated and counted. A dose-response for pathology adult worm burden was observed. Furthermore a single dose of 100 mg/kg emodepside was sufficient to clear microfilaremia. This study suggests that emodepside with its long half life warrants attention as an additional tool for drug administration strategies in filariasis.

1210

LONGTERM IMPROVEMENT OF HYDROCELE SIZE AFTER ULTRASOUND-GUIDED ASPIRATION

Alexander Y. Debrah[1], Sabine Mand[2], Henry Atawurah[3], Nana K. Ayisi-Boateng[4], Linda Batsa[5], Alexander Kwarteng[5], Jubin Osei-Mensah[5], Yusif Mubarik[5], Sabine Specht[2], Arcangelo Ricchiuto[2], Rolf Fimmers[6], Achim Hoerauf[2], **Ute Klarmann-Schulz**[2]

[1]Faculty of Allied Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany, [3]Effia Nkwanta Regional Hospital, Sekondi, Ghana, [4]University Hospital of Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [5]Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana, [6]Institute for Medical Biometry, Informatics and Epidemiology, University Hospital Bonn, Bonn, Germany

An estimated 25 million men suffer from hydrocele due to lymphatic filariasis (LF). Current treatment strategies rely on mass drug administration and, in case of hydrocele, hydrocelectomy. In previous studies it could be shown that treatment with doxycycline led to improvement or halt of progression in patients suffering from lymphedema or hydrocele. The aim of this randomized placebo-controlled study was to assess the possibility of using the combination of doxycycline treatment and ultrasound-guided

aspiration of hydrocele fluid as an alternative to hydrocelectomy. After ultrasound examination (USG), 73 men with stage 2 or 3 hydrocele (stage 1 – subclinical hydrocele, stage 2 – longitudinal and transverse diameters <1.9 and <1.6 cm, stage 3 – diameters <3.8 and <3.2 cm, stage 4 – diameters >3.8 and >3.2cm (as reported previously)) were included in the study and treated with either doxycycline 200mg or matching placebo for 6 weeks. Four months after treatment onset participants were again screened by USG and underwent an ultrasound-guided aspiration in case of hydrocele stage > 1, which was successful in 47/51 men. Follow-up examinations were carried out 4 days after aspiration, and 7, 12, 24 and 44 months after treatment onset. The primary outcome analysis (Intention-to-treat) showed an improvement (reduction of hydrocele size of at least one stage at 12 months compared to pre-treatment) in 53/73 (72.2%) participants. There was no difference between doxycycline (25/37, 67.6%) or placebo treated men (28/36, 77.8%) (p = 0.433). The per protocol analysis confirmed these results. After 24 and 44 months improvement was persistent in 67.1% (doxy 59.5%, placebo 75%; p = 0.214) and 71.2% (doxy 64.9%, placebo 77.8%; p = 0.302), respectively. Thus, following aspiration a long-term improvement of hydrocele size was observed. While spontaneous improvement cannot be formally excluded given the lack of a control group in this study, this is not likely in the light of the literature. Aspiration may therefore be considered as an alternative to circumwent the throughput limits of hydrocelectomy. Treatment with doxycycline before aspiration does not lead to an additional benefit.

1211

EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF *LOA LOA* INFECTION IN PREGNANT WOMEN IN GABON: A PROSPECTIVE COHORT STUDY

Ghyslain Mombo-Ngoma

Cermel, Lambarene, Gabon

Loa loa - the African eye worm - is a filarial pathogen occurring in Central Africa. Despite its high prevalence in affected rural communities, to date there are no data on the impact of infection during pregnancy. This study aimed to describe the characteristics of pregnant women infected with L.loa in Gabon, and the impact of the infection on pregnancy outcomes. HIV-negative pregnant women participating in a randomised controlled clinical trial assessing mefloquine as an alternative drug for intermittent preventive treatment of malaria in pregnancy (MiPPAD, NCT 00811421) were invited to participate in this cohort study. *L. loa* infections were diagnosed during the study by microscopy of peripheral and cord blood and in placental biopsies. Demographic and anthropometric characteristics, clinical and laboratory measures at delivery, placental histopathological specimens were compared between women with *L. loa* infection and those without infection. 1184 pregnant women were recruited, 194 (16.4%) presented with microfilariae of *L. loa* in peripheral blood. The relative risk (RR) for L.loa infection increased with maternal age. Women older than 30 years were more frequently infected (RR: 2.31; 95% CI: 1.28-4.17; p=0.005) and maternal age and number of previous pregnancies showed some association with filarial infection as assessed by the likelihood ratio test (p=0.056 and P=0.008, respectively). Univariate analysis demonstrated that preterm birth was more prevalent in infected women (RR: 1.76; 95%CI: 0.95-3.28) and a higher proportion of low birth weight infants (RR: 1.16; 0.75-1.8) was observed - however not reaching statistical significance. Out of 186 women with microfilariae in peripheral blood, 27 (14.5%) were observed with evidence of microfilarial invasion into the intervillous space of the placenta. No signs of further pathological alterations of the placenta were observed in histological examination. These findings suggest that loiasis occurs at high prevalence in pregnant women in Gabon and that microfilariae commonly invade the placenta. Unadjusted analysis suggests an association of loiasis with adverse birth outcome. However, at this stage it remains unclear whether this association is based on a causal link or is confounded by nutritional, socio-economic or other yet unidentified factors.

1212

EFFICACY, SAFETY AND PHARMACOKINETICS OF CO-ADMINISTERED DIETHYLCARBAMAZINE, ALBENDAZOLE AND IVERMECTIN FOR THE TREATMENT OF *WUCHERERIA BANCROFTI*

Edward K. Thomsen[1], Jessica A. Kumar[2], Nelly Sanuku[1], Manasseh Baea[1], Samson Satofan[1], Elit Maki[1], Bart Lombore[1], Delma Beaso[1], Mark Schmidt[3], Peter M. Siba[4], Gary Weil[5], James W. Kazura[2], Lawrence Fleckenstein[3], **Christopher L. King**[2]

[1]Papua New Guinea Institute of Medical Research, Maprik, Papua New Guinea, [2]Case Western Reserve University, Cleveland, OH, United States, [3]University of Iowa, Iowa City, IA, United States, [4]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea, [5]Washington University, St. Louis, MO, United States

Existing drugs against lymphatic filariasis (LF) have limited ability to kill/sterilize adult worms. This study determined whether a combination of three widely used antifilarial drugs would improve activity against killing/sterilizing adult worms compared with two drug therapy. The goal was to reduce the frequency and improve compliance of mass drug administration (MDA) compared to standard therapy. We examined the efficacy, safety, and pharmacokinetics of single-dose compared to triple-drug therapy with diethylcarbamazine (DEC), ivermectin (IVM), and albendazole (ALB) in heavily infected individuals with *Wuchereria bancrofti* from Papua New Guinea. In this single-blinded trial, twenty four adults were randomized into one of two treatment arms: DEC 6mg/kg + ALB 400mg (N=12) or DEC 6mg/kg + ALB 400mg + IVM 200ug/kg (N=12) and monitored for microfilaremia, side effects and drug levels. Individuals receiving 3 drugs had >2-log reduction in mf levels at 36 and 168 hours after treatment that was associated with one or more side effects in 11 of 12 subjects. By contrast those receiving 2-drugs had ~1-log reduction in mf levels, and 5 of 12 subjects experienced side effects. These effects included fever, lymphadenitis, elevated liver enzymes, hematuria and proteinuria. Subjective symptoms included headache, nausea, pruritis, and arthralgia. Eleven of twelve individuals receiving DEC/ALB/IVM experienced one or more side effects whereas 8 of 12 receiving DEC/ALB experienced one or more side effects. All side effects were self-limiting and resolved within 48-72h after treatment. There were no significant effects of IVM on DEC or ALB drug levels. All 12 individuals receiving 3 drugs remained amicrofilaremic one year after treatment and had 49% reduction in antigen levels, whereas all but one individual in the 2 drug regimen remained microfilaremic with ~31% reduction in antigen levels (P = 0.04). Combined treatment with DEC+ALB+IVM is safe, highly efficacious and may lead to sustained reduction in microfilaria thereby requiring fewer annual treatments.

1213

TARGETING FILARIAL ABL-LIKE KINASES: REPURPOSED, ORALLY AVAILABLE, APPROVED TYROSINE KINASE INHIBITORS (TKI) ACT AS MICRO- AND MACROFILARICIDAL AGENTS AT CONCENTRATIONS EASILY ACHIEVABLE *IN VIVO*

Elise M. O'Connell, Sasisekhar Bennuru, Cathy Steel, Thomas B. Nutman

National Institutes of Health, Bethesda, MD, United States

Global elimination of onchocerciasis and lymphatic filariasis is targeted for 2020. Given the potential for the development of drug resistance and the ongoing problem of co-incident *Loa loa* infection in Central Africa, the need for new macrofilaricides has never been greater. Thus, new orally administered drugs that are highly efficacious against the adult and microfilarial stages of the parasites are desired. Based on the genomes and transcriptomes of *L. loa*, *Wucheria bancrofti, Brugia malayi and Onchocerca volvulus*, we found that each of these filarial parasites express tyrosine kinase (TK) proteins with significant homology to the human oncogene protein product Bcr-Abl. Not only is the catalytic binding site of FDA-approved imatinib well conserved in these pathogenic filariae, but

also phylogenetically the filarial TK proteins are more closely related to human Bcr-Abl than are other parasitic helminths. To assess the antifilarial effects of imatinib and its next generation sister drugs nilotinib and dasatinib, *in vitro* killing of *B. malayi* (Bm) adult males, adult females, L3 and microfilariae (MF) were tested over a 6-day period using a wide dose range (100nM-100uM). Day 5 IC50s for Bm adult males were 58.3uM (imatinib), 10uM (dasatinib), and 49.3uM (nilotinib). IC50s for the Bm L3s were determined to be 20uM (imatinib), 17.4uM (dasatinib), and 76.4uM (nilotinib). MF IC50s were 12.1uM (imatinib), 6uM (dasatinib), and 33.5uM (nilotinib). In limited data, killing of adult females occurred within 24 hours at 75uM with complete killing by 48 hours at 10uM. Additionally, embryogenesis was markedly affected with early embryonic stages being expelled within the first 24 hours. Moreover, 3 dimensional protein modeling demonstrated how these three tyrosine kinase inhibitors (imatinib, dasatinib, nilotinib) dock at the filarial catalytic domain, thereby inhibiting its activity. Given the known safety of imatinib in humans, plans are underway to assess its efficacy in pilot clinical trials in patients infected with these pathogenic filarial parasites.

1214

THE CURIOUS CASE OF *RICKETTSIA FELIS* IN LAOS

Sabine Dittrich[1], Phonepasith Panyanivong[2], Weerawat Phuklia[2], Tippawan Anantatat[3], Paul N. Newton[1], Daniel H. Paris[1]

[1]University of Oxford, Vientiane, Lao People's Democratic Republic, [2]Lao-Oxford-Mahosot Hospital Wellcome Trust Research Unit, Vientiane, Lao People's Democratic Republic, [3]Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand

Rickettsia felis is a flea transmitted rickettsial pathogen, which has recently been identified in numerous studies all over the world, most notably in Africa where rickettsia pathogens have been regarded as rare. Case reports suggest that *R. felis* infections can have very severe manifestations including central nervous system involvement. In Laos, murine and scrub typhus diseases caused by related organisms *Rickettsia typhi* and *O. tsutsugamushi* have been identified as major bacterial causes of non-malarial fevers, including central nervous system infections. Over 9 years, ~3,500 blood and cerebral spinal fluid (CSF) samples (~2500 hospital patients) have been investigated for rickettsial pathogens by quantitative real-time PCR (qPCR). All positive samples were further typed by species-specific qPCR or DNA sequencing. *R. felis* was only identified in three patients, once in CSF and twice in blood. The rarity of *R. felis* infections triggered a closer investigation of the three patients and it became apparent that all had potentially impaired immune systems as well as infections with multiple pathogens, some of them vector-borne. Recently, *R. felis* was demonstrated in an afebrile patient in Kenya and other investigations identified the organism in a diversity of different vectors (chiggers, mosquitos, fleas). The low incidence and curious presentation in Lao, a country otherwise endemic for flea-borne rickettsiosis, combined with the findings from Africa, raise questions regarding alternative vectors of *R. felis* as well as its role as a real causative agent of disease.

1215

SCABIES AND BACTERIAL SUPERINFECTION AMONG CHILDREN --- AMERICAN SAMOA, 2011

Amanda Beaudoin[1], Laura Edison[1], Camille E. Introcaso[2], Lucy M. Goh[3], Chris Van Beneden[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Pennsylvania Hospital, Pennsylvania Center for Dermatology, Philadelphia, PA, United States, [3]Lyndon Baines Johnson Tropical Medical Center, Pago Pago, American Samoa

Scabies, a highly pruritic and contagious mite infestation of the skin, is endemic in tropical regions. Delayed treatment can lead to bacterial superinfection, and treatment of close contacts is necessary to prevent reinfestation. We described scabies incidence and superinfection among children in American Samoa (AS) to support scabies control recommendations. We reviewed 2011 pharmacy records from the only AS pharmacy to identify children aged ≤14 years with filled prescriptions for permethrin, the only scabicide available. Children's medical records were reviewed for physician-diagnosed scabies during January 1-December 31, 2011. We calculated scabies incidence, bacterial superinfection prevalence, and reinfestation prevalence during 14 days-12 months after first diagnosis. We used log binomial regression to calculate incidence ratios (IRs) for scabies by age. Medical record review identified 613 children with scabies (incidence: 31.6/1,000 children ≤14 years); 358 (58.4%) were male; 353 (57.6%) had a bacterial superinfection, and 94 (15.3%) had ≥1 reinfestation. Scabies incidence varied significantly among the 9 main island counties (range: 14.8-48.9/1,000). Children aged <1 year had the highest incidence (100.2/1,000). Children aged 0-4 years (incidence: 54.5/1,000; IR: 5.1; CI: 4.0-6.5) and 5-9 years (incidence: 27.7/1,000; IR: 2.4; CI: 1.9-3.2) had a significantly higher scabies incidence than children aged 10-14 years (incidence: 11.5/1,000). Investigating why certain AS counties have a lower scabies incidence can help support recommendations for improving scabies control in counties with a higher incidence. The high prevalence of bacterial superinfection and frequent reinfestations highlight the importance of diagnosing and treating patients and their close contacts at the first signs of infection. Interventions targeting infants and young children who have frequent close family contact should be considered.

1216

ZOONOTIC *BABESIA MICROTI* LINEAGES DO NOT DIFFER FROM THOSE THAT ARE LOCALLY ENZOOTIC

Heidi Goethert[1], Timothy Lepore[2], Victor Berrardi[3], Sam Telford[1]

[1]Tufts University School of Veterinary Medicine, N. Grafton, MA, United States, [2]Nantucket Cottage Hospital, Nantucket, MA, United States, [3]Imugen Inc., Norwood, MA, United States

Deer tick-transmitted human babesiosis due to *Babesia microti* appears to be expanding its distribution and prevalence in the northeastern United States. One hypothesis for this emergence is that "virulent" zoonotic strains have been selected in sites of longstanding transmission. We determined whether parasites infecting humans comprised a less diverse set of genetic lineages, with the alternative hypothesis being that the diversity of strains from human cases does not differ from those that are locally enzootic. We identified 9 genetic loci with tandem repeat regions that are highly variable and used these variable number tandem repeat (VNTR) markers to type parasite DNA from enzootic samples (mice and ticks) and zoonotic samples (human cases) from Martha's Vineyard and Nantucket, two sites with longstanding *B. microti* transmission. We identified 90 different VNTR genotypes from 201 samples (168 field samples and 33 human babesiosis samples); these markers appear to be variable enough to identify individual lineages. No genotypes were identified from human parasite samples more frequently than expected from the general diversity and distribution of enzootic genotypes. We conclude that humans are exposed to and become infected by any of the *B. microti* lineages that are locally enzootic and that there is no support for the hypothesis that there are specific "virulent" zoonotic lineages.

1217

HORIZONTAL TRANSMISSION OF *RICKETTSIA FELIS* BETWEEN CO-FEEDING ARTHROPODS ON VERTEBRATE HOSTS

Lisa D. Brown[1], Kaikhushroo H. Banajee[1], Fabio Del Piero[2], Kevin R. Macaluso[1]

[1]Vector-Borne Disease Laboratories, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA, United States, [2]Louisiana State University School of Veterinary Medicine, Baton Rouge, LA, United States

Rickettsia felis is the causative agent of an emerging vector-borne rickettsiosis transmitted by cat fleas, Ctenocephalides felis. Recent studies suggest that *R. felis* infections are responsible for approximately 6% of

non-malarial based fevers in hospitalized patients in Senegal and Kenya; however, transmission mechanisms required for pathogen persistence within flea populations have proved difficult to verify. Based on the variable vertical transmission efficiency associated with flea hosts, we hypothesized that maintenance of *R. felis* within the vector population is facilitated by horizontal transmission between co-feeding arthropods on a vertebrate host. In order to test this hypothesis, we developed rickettsial horizontal transmission bioassays with C3H/HeJ mice. Bioassays were conducted in three separate trials and divided into four experimental groups: bleb (intradermal inoculation of *R. felis* and uninfected fleas in feeding capsule), co-fed (*R. felis* -infected and uninfected fleas in same feeding capsule), and cross-fed (*R. felis* -infected and uninfected fleas in separate feeding capsules). Bleb and co-fed bioassays were also performed with uninfected rat fleas (Xenopsylla cheopis) and *R. felis* -infected cat fleas and uninfected rat fleas, respectively. Quantitative real-time PCR analyses (based on the rickettsial 17-kDa antigen gene) revealed that Rickettsia-uninfected cat fleas acquired the pathogen through the vertebrate host in bleb (10.0 - 20.0%), co-fed (0.0 - 40.0%), and cross-fed (0.0 - 10.0%) bioassays; uninfected rat fleas also acquired *R. felis* in bleb (0.0 - 40.0%) and co-fed (0.0 - 30.0%) bioassays. Additionally, we aimed to delineate early-phase transmission events (1, 3, 6 and 12 hrs. post infection) involved in the extrinsic incubation period by examining dissemination of *R. felis* to flea salivary glands by microscopy. Delineation of transmission mechanisms of *R. felis* is essential to fully understand the epidemiology and ecology of this emerging rickettsiosis.

1218

SCABIES AND ASSOCIATED BACTERIAL INFECTIONS

Katja Fischer, Pearl M. Swe, Martha Zakrzewski, Lutz Krause

The Queensland Institute of Medical Research, Brisbane, Australia

Scabies is a contagious superficial skin infection caused by the parasitic mite *Sarcoptes scabiei*. Although scabies infection alone is not life-threatening, it is complicated by secondary bacterial infections. Epidemiological studies indicate co-infection by *Staphylococcus aureus* and *Streptococcus pyogenes* in mite infested skin. We hypothesised that the mites alter the healthy skin microbiota and promote the growth of opportunistic pathogens. Scabies mites produce several families of different protein classes that interfere with various host complement molecules. They are secreted into the mite gut and excreted into the epidermis with the feces. We hypothesise that scabies mite complement inhibitors create a microenvironment that promotes bacterial survival. We will present whole blood bactericidal *in vitro* assay data demonstrating that scabies mite complement inhibitors increase the growth of *S. aureus* and *S. pyogenes* in a concentration dependent manner. Deposition assays show that these proteins reduce the opsonisation of bacteria resulting in reduced phagocytosis by neutrophils. Using a porcine skin *in vivo* model we tested whether mite infestation alters the healthy skin microbiota, making way for the opportunistic pathogens. We found significant changes in the epidermal microbiome, in particular a dramatic increase in pathogenic *Staphylococcus* species correlating with the onset of mite infestation persisting beyond treatment with acaricide and healing of the skin. This is a first *in vivo* study offering experimental evidence and supporting previous assumptions that scabies infection causes establishment of pathogens. Comprehending the tripartite interactions between mites, bacteria and host immune system will result in biologically important aspects of disease pathogenesis, offering avenues for alternative therapies and novel intervention strategies. This data will correct the common perception of scabies being a simple 'itch', but often the origin and driver of a complex disease involving multiple pathogens and giving rise to serious sequealae.

1219

EVIDENCE OF NON-SYSTEMIC *BORRELIA BURGDORFERI* TRANSMISSION IN THE NORTH AMERICAN VECTOR-HOST SYSTEM

Sarah L. States[1], Tanner K. Steeves[1], Denise St. Jean[1], Peter J. Krause[1], Edouard G. Vannier[2], Maria A. Diuk-Wasser[1]

[1]Yale University, New Haven, CT, United States, [2]Tufts University, Boston, MA, United States

Identifying key modes of pathogen transmission is crucial to understanding the spread of disease, and can help us better understand how pathogen variation and persistence within hosts are maintained. Lyme disease, caused by the bacterium *Borrelia burgdorferi* and transmitted by the *Ixodes scapularis* tick, is the most prevalent vector-borne disease of humans in the United States and Europe. *B. burgdorferi* exhibits broad genetic diversity at several loci, and these strains vary in their levels of invasiveness and persistence in both *Peromyscus leucopus*, the main reservoir host, and humans. A critical unresolved question is how strains that only persist for a short period in *P. leucopus* can be maintained in nature, since *B. burgdorferi* must survive in the host from the period of infection by *I. scapularis* nymphs to when most susceptible larvae of the next cohort feed, approximately two months later. Some larvae, however, feed simultaneously with nymphs, so non-systemic transmission would be an alternative transmission mode between these synchronously feeding nymphs and larvae. This mechanism has been shown to be key for the maintenance of tick-borne encephalitis virus in Europe, which only persists in the host for a few days. We determined whether this is a viable transmission mechanism in our system by infecting groups of *Peromyscus leucopus* mice with a non-invasive *B. burgdorferi* strain and another strain of unknown invasiveness that is common in our study area and has been increasing in frequency in the region. We found that non-systemic transmission is a viable mode of transmission in the North American Lyme disease system for both strains, which has not been previously confirmed. This key finding will be incorporated into mathematical transmission models to evaluate the extent to which it can explain the persistence of non-invasive *B. burgdorferi* strains in the northeastern United States.

1220

ISOLATION, CULTIVATION, AND CHARACTERIZATION OF *CANDIDATUS RICKETTSIA ASEMBOENSIS* FROM KENYAN FLEAS

Alison Luce-Fedrow[1], Alice N. Maina[1], Elkanah Otiang[2], Fredrick Ade[2], Sylvia Omulo[2], Eric Ogola[2], Linus Ochieng[2], Allen L. Richards[1]

[1]Naval Medical Research Center, Silver Spring, MD, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya

Candidatus Rickettsia asemboensis was detected molecularly in fleas collected during 2009 from Asembo, Kenya. MLST utilizing the *16S rRNA (rrs)*, 17-kD antigen gene, *gltA*, *ompA*, *ompB*, and *sca4* demonstrated that *Candidatus* R. asemboensis is closely related to *R. felis* but distinct enough to be considered for separate species classification. Despite molecular characterization of *Candidatus* R. asemboensis, the successful *in vitro* cultivation of this bacterium remained to be performed. We used *Ctenocephalides canis* and *C. felis* (dog and cat fleas, respectively) removed from dogs in Kenya to initiate the *in vitro* isolation of *Candidatus* R. asemboensis. Successful cultures were obtained from pools of dog/cat fleas using the *Drosophila melanogaster* S2 and the *Aedes albopictus* C6/36 cell lines. Cytological staining and qPCR (species-specific assay for *Candidatus* R. asemboensis) were utilized to visualize/confirm the isolation of the bacteria into both cell lines. Sequencing of fragments of the 17-kD antigen gene, *gltA*, and *ompA* has been performed and confirmed the identity of our culture isolates. Independent time course infection experiments to help define the growth kinetics of *Candidatus* R. asemboensis in the two cell lines using fresh/frozen seed material have preliminarily revealed that increases in the molecular copy numbers can

be detected during 2 weeks of culture. Infected C6/36 cells prepared for TEM were found to be heavily parasitized, and the rickettsiae appeared in round/elongated forms with the presence of double membranes and electron lucent "halos". Genome sequencing of DNA prepared from *Candidatus* R. asemboensis-infected cells has been completed and assembly of the genome is in progress. To date, we have passaged *Candidatus* R. asemboensis 7 times through S2 and C6/36 cells; and active and frozen cultures are currently being maintained. This is the first time that a *R. felis*-like organism has been maintained in culture and therefore the first time that one of them, *Candidatus* R. asemboensis, has been characterized more than just by molecular typing.

1221

THE DYNAMICS OF *PLASMODIUM VIVAX* PRIMARY INFECTIONS AND RELAPSES IN A COHORT OF CHILDREN IN PAPUA NEW GUINEA

Amanda Ross[1], Cristian Koepfli[2], Sonja Schoepflin[1], Lincoln Timinao[3], Peter Siba[3], Marcel Tanner[1], Ingrid Felger[1], Ivo Mueller[2], Thomas Smith[1]

[1]Swiss Tropical and Public Health Institute and University of Basel, Basel, Switzerland, [2]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [3]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea

The contributions of *Plasmodium vivax* infections and relapses to blood-stage infections are generally unclear in endemic areas. The dynamics are difficult to study because infections are frequently asymptomatic due to acquired immunity, individuals may harbor several different *P. vivax* infections at the same time and the density of parasites in the blood may transiently lie below the limit of detection. High-resolution genotyping allows individual infections to be distinguished. We estimate the seasonality and incidence of primary infections and relapses using data from a cohort of children in Maprik district followed up for 16 months. The children, aged one to three years at enrolment, were followed up at 2 monthly intervals. Blood samples were taken at each routine timepoint and additionally if the child was ill. Samples positive by microscopy or LDR, a molecular method for species detection, were genotyped using high-resolution capillary electrophoresis for genetic markers, *P vivax* MS16 and *P. falciparum msp2*. The number of blood-stage infections per year at risk has previously been estimated to be 15.1 (14.1,16.2) in this cohort. The data were summarized as longitudinal patterns of success or failure to detect a genotype at each routine timepoint (eg 001000001). To the frequencies of these patterns, we fit a model which included primary infection, relapse, clearance and detectibility. We assume that the seasonality of *P vivax* primary infections follows that of *P. falciparum* since they are transmitted by the same vectors. Relapses occurring during the study period can be a consequence of infections ocurring prior to the study: we assume that the seasonal pattern of *P. vivax* primary infections repeats over time. The estimated incidence of relapse decreased with time from the primary infection: 55% of relapses occurred within 4 months and 3% occurred after 12 months. Between 45% and 80% of blood stage infections arose through relapses depending on the season. The peak incidence of relapses occurred in the two month interval following the peak for primary infections. This has implications for the timing of interventions targeting different stages of the life cycle.

1222

PLASMODIUM VIVAX MORBIDITY AFTER RADICAL CURE: A TWO-YEAR COHORT STUDY IN CENTRAL VIETNAM

Thanh Pham Vinh[1], Hong Nguyen Van[1], Anna Rosanas-Urgell[2], Van Nguyen Van[3], Umberto D'Alessandro[4], Duong Tran Thanh[1], Niko Speybroeck[5], Annette Erhart[2]

[1]Vietnam National Institute of Malariology Parasitology and Emtomology, Hanoi, Vietnam, [2]Institute of Tropical Medicine Prince Leopold, Antwerp, Belgium, [3]Provincial Malaria Station (Tam Ky City), Quang Nam Province, Vietnam, [4]Medical Research Council Unit, Fajara, Gambia, [5]Université Catholique de Louvain, Bruxelles, Belgium

Plasmodium vivax malaria represents a challenge for the Vietnamese Malaria Control and Elimination Program as there is little information available on the efficacy of treatment for clinical cases and for the prevention of relapses. In order to address this question, a cohort of *P. vivax* infected patients in Central Vietnam was treated with Chloroquine (25mg/kg in 3 days) and Primaquine (0.5mg/kg/day for 10 days) and followed up for 2 years. The study was conducted between 2009 and 2011 in four rural communities of Quang Nam province. *P. vivax* infected individuals were enrolled in the cohort after individual informed consent. Treatment was directly observed and participants were monitored actively to assess treatment efficacy at Day28, then visited monthly for clinical examination and blood sampling (microscopy and PCR). A total of 260 *P. vivax* patients were enrolled in the cohort and the 240 of them who completed the 10-day treatment where include in the analysis. Most of the patients (78.7%) belonged to the M'nong ethnic group, and half of them were children<10 years. One late clinical failure and seven (2.9%) late parasitological failures were observed during the first 28 days, while 10 patients (4.2%) were positive by PCR for *P. vivax* at Day28. . About half of the participants (53.3%) had *P. vivax* recurrences detected by microscopy during the whole follow-up period, while by PCR this represented more 70%. Recurrences were mostly repeated and the number per patients ranged from 2 to 13. The incidence of *P. vivax* recurrent infections by microscopy and by PCR will be analyzed using negative binomial regression; and time to event by survival analysis and cox regression. In conclusion, after a 10-day supervised treatment with high dose primaquine, study subjects experienced a substantial number of recurrent *P. vivax* infections mainly at sub-clinical and sub-microscopic level. There is a need to develop appropriate strategies to deal with such high rate of recurrences.

1223

PLASMODIUM SP. INFECTION PREVALENCE IN TAK PROVINCE, THAILAND IS HIGHER THAN CURRENTLY ESTIMATED - SEROLOGICAL AND MOLECULAR EVIDENCE

Elisabeth Baum[1], Jetsumon S. Prachumsri[2], Jeeraphat Sirichaisinthop[3], Huw D. Davies[1], Arti Jain[1], Eugenia Lo[1], Ming-Chieh Lee[1], Liwang Cui[4], Philip Felgner[1], Guiyun Yan[1]

[1]University of California Irvine, Irvine, CA, United States, [2]Mahidol Vivax Research Center, Bangkok, Thailand, [3]Vector Borne Disease Training Center, Pra Budhabat, Thailand, [4]Pennsylvania State University, University Park, PA, United States

Malaria has been in decline in Thailand, where the reported annual parasite incidence dropped to 0.56 in 2007. The Southeast Asia International Centers for Excellence in Malaria Research has been monitoring the prevalence of *Plasmodium falciparum* (*Pf*) and *P. vivax* (*Pv*) infections of the population of several villages of Tak Province, at the Thai-Myanmar border since 2011. Both active (ACD) and passive (PCD) case detection surveys are used, relying on microscopy as diagnostic method. ACD efforts consist of weekly visits to participants' homes to record their health status, combined with quarterly mass blood surveys (MBS) of the community of a study site. PCD is carried out in malaria clinics and hospitals. We collected samples at a study site where the parasite prevalence and the rate of symptoms reported were <0.5%, and analyzed

them by qPCR and protein microarray for serology. We found a surprisingly highly level of asymptomatic infections in the community, where 11% of individuals were either *Pf*+ or *Pv*+ while experiencing no malaria symptoms for 2 months preceding and following blood sampling. Serological analysis of both infected and non-infected individuals on a microarray revealed widespread exposure to the parasite, as seropositivity rate to 458 antigens was 100% amongst community samples. Samples collected during PCD also revealed higher infection rate than estimated by microscopy, as well as serological evidence of parasite exposure in all patients, despite of infectious status at blood collection. Using the protein microarray, we identified serological markers associated with protection from malaria disease by comparing the serological profiles of disease immune and non-immune individuals, as well as markers associated with acute symptomatic infection by comparing infected and non-infected clinic patients. We conclude that *Plasmodium* sp. prevalence is higher than recently estimated in Tak, and the large number of asymptomatic infections combined with low-sensitivity of current diagnostic methods may hamper efforts to eradicate malaria in Thailand in the future.

1224

EPIDEMIOLOGY OF DISAPPEARING *PLASMODIUM VIVAX* MALARIA: A CASE STUDY IN RURAL AMAZONIA

Susana C. Barbosa[1], Amanda B. Gozze[1], Nathália F. Lima[1], Camilla L. Batista[1], Melissa Bastos[1], Vanessa C. Nicolete[1], Pablo S. Fontoura[1], Raquel M. Gonçalves[1], Susana A. Viana[1], Maria José Menezes[1], Kézia K. Scopel[2], Carlos E. Cavasini[3], Rosely S. Malafonte[1], Mônica Silva-Nunes[4], Joseph M. Vinetz[5], Márcia C. Castro[6], Marcelo U. Ferreira[1]

[1]University of São Paulo, São Paulo, Brazil, [2]Federal University of Juiz de Fora, Juiz de Fora, Brazil, [3]Faculty of Medicine of São José do Rio Preto, São José do Rio Preto, Brazil, [4]Federal University of Acre, Rio Branco, Brazil, [5]University of California San Diego, La Jolla, CA, United States, [6]Harvard School of Public Health, Boston, MA, United States

New frontier settlements across the Amazon Basin pose a major challenge for malaria elimination in Brazil. Here we describe the epidemiology of malaria during the early phases of occupation of farming settlements in Remansinho area, Brazilian Amazonia. We examine the relative contribution of low-density and asymptomatic parasitemias to the overall *Plasmodium vivax* burden over a period of declining transmission and discuss potential hurdles for malaria elimination in Remansinho and similar settings. Eight community-wide cross-sectional surveys, involving 584 subjects, complemented by active and passive surveillance of febrile illnesses between the surveys, were carried out in Remansinho over 3 years. We used quantitative PCR to detect low-density asexual parasitemias and gametocytemias missed by conventional microscopy. Mixed-effects multiple logistic regression models were used to characterize independent risk factors for *P. vivax* infection and disease. *P. vivax* prevalence decreased from 23.8% (March-April 2010) to 3.0% (April-May 2013), with no *P. falciparum* infections diagnosed after March-April 2011. Although migrants from malaria-free areas were at increased risk of malaria, their odds of having *P. vivax* infection and disease decreased by 2-3% with each year of residence in the Amazon. Several findings indicate that low-density and asymptomatic *P. vivax* parasitemias may complicate residual malaria elimination in Remansinho: (a) the proportion of subpatent infections (i.e. missed by microscopy) increased from 43.8% to 73.1% as *P. vivax* transmission declined; (b) most (56.6%) *P. vivax* infections were asymptomatic and 32.8% of them were both subpatent and asymptomatic; (c) asymptomatic parasite carriers accounted for 54.4% of the total *P. vivax* biomass in the host population; (d) over 90% of subpatent and asymptomatic *P. vivax* had PCR-detectable gametocytemias; and (e) few (17.0%) asymptomatic and subpatent *P. vivax* infections that were left untreated progressed to clinical disease over 6 weeks of follow-up or became detectable by routine malaria surveillance.

1225

SUBMICROSCOPIC GAMETOCYTE CARRIAGE: PREVALENCE AND PREDICTORS ACROSS TWO SEASONS AND THREE DISTRICTS IN SOUTHERN MALAWI

Jenna E. Coalson[1], Jenny A. Walldorf[2], Lauren M. Cohee[2], Matthias Marti[3], Regina Joice[3], Karl Seydel[4], Miriam D. Ismail[1], Nelson Chimbiya[4], Don Mathanga[5], Atupele Kapito-Tembo[6], Terrie E. Taylor[4], Miriam K. Laufer[2], Mark L. Wilson[1]

[1]University of Michigan, Ann Arbor, MI, United States, [2]University of Maryland, Baltimore, MD, United States, [3]Harvard University, Boston, MA, United States, [4]Blantyre Malaria Project, Blantyre, Malawi, [5]Malaria Alert Centre, Ann Arbor, MI, United States, [6]Malaria Alert Centre, Blantyre, Malawi

As the transmissible stage of *Plasmodium*, gametocytes are critical to population-level malaria dynamics, though the epidemiology remains poorly characterized. Because gametocytes comprise a small proportion of the parasite burden in infected individuals, detection requires highly sensitive molecular methods. A *P. falciparum* stage-specific quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay was used to test for gametocytemia in samples collected through the Malawi International Center of Excellence for Malaria Research. The study involves post-rainy and post-dry season cross-sectional sampling of individuals from 900 households across urban/low, semi-rural/moderate, and rural/high transmission areas. Peripheral blood was collected as dried spots on filter paper for PCR and preserved in RNAprotect™ (Qiagen) for qRT-PCR. Samples positive for *P. falciparum* lactate dehydrogenase by PCR were subsequently tested using qRT-PCR to detect gametocyte carriage. *P. falciparum* infection prevalence was 8.1% in 617 samples from dry season 2012 and 18.8% in 795 samples from rainy season 2013. Gametocytes were found by qRT-PCR in 3.4% and 9.4% of the population, respectively. The prevalence of gametocytemia among school-aged children (6-15 yrs) was 8.3% after the dry season and 16.4% after the rainy season, compared with only 2.1% and 6.0% among young children (≤5 yrs) and 0.7% and 5.5% among adults (>15 yrs). The proportion of infections that were gametocytemic was slightly higher in areas with lower asexual parasite prevalence, and in school-aged children relative to adults and young children. Specifically, gametocytes were detected in 56.4% of infections in school-aged children, 46.7% of those in young children, and 35.1% of those in adults (X^2, p=0.03). Additional predictors of gametocytemia will be presented, with comparisons to gametocyte detection by microscopy. School-aged children are a key reservoir for *P. falciparum* transmission, and may represent a strategic target for intervention.

1226

THE PREVALENCE OF MALARIA AT FIRST ANTENATAL VISIT IN PREGNANT WOMEN IN BLANTYRE, MALAWI FROM 2009-2013

Sarah Boudova[1], Titus Divala[2], Patricia Mawindo[2], Lauren Cohee[1], Linda Kalilani-Phiri[2], Phillip Thesing[2], Terrie Taylor[2], Miriam Laufer[1]

[1]University of Maryland, Baltimore, MD, United States, [2]Blantyre Malaria Project, Blantyre, Malawi

Preventing malaria during pregnancy is important for the health of mothers and newborns. Interventions which include distribution of bed nets and administration of intermittent preventive treatment (IPT) typically occur at the first antenatal visit, usually in the second or third trimester of pregnancy. During the course of our ongoing studies of malaria among pregnant women in Malawi, a universal bed net campaign occurred in the middle of 2012. We hypothesized that this intervention would decrease the prevalence of malaria among pregnant women at their first antenatal visit. We conducted quantitative PCR (qPCR) from dried blood spots collected at the first antenatal care visit (prior to administration of IPT) from women who were in their first or second pregnancy and less

than 28 weeks gestation by clinical assessment. Overall, 145/753 (19.2%) women tested for malaria at their first antenatal visit were infected. By year, the malaria infection rates were 22.0% in 2009, 23.2% in 2010, 15.7% in 2012 and 8.5% in 2013. While declines between other years did not reach statistical significance, the odds of malaria infection at the time of first antenatal visit in 2013 as compared to 2009 were 0.3 (95% CI: 0.1 - 0.9). We will continue to analyze samples from the ongoing clinical trial. Rates of malaria at first antenatal visit declined from 2009 to 2013 with the most pronounced decline in 2013 after completion of the bed net campaign. Nevertheless, infection in this cohort is still common. These first and second trimester infections may cause maternal anemia and placental malaria resulting in adverse maternal and fetal outcomes. Infection early in pregnancy may also contribute to malaria transmission as pregnant women represent a significant untreated reservoir of parasites. Universal bed nets appear to have moderate success in preventing malaria early in pregnancy and our findings support continued efforts to target women early in pregnancy and possibly all women of childbearing age.

1227

A COMPARISON OF HEMOLYTIC POTENTIAL OF THREE ANTI-MALARIALS ON NORMAL AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE-DEFICIENT INFANTS

Eugenie A. Poirot[1], Eric Vittinghoff[2], Deus Ishengoma[3], Michael Alifrangis[4], Ilona Carneiro[5], Ramadhan Hashim[6], Jacklin Mosha[6], Samwel Gesase[3], Daniel Chandramohan[5], Roly Gosling[1]

[1]Malaria Elimination Initiative, Global Health Group, San Francisco, CA, United States, [2]University of California San Francisco, School of Medicine, San Francisco, CA, United States, [3]National Institute for Medical Research, Tanga Medical Research Centre, Tanga, United Republic of Tanzania, [4]University of Copenhagen, Center for Medical Parasitology, Copenhagen, Denmark, [5]London School of Hygiene & Tropical Medicine, London, United Kingdom, [6]National Institute for Medical Research, Mwanza Medical Research Centre, Mwanza, United Republic of Tanzania

Chlorproguanil-dapsone (CD) has been linked to hemolysis in G6PD-deficient individuals but there are no data for children <1 year. Therefore, the present analysis sought to assess incidence of hemolysis in G6PD-deficient and normal infants treated with CD, sulfadoxine-pyrimethamine (SP), and mefloquine (MQ), using data from a double-blind, placebo-controlled trial of Intermittent Preventive Treatment in infants (IPTi). Hemoglobin (Hb) measurements were made at IPTi doses, regular follow-up and emergency visits. G6PD genotype was determined at 9 months looking for SNPs for the A- genotype at coding position 202. Hemolysis was defined as absolute change in Hb, ≥15% decrease in Hb, or post-dose Hb measurement <8 g/dL. These outcomes were assessed using - 1) a single follow-up Hb on day 7 after an IPTi dose; 2) Hb obtained 1 to 14 or 28 days after each IPTi dose; and 3) all follow-up Hb. A total of 1557 (64%) children had valid G6PD results - 1324 were G6PD normal, 114 homo/hemi (HH), and 119 heterozygous (HET). Of these, 329 children in the active arms had Hb measured on day 7. A strong association of HH genotype with Hb < 8 g/dL on day 7 was seen (OR = 6.65, 95% CI 1.67-26.6, p = 0.007). Similarly, an adjusted linear model for absolute changes in Hb between days 0 and 7 among children in the three active arms showed a statistically greater decline in HH children (-0.56 g/dL, 95% CI 0.01-1.12, p=0.06). However, using all follow-up measurements, Hb declined less in HH infants (0.24 g/dL, 95% CI 0.02-0.46, p=0.03). Likewise, we found lower prevalence of ≥15% declines in HH infants. There was no evidence for an effect of G6PD status on absolute declines or declines of >=15% for changes within 14 or 28 days of the IPTi dose. Finally, while we did find greater declines in Hb in the CD arm compared to both the SP and MQ arms, there was no evidence of a drug-genotype interaction. These results could be explained by low statistical power, genotypes other than A- being common in the study area or that CD did not result in acute hemolysis in G6PD-deficient children. Our ability to assess drug-induced G6PD deficiency-related risk of hemolysis remains challenging and will require creative approaches for eliciting, assessing and recording safety data in this important patient population as drugs with hemolytic potential, such as the 8-aminoquinolines, primaquine and tafenoquine are more commonly used.

1228

NOVEL SERO- EPIDEMIOLOGICAL TOOL FOR ASYMPTOMATIC *LEISHMANIA DONOVANI* INFECTION

Aarthy C. Vallur[1], Caroline Reinhart[1], Hong Liang[1], Yasuyuki Goto[2], Dinesh Mondal[3], Malcolm Duthie[1], Steven G. Reed[1]

[1]Infectious Diseases Research Institute, Seattle, WA, United States, [2]University of Tokyo, Tokyo, Japan, [3]International Centre for Diarrhoeal Diseases Research, Bangladesh, Dhaka, Bangladesh

Visceral leishmaniasis (VL) is a serious disease that threatens 200 million people living in endemic areas across the Indian subcontinent, Mediterranean basin, East Africa and South America. Though diagnosis of VL disease has been made simple and cost- effective through the availability of standard serological tests, similar tools do not exist for asymptomatic infection detection, which is 4-20 times more likely than symptomatic VL disease in endemic areas. A serological test for asymptomatic infection is necessary for designing surveillance and elimination programs, already underway in the Indian sub- continent. We identified 12 novel VL- specific tandem repeat antigens by bioinformatics and one by mass spectrometry of VL patient samples. All 13 antigens were expressed recombinantly and down selected by ELISA based on their agreement and complementation of with DAT on a panel of sera from a hyperendemic district of Bangladesh, indicating those at highest risk of being asymptomatically infected. A conserved protein rKR95 and a tandem repeat antigen rTR18 together agreed at 92% with DAT on asymptomatic sera with robust signals and low background. The antigens were also able to detect 26% of DAT negative sera, proving that they are highly sensitive for asymptomatic infection. rKR95 and rTR18 have utility in a sero-epidemiological tool for asymptomatic infection in endemic regions distinct from one used to confirm disease, individually or as fusion antigens. Such a test for asymptomatic infection will have high impact on achieving active detection, a primary goal of larger surveillance and elimination programs.

1229

RISK FACTORS FOR VISCERAL LEISHMANIASIS IN GEDAREF STATE, SUDAN

Yolanda K. Mueller[1], Fabienne Nackers[1], Niven Salih[2], Mousab S. Ali[3], Mubarak Ilnour[4], Omer Hammam[2], Ann Mumina[2], Atia A. Atia[2], Jean-Francois Etard[1], Koert Ritmeijer[5], Francois Chappuis[6]

[1]Epicentre, Paris, France, [2]Doctors Without Borders, Geneva, Switzerland, [3]Federal Ministry of Health, Khartoum, Sudan, [4]State Ministry of Health, Gedaref, Sudan, [5]Doctors Without Borders, Amsterdam, Netherlands, [6]Geneva University Hospital, Geneva, Switzerland

Risk factors for visceral leishmaniasis (VL) in areas where P. orientalis is the main vector remain unclear, limiting the development of relevant control strategies. We conducted a case-control study to identify determinants of clinical VL in 24 villages of Tabarak Allah hospital's catchment area, Gedaref State, Sudan. From September 2012 to July 2013, we recruited 198 patients newly diagnosed with probable or confirmed primary VL. Using spatial sampling, we randomly recruited 801 controls with a distribution of age, sex and village of residence proportionate to the distribution of the target population. Controls were free of VL symptoms, previous VL treatment and had a negative VL rapid test. A questionnaire was used to collect information on the demographic and socio-economic characteristics of the participants, their travel history, day and evening activities, and the characteristics of their house, yard and surroundings. In a multivariate logistic regression model, VL risk increased with household size, sleep location (outside the yard, not in the farm), evening outdoor activities in the rainy season (playing, watching TV, radio listening), use of ground nut oil as animals' repellent, presence of dogs in the yard at night, Acacia nilotica in the yard's immediate surroundings and of a forest at eye

range. VL risk appeared to decrease with the use of drinking water sources other than the village water tank, increasing distance from the adjacent house yard, and with the presence of animals other than dogs in the yard at night. Children and men were at higher risk of VL as well as individuals reporting VL patient(s) in their household in the previous year. In contrast with previous studies, housing factors, mosquito-net use, black cotton soil, ethnicity, socioeconomic index, presence of Balanites aegyptica and Azadirachta indica in the yard were not independent VL determinants. Although our results do not provide evidence of causality, they provide useful suggestions for the development of relevant VL preventive measures as well as for guiding further studies.

1230

PREVALENCE OF *TRYPANOSOMA CRUZI* INFECTION AMONG BOLIVIAN IMMIGRANTS IN THE CITY OF SAO PAULO, BRAZIL, PRELIMINARY REPORT

Expedito J. Luna[1], Celia R. Furucho[1], Rubens A. Silva[2], Dalva M. Wanderley[2], Ruth M. Leite[2], Vera L. Freitas[1], Cassio Silveira[3], Luzia Martinelli[3], Sonia R. Almeida[3], Rosario Quiroga[1], Karina Kiso[3], Noemia B. Carvalho[1], Lia M. Silva[1], Fernando M. Aith[1], Nivaldo Carneiro, Jr.[3], Maria A. Shikanai-Yasuda[1]

[1]Universidade de Sao Paulo, Sao Paulo, Brazil, [2]Secretaria Estadual de Saude de Sao Paulo, Sao Paulo, Brazil, [3]Faculdade de Ciencias Medicas da Santa Casa de Sao Paulo, Sao Paulo, Brazil

With the urbanization of the population of developing countries and the process of globalization, Chagas disease has become an emerging disease in urban areas of endemic and non-endemic countries. The aim of the present study was to determine the prevalence of *Trypanosoma cruzi* infection in Bolivian immigrants living in Sao Paulo Metropolitan Area. Approximately 300,000 Bolivian immigrants live in it. In 2006, PAHO estimated the prevalence of Chagas disease in Bolivian general population and among candidate blood donors in 6.8% and 8.0% respectively. Vectorial transmission of Chagas disease has been interrupted in Brazil since 2006. This prevalence survey was undertaken in a sample of 633 volunteers (being 111 children below 10 years of age), randomly selected from the clientele of primary care units located in the central districts of the City of Sao Paulo, Brazil. Inclusion criteria were the agreement to respond to a semi-structured questionnaire and to collect blood for serology after signing the informed consent form. Infection was detected by two different ELISA assays with epimastigote antigens (Bioschile and Biomeriéux), followed by immunoblot with trypomastigote antigens as confirmatory test. The prevalence of infection in 598 individuals so far analyzed was 4.68%. Among children less than 10 year old the prevalence was 2.76% and among the older than 10 years, 5.13%. This is, to our knowledge, the first information on the prevalence of infection among the Bolivian immigrant community in the City of Sao Paulo and represents a challenge to primary care clinics to manage chronic Chagas disease, its vertical transmission, as well as to investigate parasite reactivation in patients under immunossupression in tertiary health care. Additionally, these data will be useful in planning and evaluation of the surveillance and control program of Chagas disease transmission by blood derivatives and organ transplants.

1231

HUMAN MIGRATION DRIVES THE DISPERSAL OF EPIZOOTIC CHAGAS DISEASE: THE CASE OF HIGHLAND BOLIVIA

Louisa A. Messenger[1], Lineth Garcia[2], Michael A. Miles[1], Martin S. Llewellyn[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Universidad Mayor de San Simón, Cochabamba, Plurinational State of Bolivia

An improved understanding of the interactions between natural parasite populations and their environment is crucial to establish the epidemiological risk associated with epidemic pathogenic genotypes. *Trypanosoma cruzi*, the aetiological agent of Chagas disease, is an ancient and widespread zoonosis distributed throughout the Americas. TcI is the most abundant genetic lineage; it is the principal cause of human chagasic cardiomyopathy and is ubiquitous among sylvatic transmission cycles. Multiple molecular markers consistently identify high levels of diversity within sylvatic TcI populations, and divergent, but genetically homogeneous, strains isolated from human infections. However, current understanding of the genetic determinants that drive natural *T. cruzi* diversification is incomplete. We performed high resolution nuclear and mitochondrial genotyping of contemporaneous sylvatic TcI (n=199 biological clones), isolated from a range of triatomine and mammalian hosts across Bolivia. We detected two distinct sylvatic transmission cycles in adjacent highland and lowland areas. Highland Bolivian strains were characterized by reduced genetic diversity and heterozygosity (Ar= 1.92-2.22, FIS=-0.241-0.026) compared to lowland areas (Ar = 3.40-3.93, FIS=0.176). We observed equivalent levels of subdivision among highland areas spanning >465 km (FST = 0.084, p=0.0032) and between lowland populations across 155 km (FST = 0.087, p<0.001). Measurements of isolation by distance detected greater parasite dispersal among geographically disparate, but heavily populated, highland areas (RXY= 0.053, p=0.142) than between proximate, sparsely populated, lowland foci (RXY= 0.209, p<0.001). Importantly, human isolates from highland Bolivia, were closely related to sylvatic strains circulating in the same area. Overall, the most parsimonious explanation for our results is a founder event in highland Bolivia with long-range anthropogenic dispersal of parasites across an ecological cline. We discuss the important role of humans as an abundant, but often neglected, vector of *T. cruzi* and consider their impact on the emergence of epizootic Chagas disease.

1232

TEMPORAL AND REGIONAL TRENDS OF FORCE INFECTION OF CHAGAS DISEASE IN COLOMBIA

Zulma M. Cucunubá[1], Pierre Nouvellet[2], Gabriel- Jaime Parra[3], Victor M. Angulo[3], Lesong Conteh[2], María-Gloria Basáñez[2]

[1]Imperial College London and Red-Chagas Colombia, London, United Kingdom, [2]Imperial College London, London, United Kingdom, [3]Red Chagas Colombia, Bogotá, Colombia

In the context of Chagas disease, the impact of vector control strategies in endemic zones is often measured via age-specific seroprevalence surveys, for which interpretation is complicated by secular changes in exposure, resulting in complex age profiles. Reconstruction of temporal trends in the force of infection (FOI) from prevalence data can help to improve our understanding of the transmission dynamics and to plan cost-effective national strategies for diagnosis and treatment. Using 52,712 registries, this study aimed to estimate the trends in the FOI (incidence) for Chagas disease in 14 endemic departments. Given the range of surveys from 1996 to 2011, the data accounted for exposure history between 1920 and 2011. Various catalytic models were fitted to estimate either a constant FOI as a function of exposure time, or a modified FOI at a specific (estimated) time, plus a parameter that accounts for other transmission routes. Parameters were estimated through Markov Chain Monte Carlo (MCMC) methods. Using the simple model, the FOI varied between 0.04 x10-3 and 0.03 person/year across all locations. The Sierra Nevada de Santa Marta region (northern Colombia) showed the highest FOI (0.023; 95% CI 0.02-0.026). Among high endemic zones, the FOI varied from 0.004 to 0.026 in the late 1990's, and between 0.001 and 0.004 in the 2009/2011 surveys. For two surveys, a change in FOI was evident; in Casanare (eastern Colombia), from 0.07 (0.014-0.28) before 1972 to 0.001 (0.0009-0.001) after 1972, and in Santander (east-centre), from 0.009 (0.002-0.08) before 1981 to 0.001 (0.0003-0.002) afterwards. These results provide evidence on the temporal and regional variation patterns in Chagas disease incidence in Colombia. Marked transitions around the 1970's and 1980's have likely resulted from control interventions, improved housing conditions, or migration movements.

Ongoing modelling analyses, allowing more flexible variations in the FOI through time, will be discussed to provide improved and updated predictions of Chagas disease prevalence and incidence in Colombia.

1233

EVALUATION OF INDOOR INSECTICIDE SPRAYING (IRS) PROGRAM, AN INTEGRATED APPROACH FOR VECTOR CONTROL IN VISCERAL LEISHMANIASIS

Paritosh Malviya[1], Shyam Sundar[1], Marleen Boelaert[2], Jean-Pierre van Geertruyden[3]

[1]Banaras Hindu University, Varanasi, India, [2]Institute of Tropical Medicine, Antwerp, Belgium, [3]University of Antwerp, Antwerp, Belgium

Human visceral Leishmaniasis (VL) or commonly known as kala-azar is vector born infectious disease transmitted by *Phlebotomine argentipes* sandflies. The disease is highly endemic in Bihar state of India and is fatal if left untreated. VL is a neglected tropical disease and Indian government targets to eliminate it from the region by 2018. Active case surveillance and vector control by indoor residual spraying (IRS) using DDT are two ways to target the elimination. In the district of Muzaffarpur in Bihar, during our evaluation surveys we found IRS coverage was only 17% in 2010 which increased to 70% in 2013. However, in the villages with 100% coverage sand fly density did not reduce significantly. There were several ditches between the planning and monitoring of IRS program. We observed the use of low concentration solution, poor quality of insecticide and walls were not sprayed up to adequate height at places. Peri-domiciliary areas were inadequately sprayed. IRS was done only once in a year as against twice per year as recommended in the program guidelines. We did not find any trouble and discomfort in the community by the use of insecticides. However people felt IRS ineffective because it did not reduce the density of malaria mosquitoes. Some of the households did not allow spraying as it would disturb their life for at least one day or no male member was present at that time. Some households allowed spraying only a part of their house. Some households did complaint that the spray team did not visit their house even after request. We also sensed IRS as a means of fulfilling political agendas of village chief or local leaders. There are some other perceptions and barriers in the community against the acceptance of IRS program. These perceptions vary from community to community, from illiterate to educated ones, from lower to upper caste and from poor to rich. To understand more on these perceptions we are conducting a qualitative study in the selected villages of the district. In-depth interviews and focused group discussions are being conducted among villagers, community leaders, health providers and program in-charge. Participants represent wider section of the community in terms of socio-economic status, education, and caste. The study is expected to be completed by the end of 2014.

1234

TRENDS OF MORTALITY SECONDARY TO CHAGAS DISEASE AND IMPLICATIONS OF REPORTING ON ESTIMATES

Carlos A. Valencia-Hernández[1], Zulma M. Cucunubá[2]

[1]National Health Institute, Bogotá, Colombia, [2]Imperial College London, London, United Kingdom

In Colombia is estimated that about 436. 000 people are infected with *Trypanosoma cruzi* and 140.000 have the cardiologic sequelae. This study aims to explore the historical trends of mortality secondary to Chagas disease (CD) and tries to explain the variations of this pattern through the 35 years of existence of the mortality reporting system. The national registry of deaths is based on death certificates, which are filled by Doctors when a patient dies, and then processed by the National Statistics Department, where direct cause and associated causes of death are classified. We use the method of associated causes of death, taking the total available registries (1979-2011). By using the direct method, we estimated crude and adjusted death rates do to CD at National, Departmental and Municipality levels. The patterns of death rates were estimated using joinpoint regression techniques. For the described period, a total of 5.349.628 deaths were registered and 1957 of those (proportional mortality rate of 0.03%) corresponded to CD. 6 departments registered 80% and 7 municipalities 60% of the deaths; 1 of these 6 departments (Capital District) is not an endemic zone. Death rates in men were almost twice the rates in women. Crude death rates increased from 0.03 per 100.000 inhabitants in 1979 to 0.23 in 2011. Adjusted death rates showed a similar increase. The rates increased with age, being the highest mortality in the group between 60 and 70 years. Regarding the joinpoint regression analysis, we observed a progressive increment for the period 1979-2008, and a final decrease in the period 2008-2011. The concentration of deaths in certain non-endemic regions could represent a consequence of migration from rural to urban areas. The higher rates in certain age groups could be the result of a cohort effect due to higher prevalence in previous decades. We found evidence of under-reporting of deaths due to CD by comparing known deaths in outbreaks of CD with the reported ones in the same year. Changes in the reporting system through time and its implications are discussed.

1235

THE PREVALENCE AND ORIGINS OF ARTEMISININ RESISTANT FALCIPARUM MALARIA IN MYANMAR

Shannon Takala-Harrison[1], Myat Phone Kyaw[2], Kay Thwe Han[2], Ye Myat Kyaw[2], Kyin Hla Aye[2], Khin Phyu Pyar[2], Kara Moser[1], Christopher G. Jacob[1], Matthew Adams[1], Shay M. Hampton[1], Amy Wang[1], Tin Maung Hlaing[2], Ye Htut[2], Myaing M. Nyunt[1], Christopher V. Plowe[1]

[1]University of Maryland School of Medicine, Baltimore, MD, United States, [2]Department of Medical Research (Lower Myanmar), Yangon, Myanmar

Mutations in a kelch protein encoded on *Plasmodium falciparum* chromosome 13 (K13) have been shown to confer resistance to artemisinins in parasites in Southeast Asia. Mapping the prevalence and distribution of K13 mutations, and ascertaining whether resistance is spreading or emerging independently, is important to inform strategies to contain and eliminate resistant parasites. In this study we sequenced the K13 gene in samples from sites throughout Myanmar, including little-studied areas in southern, central and western Myanmar, and estimated the prevalence of K13 mutations at each site. Parasites from each site were genotyped at 33,716 SNPs using a DNA microarray, and haplotypes defined by single nucleotide polymorphisms in linkage disequilibrium with the K13 gene were used to determine the origins of K13 mutations identified at each site. Preliminary analyses suggest that different K13 mutations predominate at different study sites. Haplotype analysis indicates the independent emergence of K13 mutations in Myanmar, including the 580Y and the 574L mutations, which have been observed in other areas of Southeast Asia. Haplotype analysis for all sites will be presented and implications for resistance surveillance will be discussed.

1236

ARTEMISININ RESISTANCE AND THE EMERGENCE OF "SOFT" SWEEPS IN MALARIA PARASITE POPULATIONS

Tim Anderson[1], Shalini Nair[1], Marina McDew-White[1], Ian Cheeseman[1], Fatma Bilgic[1], Rose McGready[2], Elizabeth Ashley[3], Aung Pyae Phyo[2], François Nosten[2]

[1]Texas Biomedical Research Institute, San Antonio, TX, United States, [2]Shoklo Malaria Research Unit, Mae Sot, Thailand, [3]Centre for Tropical Medicine, Nuffield Department of Medicine, Oxford, United Kingdom

Textbook "hard" selective sweeps, in which single resistance alleles have rapidly spread through malaria parasite populations driven by chloroquine and pyrimethamine treatment, have had a powerful influence in shaping our understanding of drug resistance evolution in malaria. These examples have guided the strategies used to search for novel resistance loci; but are they typical, atypical, or even misleading? The recent discovery of a novel artemisinin resistance gene, kelch, in SE Asia, in which resistance alleles

clearly have multiple independent origins, supports a radically different model of resistance evolution. In such "soft" selective sweeps, resistance alleles spread on multiple different genetic backgrounds, so are expected to have a more subtle impact on patterns of flanking genetic variation. We examined the emergence of artemisinin resistance in real time to empirically determine the impact of spreading resistance on patterns of flanking genetic variation. We characterized the emergence and spread of kelch mutations (from 0% in 2001 to 70% frequency in 2013), in malaria patients attending clinics run by the Shoklo Malaria Research Unit in Western Thailand over a thirteen year period. We describe 26 independent mutations within the gene's coding sequence, each associated with reduced parasite clearance rate. We then use genotyping data from 41 SNPs targeting 470kb flanking the kelch gene in 1,577 infections to characterize the emergence of a "soft selective sweep" surrounding this gene. Based on these data, and recent models of adaptation, we argue (1) that the patterns of variation surrounding the kelch gene are likely to be common in the early stages of drug resistance in general, (2) that more sophisticated strategies are needed for identifying selective sweeps that are able to detect both "soft" and "hard" selective events, and, (3) that SNP-by-SNP methods for conducting association studies are likely to be grossly underpowered for discovery of novel drug resistance genes.

1237

DISSECTING THE GENETIC BASIS OF EMERGING ARTEMISININ RESISTANCE IN *PLASMODIUM FALCIPARUM*

Judith Straimer[1], Nina F. Gnadig[1], Benoit Witkowski[2], Chanaki Amaratunga[3], Arba Pramundita Ramadani[4], Valentine Duru[4], Mélanie Dacheux[1], Nimol Khim[2], Lei Zhang[5], Edward Rebar[5], Philip D. Gregory[5], Fyodor Urnov[5], Odile Mercereau-Puijalon[6], Francoise Benoit-Vical[4], Rick M. Fairhurst[3], Didier Menard[2], David A. Fidock[1]

[1]Columbia University, New York, NY, United States, [2]Institut Pasteur du Cambodge, Phnom Penh, Cambodia, [3]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [4]Centre National de la Recherche Scientifique, Toulouse, France, [5]Sangamo BioSciences, Richmond, CA, United States, [6]Institute Pasteur de Paris, Paris, France

In recent years, the global deployment of artemisinin (ART)-based combination therapies (ACTs) has contributed substantially to reducing the burden of malaria throughout tropical regions. Thus, alarms were sounded in 2008 when efficacy studies in Western Cambodia documented the emergence of ART resistance in *Plasmodium falciparum*. *In vitro* selection of ART-resistant parasites and association-based field studies suggest that mutations in the propeller domain of the kelch gene (PF3D7_1343700) might be central to resistance. Prevalence of these mutations associates strongly with slow parasite clearance rate in patients as well as elevated survival of drug-exposed ring-stage parasites *in vitro*. To confirm the kelch propeller domain as a candidate ART resistance marker, we have tested the hypothesis that mutations in this gene constitute a major determinant of emerging ART resistance across Cambodian *P. falciparum* parasites. We genetically modified the kelch propeller domain in clinically or *in vitro* defined ART-resistant or -sensitive Cambodian Pf parasites using the highly efficient process of zinc finger nuclease (ZFN)-based gene editing. Parasites were transfected with a single plasmid that expressed a kelch-specific ZFN pair, a selectable marker and a kelch donor sequence containing propeller domain mutations observed in Western Cambodia. This approach allowed for discrete editing of the kelch locus and the generation of isogenic parasites lines. Our study has focused on the *in vitro*-selected M476I mutation and the C580Y, R539T and Y493H mutations that predominate in regions with high prevalence of ART resistance in western Cambodia. These mutations were introduced into drug-sensitive parasites or removed from drug-resistant parasites. The aim of this work is to determine whether and to which degree these mutations mediate resistance by using a ring-stage survival assay. We will show data from a comprehensive study of genetically edited laboratory lines and clinical isolates that assess the role of kelch-propeller domain polymorphisms in emerging ART resistance.

1238

EMERGENCE OF HIGH-LEVEL, STABLE ARTEMISININ RESISTANT *PLASMODIUM FALCIPARUM* UNDER ARTESUNATE PRESSURE *IN VIVO*, WITH QUININE CO-RESISTANCE

Rajeev Tyagi[1], James Gleeson[1], Ludovic Arnold[1], Eric Prieur[1], Laurent Decosterd[2], Jean-Louis Pérignon[1], **Piero L. Olliaro**[3], Pierre Druilhe[1]

[1]Institut Pasteur and Vac4All Initiative, Paris, France, [2]Division of Clinical Pharmacology - Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, [3]World Health Organization, Geneva, Switzerland

Delayed parasite clearance among patients treated with artemisinins in Southeast Asia indicates that artemisinin resistance is evolving, but still limited in strength. We capitalized on the development of a laboratory model, which harbors high *Plasmodium falciparum* loads, to investigate the effect of progressive *in vivo* artesunate drug-pressure on *P. falciparum*. Single flash-dose and two-day regimens of artesunate were applied to *P. falciparum* (PAM) in a humanized NOD/SCID IL-2Rγ-/- mouse model; *in vitro* drug-sensitivities (IC50) were monitored in parallel. *P. falciparum* rapidly evolved high-level, stable artemisinin resistance against both regimens up to extreme, near-lethal, doses of artesunate (240mg/kg). The selection of artemisinin resistance was reproducible, occurring in 80% and 41 % of mice treated with single and two-day regimens, respectively. *In vitro* response proved ineffective as a marker of early resistance: IC50 remained stable while resistance increased *in vivo* to doses of 30mg/kg artesunate. Later, when *in vivo* resistance strengthened further, artesunate IC50 increased to 82.8nM (95%CI 58.2nM - 117.8nM) from a sensitive level of 10.5nM (95%CI 9.0nM - 12.3nM), and finally shifted ten-fold to 99nM. Emergence of artemisinin-resistance in this African strain was associated with selection of the MAL13-1718319(T) mutation, which is significantly associated with clinical artemisinin resistance in Southeast Asia, and found in a gene that encodes a putative DNA-repair protein (RAD5 homologue). Remarkably, despite exclusive exposure to artesunate, resistance to several quinolone antimalarials emerged; of particular concern resistance to quinine, the second-line treatment for severe malaria, was documented both *in vivo* (3 doses of 73 mg/kg IV over 24 h) and *in vitro* (IC50 = 214 nM). *P. falciparum* has the potential to evolve extreme artemisinin resistance and more complex patterns of multi-drug resistance than anticipated. If resistance in the field advances even partially along this trajectory, we could be faced with an unprecedented health crisis.

1239

K13 PROPELLER POLYMORPHISM IN COMMUNITIES OF THE *PLASMODIUM* DIVERSITY NETWORK: A NETWORK FOR INVESTIGATING AND USING *PLASMODIUM* GENETIC DIVERSITY TO INFORM MALARIA ELIMINATION POLICIES IN SUB-SAHARAN AFRICA

Edwin Kamau[1], Susana Campino[2], Lucas Amenga-Etego[3], Deus Ishengoma[4], Kimberly Johnson[5], Dieudonne Mumba[6], Yavo William[7], Bouyou Marielle[8], Tobias Apinjoh[9], Lemu Golassa[10], Milijaona Randrianarivelojosia[11], Ben Andagalu[12], Oumou Maiga-Ascofare[13], Ngwa Alfred Amambua[14], Paulina Tindana[3], Anita Ghansah[15], Bronwyn MacInnis[2], Dominic Kwiatkowski[2], **Abdoulaye Djimde**[16]

[1]KEMRI/United States Army Medical Research Unit – Kenya, Kisumu, Nairobi, Kenya, [2]Wellcome Trust Sanger Institute, Hinxton, United Kingdom, [3]Navrongo Health Research Centre, Navrongo, Ghana, [4]National Institute for Medical Research, Tanga, United Republic of Tanzania, [5]University of Oxford, Oxford, United Kingdom, [6]Université de Kinshasa, Kinshasa, Democratic Republic of the Congo, [7]National Institute of Public Health, Abidjan, Côte D'Ivoire, [8]Universite des Sciences de la Sante, Libreville, Gabon, [9]University of Buea, Buea, Cameroon, [10]Addis Ababa University and Armauer Hansen Research Institute, Addis Ababa, Ethiopia, [11]Institut Pasteur de Madagascar, Antananarivo, Madagascar, [12]KEMRI/United States Army Medical Research Unit, Nairobi, Kenya, [13]University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali, [14]Medical Research Council, Gambia Unit, Banjul, Gambia, [15]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [16]University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali and Wellcome Trust Sanger Institute, Hinxton, United Kingdom, Bamako, Mali

Artemisinin resistance has been confirmed in South-East Asia. A concerted effort at surveying *Plasmodium* genetic diversity is required for tracking the emergence and potential spread of artemisinin resistance to sub-Saharan Africa. This comes with its underlying scientific, ethical, and practical challenges. The *Plasmodium* diversity network (PDN) is bringing together researchers from malaria endemic sub-Saharan African countries and making use of relevant existing consortial frameworks to address these challenges. The PDN includes scientists from biomedical research Institutions in 11 sub-Saharan African countries i.e. Cameroun, Côte d'Ivoire Democratic Republic of Congo (DRC), Ethiopia, Gabon, The Gambia, Ghana, Kenya, Madagascar, Mali and Tanzania. With the recent identification of K13 propeller as a major molecular marker for artemisinin resistance, we are investigating the presence and prevalence of its polymorphisms at the PDN study sites. Samples, either in the form of dried blood spots or extracted DNA, have been collected from falciparum malaria infections in PDN sites in each of the above listed countries. Capillary sequencing was performed at the Wellcome Trust Sanger Institute and SNPs called using 3D7 as the reference genome. This primary African K13 SNP survey includes 100 samples from each PDN site. Data analysis on a total of 1200 processed samples is underway and preliminary results will be presented at the meeting. This study will provide a sub-Saharan Africa-wide map of K13 propeller polymorphisms and assess the presence of mutations associated with artemisinin resistance in South-East Asia

1240

IDENTIFICATION OF DRUG RESISTANCE LOCI USING GENOME-WIDE SCANS

Amanda K. Lukens[1], Andrew H. Lee[2], Daniel J. Park[3], Asako Tan[4], Lisa Checkley[4], Michael T. Ferdig[4], David A. Fidock[2], Dyann F. Wirth[5], Sarah K. Volkman[5]

[1]The Broad Institute, Cambridge, MA, United States, [2]Columbia University, New York, NY, United States, [3]Harvard University, Cambridge, MA, United States, [4]University of Notre Dame, Notre Dame, IN, United States, [5]Harvard School of Public Health, Boston, MA, United States

Through rapid genetic adaptation, the *Plasmodium falciparum* parasite is able to develop resistance to antimalarial drugs, thwarting global health efforts. In recent years, genome-wide scans such as Quantitative Trait Locus (QTL) mapping and Genome Sequencing Association Studies (GSAS) have provided critical hypothesis-generating tools in the effort to understand the mechanisms by which this occurs. We have applied these tools in a population genetics approach to identify the targets of highly potent bioactives that can be powerful probes of parasite-specific biological processes. Here we present studies of a benzothiazepine amide chemotype, IDI-3783, discovered to have nanomolar activity in phenotypic whole cell assays against the chloroquine resistant (CQ^R) parasite line Dd2. Strikingly, dose-response to the compound is significantly reduced in chloroquine sensitive (CQ^S) parasites. To study the genetic basis of the inverse relationship between CQ and IDI-3783 response, the parents and progeny from the Dd2 x HB3 cross were used to genetically map the locus responsible for the phenotypic difference. QTL mapping identified a significant peak on chromosome seven. Further refinement of this signal with GSAS analysis of 40 culture adapted clinical isolates indicated a role for PfCRT haplotypes in dose response to these compounds. Independent experiments lead to the generation of *in vitro* selected Dd2 parasites that were 500-fold less sensitive to IDI-3783. Whole genome sequencing identified target mutations in PfCRT, further validating the role of this locus in IDI-3783 mode of action. Interestingly, the IDI-3783 resistant mutants also demonstrated a marked reduction in CQ EC_{50} rendering them susceptible to the drug despite retaining an otherwise CQ^R PfCRT haplotype. A ZFN-edited Dd2 cell line was then generated confirming the role of these PfCRT mutations in IDI-3783 and CQ dose response. These studies demonstrate the power of genome-wide scans in understanding the underlying genetic basis for drug resistance. As novel antimalarial chemotherapies are developed, understanding their resistance mechanisms will be critical in extending the useful lifetime of new drugs and combating this devastating disease.

1241

REVERSAL OF CHLOROQUINE RESISTANCE IN *PLASMODIUM FALCIPARUM* IN FRENCH GUIANA: AN ORIGINAL EVOLUTIONARY PATHWAY FROM LOW ENDEMIC SETTINGS AND IMPLICATIONS FOR RESISTANCE SURVEILLANCE IN SOUTH AMERICA

Stéphane Pelleau[1], Eli L. Moss[2], Satish Dhingra[3], Béatrice Volney[1], Jessica Casteras[1], Sarah K. Volkman[4], Dyann F. Wirth[4], Eric Legrand[1], David A. Fidock[3], Daniel E. Neafsey[2], **Lise Musset**[1]

[1]Intitut Pasteur de la Guyane, Cayenne cedex, French Guiana, [2]Broad Institute, Cambridge, MA, United States, [3]Columbia University, New York, NY, United States, [4]Harvard School of Public Health, Boston, MA, United States

In French Guiana, *Plasmodium falciparum* is endemic but transmitted at a low level. For several years the number of malaria cases has decreased in this region, which is now considered in a pre-elimination phase. This region, where drug resistance can appear de novo and *in situ*, is therefore an excellent model to study parasite evolution in low-endemicity settings. After decades of treatment with chloroquine (CQ), this drug was abandoned in 1995 due to high prevalence of *in vitro* CQ resistance (>90%). This was associated with the presence of the K76T mutation in

the pfcrt gene, encoding the typical South American haplotype SVMNT. Twenty years after CQ withdrawal, 70% of the isolates have regained *in vitro* susceptibility to CQ. This is not due to the reemergence of the wild-type pfcrt K76 allele, as the mutant K76T allele is fixed within the parasite population. This creates an apparently paradoxical genotype/phenotype association, indicating that *P. falciparum* populations circulating in French Guiana have acquired a novel mechanism of drug response unique to this region. Here we present the results of a genome-wide association study performed on parasites collected in French Guiana between 2009 and 2012 presenting contrasting CQ phenotypes. This allowed identification of a new mutation strongly associated with CQ susceptibility. The causal relationship has been established by gene editing in the 7G8 Brazilian strain which is responsible for a 20-fold decrease of CQ IC50. In order to date and evaluate the extent of this phenomenon, a retrospective analysis was conducted on 400 isolates from 1997 to 2013. Results showed that the new susceptible allele emerged in the early 2000s and rapidly spread thereafter throughout the population. The significant impact of this genotype on other antimalarial drug responses will be presented at the meeting. In conclusion, the utility of the mutation pfcrt K76T to monitor chloroquine resistance is severely reduced in French Guiana and potentially the greater Amazon basin region because of the presence of other mutations that render parasites CQ susceptible. This situation underlines the necessity to regularly validate molecular markers with *in vitro* drug assays before using it erroneously. It also illustrates how in these low-transmission settings where resistant alleles can reach fixation, parasites are able to follow original evolutionary paths.

1242

NEGATIVE CROSS RESISTANCE: A PRACTICABLE MEANS OF RESTORING PYRETHROID-SUSCEPTIBILITY TO VECTORS OF MALARIA

Michael White[1], Ian Denholm[2], John Marshall[1], **Gregor Devine**[3]

[1]MRC Centre for Outbreak Analysis and Modelling, Imperial College, London, United Kingdom, [2]University of Hertfordshire, Hatfield, United Kingdom, [3]QIMR Berghofer Medical Research Institute, Brisbane, Australia

Insecticide-treated nets and indoor residual spray programs for malaria control are almost entirely dependent on the use of pyrethroid insecticides. The ubiquitous exposure of *Anopheles* mosquitoes to this chemistry has selected for resistance in a number of populations. This threatens the sustainability of our most effective interventions but no operationally practicable way of conserving pyrethroid-susceptibility has yet been suggested. Combinations of pyrethroid nets or spray formulations with other insecticide classes are generally believed to have little impact on the frequency of pyrethroid-resistant genes if that resistance is already present in the target population. One interesting exception that we are exploring involves the co-application of a powerful chemosterilant (pyriproxyfen or PPF) to bed nets or resting surfaces that are usually treated only with pyrethroids. Resistant mosquitoes that are unaffected by the pyrethroid component of a PPF / pyrethroid co-treatment remain vulnerable to PPF. This chemosterilant has a far greater impact on pyrethroid-resistant mosquitoes than on susceptible ones because of their differential behavioural responses at co-treated surfaces. This imposes a specific fitness cost on pyrethroid-resistant phenotypes. The development of a pyrethroid / pyriproxyfen co-treated net was announced in early 2014 but its potential as a resistance management tool was not closely examined. We demonstrate the full potential of the combination, and a phenomenon called "behaviourally-mediated negative cross-resistance" using a mathematical model supported by empirical data on mosquito behaviour. The technique promises to select against pyrethroid-resistant genes and conserve pyrethroid-susceptibility and the sustainability of an insecticide class that is essential for malaria control.

1243

AUTO-DISSEMINATION OF PYRIPROXYFEN FOR CONTROL OF AFROTROPICAL MALARIA VECTORS

Oscar Mbare[1], Steve Lindsay[2], Ulrike Fillinger[3]

[1]International Centre of Insect Physiology and Ecology, Mbita, Kenya, [2]Durham University, Durham, United Kingdom, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom

Novel strategies for larvicide application to *Anopheles* larval habitats are required to minimize intervention costs. Recently adult *Aedes* mosquitoes have been used to transfer a persistent pupicide (pyriproxyfen) to preferred aquatic habitats to inhibit larval development. However pyriproxyfen (PPF) also sterilizes female mosquitoes exposed to the chemical. The aims of this study were to determine if: (1) PPF sterilizes *Anopheles gambiae* s.s. (2) sterilized females can transfer PPF to oviposition substrate (3) the best point in time to contaminate *An. gambiae* s.s. with PPF for use as agents to transfer the chemical to oviposition substrate. Female *An. gambiae* s.s. were exposed to jars treated with 2.6 mg PPF/m^2 at 48 hour before, 24 hour before, 0.5 hour before, 0.5 hour after, 24 hour after, 48 hour after and 72 hour (on the day of egg-laying) after bloodmeal. Control females were exposed to acetone-coated jar 0.5 hour before bloodmeal. Sterilization effects of PPF was assessed by providing individual females in cages with an oviposition cup for egg-laying 72 hours after bloodmeal. The number of eggs laid by individual females and number of larvae hatched per female were counted. Transfer of PPF to oviposition substrate was assessed by introducing late instar *An. gambiae* s.s. larvae into all oviposition substrate. Both the sterilizing effect and transfer of PPF was dependent on the time of exposure to PPF in reference to bloodmeal. Success to lay eggs was reduced by 85%-88% in females exposed to PPF between 24 hour before and after bloodmeal. Egg-production at this same time intervals was reduced by 15%-31% while larval hatching was overally reduced by 91%. Greater reductions in adult emergence of introduced larvae occurred with increasing time of exposure to PPF after bloodmeal. Sixty-five percent emergence inhibition occurred in oviposition substrate in which females exposed closer to oviposition laid eggs. This study indicates that PPF exhibits great potential for reducing the population of malaria vectors and reduce the huge labour costs for larviciding.

1244

EVIDENCE FOR POLYGENIC INSECTICIDE RESISTANCE IN THE MALARIA MOSQUITO, *ANOPHELES COLUZZII*

Bradley J. Main

University of California Davis, Davis, CA, United States

Starting in 2006, admixed individuals were identified between the sympatric malaria mosquitoes *Anopheles gambiae* and *A. coluzzii* coincident with a major insecticide treated bed net campaign in Mali. We performed whole genome sequencing to reveal all major genomic changes that occurred in *A. coluzzii* post-2006. We confirmed the introgression of *kdr-w* on chromosome 2L from *A. gambiae* into *A. coluzzii* and documented a previously unreported selective sweep on standing variation at the candidate insecticide resistance gene *CYP9K1* (cytochrome monooxygenase; P450) on the X chromosome. The selected *CYP9K1* allele (*cyp-l*) has two regulatory SNPs and appears to have higher copy number than pre-2006 *A. coluzzii*. Although selection acted independently on *kdr-w* and *CYP9K1*, *A. coluzzii* individuals with the combination of *cyp-l* and *kdr-w* alleles have increased in relative frequency in the population from 60 to 92%, suggesting an additive fitness advantage in the presence of concerted insecticide use. Thus, adaptation to increased insecticide exposure in the malaria mosquito involves the accumulation of multiple beneficial alleles from both within and between species.

1245

DEFINING VECTOR CONTROL STRATEGIES FOR CONTROLLING MALARIA TRANSMISSION USING NEW TYPES OF COMBINATION LLIN AND IRS IN AREAS OF PYRETHROID RESISTANCE

Mark Rowland, Natacha Protopopoff, Phillipa West, Immo Kleinschmidt

London School of Hygiene & Tropical Medicine, London, United Kingdom

The development of pyrethroid-resistant *Anopheles gambiae* across Africa is a serious threat to malaria control. To combat the threat and to reduce malaria transmission long-lasting insecticidal nets (LLIN) are being used together with indoor residual spraying in many endemic areas. New types of non-pyrethroid, wash-resistant LLINs are urgently needed. Several LLIN products incorporating either PBO synergist or combinations of insecticide are in final stages of development and undergoing laboratory and experimental hut trials. Before adopting the new products or incorporating them into national malaria control strategies it is necessary to test them at community level, ideally in cluster randomised trials to evaluate their impact on malaria transmission and mosquito populations and for their potential to select for pyrethroid resistance. In northwest Tanzania *An. gambiae* is highly resistant to pyrethroids and malaria transmission was intractable. To meet the problem pyrethroid LLINs were distributed to all households and IRS with bendiocarb was sprayed using a cluster randomised design. The encouraging results of the trial on malaria transmission and on resistance will be described. However, to further reduce malaria transmission it is necessary to use new generation nets and IRS formulations whose properties and effects on resistant mosquitoes in hut trials will be discussed. To meet the continuing challenge a LLIN incorporating PBO and long lasting IRS formulation based on pirimiphos methyl are being trialled in northwest Tanzania using a unique factorial design which assesses their effects separately and together on transmission rates and development of resistance. This will help define the different control strategies to adopt in areas of high transmission, in epidemics, and in areas of lower transmission with and without resistance.

1246

BEHAVIOR MODIFYING EFFECTS DURING AND AFTER EXPOSURE OF PYRETHROID-SUSCEPTIBLE AND RESISTANT *ANOPHELES GAMBIAE* TO SPATIAL REPELLENTS: POTENTIAL FOR MALARIA PARASITE TRANSMISSION CONTROL

Welbeck Achille Oumbouke

London School of Hygiene & Tropical Medicine/CREC, Cotonou, Benin

Tackling malaria vectors indoor with Insecticide Treated Nets (ITNs) and Indoor residual Spraying (IRS) is leading to behavior change of vectors and build up of residual outdoor malaria. Spatial repellents (SRs) could be the solution but they would not be approved for malaria control until appropriate design and measurable entomological endpoints are established to show their potential to control malaria transmission in diverse epidemiological settings including areas with high pyrethroid resistance. In Benin, we used Semi-Field Tunnel (SFT) initially developed by Dr. Moore and colleagues, to assess the repellence range (up to 65m) and toxicity that pyrethroid-based coils (transfluthrin and metofluthrin) used by man would induce against pyrethroid-susceptible and resistant *Anopheles gambiae* bearing the knock down resistance (kdr) gene. The ability of mosquitoes to take subsequent blood meal after surviving the SFT space containing vapors of the SRs was evaluated using a bioassay cage containing a shaved animal. Metofluthrin and transfluthrin coils induced high repellence of both pyrethroid-susceptible and resistant *An. gambiae* (>70%) at close range (5-10m) to the source but on the longer term, i.e. distance, metofluthrin protected the best, offering >40% protection at 65m compared to only 10% with transfluthrin. With either product, repellence rates of pyrethroid-susceptible *An. gambiae* were similar to that of the resistant strain and the trends at all distance range were not distinguishable. Pyrethroid-resistant individuals recovered from SR exposure and blood-fed on the animal sooner than their susceptible counterpart. Forty hrs after exposure, between 40-60% of susceptible *An. gambiae* were unable to bloodfeed compared to nearly 100% feeding success with resistant *An. gambiae*. Transfluthrin but not so for metofluthrin, delivered sublethal deposit, killing no greater than 25% of both pyrethroid- susceptible and resistant *An. gambiae* at all range. The data supports the hypothesis that Metofluthrin and transfluthrin coils have potential for malaria transmission control and suggest that they would do so by creating a vector-free space, even in areas with pyrethroid resistance. The need to develop SRs with different mode of action to complement pyrethroids on nets or IRS is becoming urgent, and should be put on a par with the seeking of novel insecticides or vaccines.

1247

MICROENCAPSULATED DEET AS AN INDOOR RESIDUAL SPRAY TREATMENT FOR CONTROL OF *ANOPHELES ARABIENSIS*

Jovin Kitau[1], Richard Oxborough[2], Johnson Matowo[1], Franklin Mosha[1], Stephen M. Magesa[3], Mark Rowland[2]

[1]Kilimanjaro Christian Medical University College, Moshi, Kilimanjaro, United Republic of Tanzania, [2]Department of Disease Control, London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]RTI International, Global Health Division, Nairobi, Kenya

As scale up of LLIN continues, a shift in the sibling species composition of the *Anopheles gambiae* complex is being reported over increasingly large areas, with *An. arabiensis* predominating, and *An. arabiensis* is likely to become an increasingly important vector in sustaining transmission of residual malaria. Because of increasing resistance, it is important that alternative chemical classes are evaluated to complement the existing insecticides and that these are tested against *An. arabiensis*. Microencapsulated DEET CS formulation has been evaluated alongside other standard residual vector control chemicals - lambdacyhalothrin CS, permethrin EC, pirimiphos methyl CS and DDT WP in an experimental hut trial. The chemicals were sprayed on plywood panels attached to experimental hut walls to assess the efficacy against pyrethroid resistant, wild free-flying *Anopheles arabiensis*, in terms of chemical-induced mortality, blood-feeding inhibition and chemical-induced exit from huts while rotating the treatments between huts. A sub-sample of fed mosquitoes was analyzed by ELISA to determine their blood meal sources. The overall mortality of *An. arabiensis* collected in huts with all treatments (76-86%) was significantly greater than the mortality in unsprayed control huts (8%, P<0.001). Mortality in DEET sprayed huts (82%) was significantly higher than in lambdacyhothrin sprayed huts (76%, P=0.043) and similar to pirimiphos methyl sprayed huts (86%, P=0.204). Blood feeding was higher in unsprayed control hut (34%) than other sprayed huts (19-22%, P<0.002). DEET (44%) provided equivalent mosquito blood feeding inhibition to DDT and lambacyhalothin. Exiting rates were higher from DEET (98%), lambda-cyhalothrin (98%) and permethrin (96%) than from the unsprayed control huts (80%, P<0.01). This trial has demonstrated the potential of microencapsulated DEET to provide substantial protection as an IRS treatment against *An. arabiensis*.

1248

ADAPTATIONS OF *ANOPHELES GAMBIAE* TO BREEDING IN POLLUTED WATER: CHALLENGES TO URBAN MALARIA CONTROL

Dziedzom K. de Souza, Jewelna Osei-Poku, Samuel Dadzie, Michael D. Wilson, Takashi Suzuki, Daniel A. Boakye

Noguchi Memorial Institute for Medical Research, Legon-Accra, Ghana

Urban malaria is a major emerging health problem in sub-Saharan Africa. It is estimated that 800 million people will live in African cities by 2025. Thus, urbanization has a great impact on the composition of the vector system and malaria transmission dynamics. The main vectors of malaria in sub-Saharan Africa, the *Anopheles gambiae*, normally breed in clean water

sources. However, there is growing evidence suggesting the adaptation of Anopheline species to polluted breeding habitats in urban settings. In Ghana, *An. gambiae* has been found breeding in much polluted water bodies leading to an increase in urban malaria cases. This adaptation may pose challenges to the already underfunded malaria control programs. Thus, this study aims at understanding the molecular and genetic basis of this adaptation and evaluating the differences and expression of genes involved in insecticide detoxification in *An. gambiae* s.s. Three Cytochrome P450 genes (CYP_6P3, CYP_4H19 and CYP_4H24), one Glutathione S-transferase gene (GSTD_1-4) and one ABC Transporter gene (ABCC_11), were analysed to determine their expression levels in the larval and adult populations in 5 selected breeding sites, in urban Accra, Ghana. The results revealed that generally the fold expression of these genes was higher and significant in the larvae compared to the adults. The fold expressions, however, varied between sites. With the exception of GSTD 1-4, the expression of the other genes was significantly higher in the most polluted site compared to the other sites. Also, there was significant correlation between ABCC_11, GSTD 1-4, CYP_4H24 and most water quality parameters of the study sites. Analysis of enzyme activity of α-esterase, monooxygenases, glutathione S-transferase and acetylcholinesterase revealed higher and significantly different enzyme activity in larval and adult populations. These results suggest that detoxification enzymes could be involved in adaptation to polluted breeding sites. While the increased enzyme activities observed could be due to functional plasticity, it has been hypothesized that such an adaptive plasticity might continuously evolve to maximize the adaptation of mosquito larvae to breeding sites that are chemically changing. The results may also suggest that perhaps some other mechanisms are involved, which require further studies.

1249

IMPROVING SOCIOECONOMIC EQUITY IN INSECTICIDE-TREATED BEDNETS (ITNS) ACCESS, OWNERSHIP AND USE IN RWANDA FROM 2000-2010

Lia S. Florey[1], Cameron Taylor[1], Rebecca Winter[1], Ana Claudia Franca-Koh[1], Ana Claudia Franca-Koh[2], Samantha Herrera[2], Jui Shah[2], Yazoume Ye[2]

[1]ICF International - The DHS Program, Rockville, MD, United States, [2]ICF International - MEASURE Evaluation, Rockville, MD, United States

Enabled by significant increases in funding for malaria control, remarkable scale-up of malaria control interventions has occurred over the past decade in sub-Saharan Africa. Despite high national coverage with interventions such as insecticide-treated bednets (ITNs), distribution is not always equitable across the population, with coverage varying by households' socioeconomic status. In Rwanda, ownership of ITNs increased substantially from 15% to 82% between 2005 and 2010. Similarly, use of ITNs by children less than five years of age and by pregnant women rose from 4% to 70% and from 4% to 72%, respectively, from 2000 to 2010. The percentage of households owning at least one ITN for every two household members (access) increased from 3% to 39% from 2000 to 2010. To assess the equity of ITN access, ownership and use in Rwanda from 2000 to 2010, data on household wealth and ITNs from Demographic and Health Surveys in 2000, 2005 and 2010 were used to compute Lorenz Concentration Curves and Indices. Concentration Index (C-Index) values range between -1 and 1 with a value of 0 representing perfect equality. Results show drastic improvements in equity of ITN ownership over time (C-Index: 0.35 in 2005 compared to 0.07 in 2010), household ITN access (C-Index: 0.42, 0.12, 0.06 in 2000, 2005 and 2010, respectively), ITN use in children less than five years (C-Index: 0.66, 0.33 and 0.05 in 2000, 2005 and 2010, respectively) and ITN use in pregnant women (C-Index: 0.70, 0.25 and 0.03 in 2000, 2005 and 2010, respectively). Results suggest that ITN distribution programs in Rwanda have achieved increasing equity over time such that by 2010, levels of ITN access, ownership and use were similar across households of all wealth quintiles. This may be due, in part, to the shift in distribution from target populations to mass campaigns.

1250

STRUCTURED SUPERVISION VISITS USING TABLETS FOR IMPROVEMENT OF MALARIA CASE MANAGEMENT IN SENEGAL

Julie I. Thwing[1], William Olander[2], Medoune Ndiop[3], Moustapha Cisse[3], Mame Birame Diouf[4], Alioune B. Gueye[3], Ibrahima Diallo[3], Mady Ba[3]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Independent Consultant, Dakar, Senegal, [3]Senegal National Malaria Control Program, Dakar, Senegal, [4]United States Agency for International Development, Dakar, Senegal

Improving the quality of malaria case management in public health facilities in Senegal is an ongoing challenge, with 32 hospitals, 89 health centers, and 1247 health posts. The National Malaria Control Program (NMCP) instituted health facility supervision visits to improve malaria services, supervising over 800 structures annually. During each visit, a lengthy paper form is completed, which includes rapid diagnostic test technique, consultation observation, register abstraction of case management of uncomplicated and severe malaria, and stock management. Supervisors are chosen from a cadre of health officers who have attended training in malariology conducted by the NMCP. Health personnel and district health teams receive feedback based on a synthesis of the results. However, are not entered electronically and capacity is limited to perform detailed analysis of the data collected. The NMCP piloted the use of Android tablet computers to facilitate improved data collection and analysis of supervision visits. The supervision form was programmed using Open Data Kit, which provided internal data checks, automatically calculated scores, and collected Global Positioning System (GPS) coordinates for each facility. After completion of each round of supervision, data are downloaded at the NMCP and analyzed. The test phase included 4 hospitals, 4 epidemic surveillance sites, 4 health centers and 3 health posts. While none were out of stock of all dose-packs of artemisinin-based combination therapy (ACT), stockouts of the infant dose, the 1-5 year dose , the 6-13 year dose, and the adult dose affected 7%, 40%, 7%, and 70% of facilities, respectively. All facilities had rapid diagnostic tests (RDT) in stock. Data were missing regarding RDT performance in 10% of febrile patients, but an RDT was performed in 95% of the suspect cases for which data were available. Of patients with a positive RDT, 92% were documented to have received an ACT. Of severe cases, 93% of patient < 5 years and 69% of patients ≥5 years were judged to have been managed correctly. During 2014, tablets will be used to conduct supervision visits nationwide, reducing time required to compile reports, and enabling rapid feedback, in-depth analysis of case management, mapping of indicators, and increased capacity to identify and correct deficiencies. This provides a powerful tool for monitoring malaria case management and an evidence base for continuous quality improvement.

1251

INCREASING HUMAN RESOURCES FOR MALARIA PROGRAM IMPLEMENTATION IN LOW RESOURCE SETTING: THE IMPACT OF A MALARIOLOGY COURSE FOR PUBLIC HEALTH PROVIDERS IN SENEGAL

Alioune B. Gueye[1], Medoune Ndiop[1], Julie I. Thwing[2], Mame Birame Diouf[3]

[1]Senegal National Malaria Control Program, Dakar, Senegal, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]United States Agency for International Development, Dakar, Senegal

The Senegal National Malaria Control Program (NMCP) aggressively scaled up malaria control interventions during 2007-2010 and is now moving toward pre-elimination. However, the NMCP did not have the human resource capacity to implement and supervise the interventions, particularly correct case management, throughout the public health system of 32 hospitals, 76 district health centers, 1,247 health posts, 2,162

health huts, and 1992 home-based care providers. In order to address this challenge, the NMCP developed an annual malariology course to train public health personnel, with a focus on district health management teams. Each year, senior and middle management health personnel are invited to attend three and two week residential courses, respectively, in malariology, including planning, implementation, monitoring and evaluation. Since 2008, 50 senior and 65 middle managers have been trained, with technical support from WHO and the school of public health, at a cost of $153 per day for senior managers and $107 per day for middle managers. The NMCP recruits from this pool of trained managers for many activities, including training providers on guidelines for malaria prevention and case management, supervision of over 800 health facilities and their providers at all levels in biannual sessions of 21 days each, and periodic assessment of quality of case management. District health officials trained by the NMCP were crucial to the successful adoption of rapid diagnostic tests, enabling Senegal to test over 85% of suspected cases by the second year of implementation. During 2013, they supervised a total of 2,116 providers, seeing an average of 8 providers per working day. Currently, they are training personnel in the newly revised case management guidelines, including pre-referral treatment with rectal artesunate and treatment of severe disease with parenteral artesunate. The Senegal NMCP has trained a critical mass of district-level managers in malaria, who have facilitated the implementation, monitoring and supervision of malaria control activities in a context of a shortage of human resources, and whose contribution to the success of malaria control efforts in Senegal has been consistent and cannot be underestimated. This approach is recommended for other low resource malaria endemic countries struggling with lack of qualified personnel to implement malaria control efforts.

1252

FACILITATING HARMS DATA CAPTURE BY NON-CLINICIANS UTILIZING A NOVEL DATA COLLECTION TOOL DEVELOPED BY THE ACT CONSORTIUM - RESULTS OF TESTING IN THE FIELD

Cheryl Pace[1], Sham Lal[2], Victoria Ewing[3], Anthony K. Mbonye[4], Richard Ndyomugyenyi[4], David G. Lalloo[1], Dianne J. Terlouw[3]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Malawi-Liverpool-Wellcome Trust Clinical Programme, Blantyre, Malawi, [4]Ministry of Health, Kampala, Uganda

In recent years, rapid diagnostic tests and enhanced delivery strategies have improved access to efficacious antimalarials by those who need them. As case management improves and preventative measures such as Mass Drug Administration (MDA) are considered in order to reduce both disease burden and transmission, the potential acceptable risk-benefit ratio to end users has shifted; monitoring the safety of these drugs, however, has been slow to rise on the public health agenda. Whilst novel strategies for improving access to antimalarials and disease burden surveillance are being employed, we still rely on the traditional weak, inefficient and in many places virtually non-existent pharmacovigilance system of clinician-led reporting within the context of the conventional healthcare setting. As antimalarials are increasingly being provided via non-conventional routes and by lower-level healthcare workers, the importance of equipping these workers with the appropriate tools to monitor and report on possible drug-related patient-experienced harms becomes paramount. Traditional pharmacovigilance data collection forms are complex and challenging to use by non-clinicians. The ACT Consortium developed data collection tools to allow lower-level healthcare workers to collect high quality harms data within a variety of contexts such as research studies and programmatic, real-life settings. These tools use a pictorial storyboard to convey the need for data collection to a low-literacy level population. A diary captures drug administration and event data in chronological relation to each other with minimal interpretation required by the data collector, thereby making it suitable for use by lower-level healthcare staff. These tools were used and tested by non-clinical data collectors within ACT Consortium projects and preliminary analysis of the results show that the harms data collected are comparable to those collected within the same and similar clinician-led studies.

1253

IMPACT OF SCHOOL-BASED PROGRAM OF MALARIA DIAGNOSIS AND TREATMENT ON SCHOOL ATTENDANCE IN SOUTHERN MALAWI

Katherine E. Halliday[1], Don Mathanga[2], Stefan Witek-McManus[1], Austin Mtali[3], Doreen Ali[4], John Sande[4], Reuben Mwenda[5], Charles Mazinga[6], Tiyese Chimuna[7], Natalie Roschnik[8], Simon Brooker[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Malaria Alert Centre, College of Medicine, Blantyre, Malawi, [3]Save the Children International, Zomba, Malawi, [4]National Malaria Control Program, Lilongwe, Malawi, [5]Health Technical Support Services-Diagnostics, Ministry of Health, Lilongwe, Malawi, [6]Ministry of Education, Science and Technology, Lilongwe, Malawi, [7]Save the Children International, Lilongwe, Malawi, [8]Save the Children International, Washington, DC, United States

Whilst real progress in the goal of education for all has been made in sub-Saharan Africa, evidence indicates children who suffer from ill health are less likely to attend and complete school. Malaria is an important cause of morbidity in school children and a significant contributor to school absenteeism. To address the burden of malaria in this age group, the Malawian National Malaria Control Programme, with support from Save the Children, are currently implementing a programme of school-based malaria case management in Southern Malawi. A cluster randomised trial in 58 schools in Zomba is evaluating the impact of this programme whereby malaria rapid diagnostic tests (mRDTs) and artemisinin-based combination therapies (ACTs) to diagnose and treat uncomplicated malaria have been placed in primary schools, as part of basic first aid kits [Learner Treatment Kits (LTKs)]. Head teachers and two additional teachers in the schools were trained in the use of mRDTs and the additional contents of the kits at a 7-day residential workshop. Twenty nine schools were randomly selected to receive the LTKs and a further twenty nine schools were selected to serve as the control. Baseline findings indicated 60.0% (95% CI: 56.2-63.7%) children in this region were infected with *Plasmodium falciparum*, while the prevalence of anaemia was 32% (95% CI: 29.2-35.5%). We present data from teachers' treatment registers describing uptake of the malaria diagnostic service by learners, including the number of malaria cases diagnosed by teachers. Treatment and referral practices will also be reported. Additionally, we present preliminary results on the impact of LTKs, comprising malaria diagnosis and treatment and basic first aid, on the principal outcome of school attendance. To our knowledge, this is the first instance in which school teachers have been trained to perform mRDTs and provide treatment on the basis of parasitological confirmation. Such a programme could provide a valuable complementary service to facility- and community-based roll out of mRDTs.

1254

THE ECONOMIC VALUE OF THE POST-TREATMENT PROPHYLACTIC EFFECT OF FIRST-LINE ANTIMALARIAL TREATMENTS ACROSS DIFFERENT MALARIA TRANSMISSION SETTINGS

Johannes Pfeil[1], **Yesim Tozan**[2]

[1]University of Heidelberg, Heidelberg, Germany, [2]New York University, New York, NY, United States

Recent trials show that dihydroartemisinin-piperaquine (DHAPQ) is as efficacious and safe as artemether-lumefantrine (AL) in treating uncomplicated childhood malaria and has a longer post-treatment prophylactic (PTP) effect compared to AL, reducing the risk of re-infection in children. In a recent cost-effectiveness analysis, we showed that DHAPQ was superior to AL from both the clinical and economic perspectives for treatment of uncomplicated childhood malaria in high transmission

settings (*in press at Plos One*). From a clinical and economic perspective, the benefits of post-treatment prophylaxis are, however, expected to become more significant with increasing transmission intensity and, conversely, less significant with decreasing transmission intensity. Our aim in this analysis is to assess the economic value of the PTP benefit conferred by DP compared to AL across different transmission settings. We base our analysis on primary clinical outcome data from a multi-centre clinical trial of ACTs that was conducted in Asian (n=998) and African children (n=1,698) in a wide range of malaria transmission settings (data provided by Sigma Tau I.F.R. SpA). Using the Markov model we developed for the previous analysis, simulating the progression of malarial disease and the risk of recurrent malaria, we estimate the mean incremental costs and health outcomes of the two treatment strategies per child over one year from the provider perspective in transmission settings stratified as low, moderate and high. We employ probabilistic sensitivity analysis to account for uncertainty in key model parameters. Our preliminary results show that the economic value of the PTP effect of DHAPQ over AL is significant in moderate to high transmission settings, using maximum manufacturer drug prices for ACTs set by the Global Fund. Our full analysis will report how the extent of this benefit varies by local malaria endemicity and antimalarial drug prices. Our findings should help inform the policy discussions on the choice of optimal malaria treatment strategy across endemic settings.

1255

THE 'PADDY PARADOX' REVISITED: HOW RICE FARMING IMPACTS ON HOUSEHOLD ECONOMIC STATUS AND MALARIA RISK IN EASTERN RWANDA

Alexis Rulisa[1], Fred Kaatera[1], Chantal Ingabire[1], Emmanuel Hakizimana[1], Michele Van Vugt[2], Leon Mutesa[3], Luuk Van Kempen[4]

[1]Rwanda Biomedical Center, Kigali, Rwanda, [2]Academic Medical Center, Amsterdam, Netherlands, [3]University of Rwanda, Huye, Rwanda, [4]Radboud University Nijmegen, Nijmegen, Netherlands

Economic activities may entail negative externalities for public health, which is particularly problematic in poverty-stricken areas. The case of rice farming in eastern Rwanda fits this description, as it provides breeding sites for malaria-infested mosquitoes but at the same time generates cash income and improves nutritional standards locally. We add to the evidence base on the 'paddy paradox' by studying a case in Eastern Rwanda (Ruhuha district of Bugasera province). The study unpacks the impact of rice cultivation on malaria incidence by comparing households that differ in their involvement in rice cultivation and proximity to the marshlands that host the rice fields. To this purpose, a large-scale survey was conducted among more than 4,000 households (comprising 17,000 individuals) in the area from June to December 2013. Data on household demographics, economic status, malaria prevention efforts as well as health-seeking behavior has been collected. All household members have also been screened for malaria parasitemia and anemia, and a malnutrition assessment was carried out for under-five children. In addition, qualitative data was collected through nine focus group discussions. It is shown that rice farming is positively and significantly associated with households' wealth, food security, health insurance status, and protection against malaria. At the same time, it is confirmed that rice farming practices increase the risk of malaria transmission through expanded mosquito populations. Rice fields are the main breeding site in the area. Households located nearby the marshlands where rice is cultivated are the most affected by malaria. For those households who generate income from rice production directly, the income effect dominates, resulting in a lower disease burden from malaria. By contrast, households in communities that are located close to the rice cultivation areas but who do not participate in this economic activity, face a higher malaria burden. Rice farming leads to private benefits in the economic domain, which spills over into the health domain, but at the same time creates a public health risk. As a result, the 'paddy paradox' hypothesis is confirmed at the level of rice-producing households, but rejected at the wider community level. Hence, strategies need to be developed that are able to tap the private benefits of rice cultivation and re-direct these to fund collective action against malaria.

1256

SERUM 8,12-ISO-IPF2α-VI ISOPROSTANE MARKER OF OXIDATIVE DAMAGE AND COGNITION DEFICITS ASSOCIATED WITH CASSAVA CYANOGENIC POISONING

Desire Tshala-Katumbay[1], K. Kambale[2], L. D. Okitundu[2], B. M. Bumoko[2], Michael J. Boivin[3], D. N. Mumba[2], J. J. Tamfum-Muyembe[2], J. P. Banea[2]

[1]Oregon Health and Sciences University, Portland, OR, United States, [2]University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [3]Michigan State University, East Lansing, MI, United States

We sought to determine whether motor (konzo) and cognitive deficits associated with cassava (food) cyanogenic poisoning were associated with high levels of F2-isoprostanes, well-established indicators of oxidative damage. Levels of serum F2-isoprostanes were quantified by LC-MS/MS and anchored to measures of motor proficiency and cognitive performance assessed through BOT-2 or KABC-II neuropsychological testing of 40 Congolese children [21 with konzo and 19 presumably healthy controls, overall mean age (SD): 9.3 (3.2) years]. Cyanogenic exposure was ascertained by levels thiocyanate (SCN) in plasma and urinary. Overall, levels of plasma SCN ranged from 91 to 325 µmol/l or 172 to 1032 µmol/l in plasma or urine, respectively. Levels of isoprostanes (ng/ml) ranged from 01 to 0.8 (Isoprostane-III), 0.8 to 8.3 (total Isoprostane-III), 0.1 to 1.5 (Isoprostane-VI), 2.0 to 9.0 (total Isoprostane-VI), or 0.2 to 1.3 ng/ml (8,12-iso-iPF2α-VI isoprostane). Children with konzo poorly performed both at the BOT-2 and KABC-II testing ($p<0.01$) Within a regression model controlling for age, gender, and other biochemical variables, 8,12-iso-iPF2α-VI isoprostane (ng/ml) was significantly related to overall cognitive performance (Mental Processing Index) on the KABC-II ($\beta = -32.36$ (-51.59 to -13.03 95% CI; $P<0.001$). A regression model including age, gender, motor proficiency impairment, serum albumin and triclyceride levels, and 8,12-iso-iPF2α-VI isoprostane in 20 konzo children explained over 85% of variation in the overall Mental Processing Index, but was not significant in explaining the overall motor proficiency impairment . These findings suggest that brain/behavior injury associated with cassava poisoining is mediated in part by oxidative stress injury. We conclude that 8,12-iso-iPF2α-VI isoprostane is a sensitive biomarker of the neuropathogenic mechanisms mediating brain injury in konzo, and can be used to monitor the impact of interventional trials to prevent or mitigate the neurotoxicity effects of cassava cyanogenic poisoning.

1257

LEAD AND IRON INTERACT TO INCREASE *PLASMODIUM FALCIPARUM* PARASITEMIA IN BENINESE INFANTS

Violeta Moya-Alvarez[1], Michael Osei Mireku[1], Smaila Ouedraogo[2], Manfred Accrombessi[2], Michel Cot[3], Florence Bodeau-Livinec[4]

[1]Institut de Recherche pour le Développement, EHESP, Paris/Benin, France, [2]Institut de Recherche pour le Développement, Benin, France, [3]Institut de Recherche pour le Développement, Paris/Benin, France, [4]Ecole des Hautes Etudes en Santé Publique, Rennes, France

Lead poisoning is a major public health challenge in West Africa. It hinders the correct neurocognitive development of infants and it entails impaired growth and learning disorders as well as kidney damage and anaemia. In Benin over 80% of the children are anemic. Malaria, another major cause of anemia, is the first cause of infant morbidity and mortality. However, the interaction of lead poisoning, anaemia and malaria has not been investigated so far. By analyzing the effect of lead levels on *P. falciparum* parasitemia we aim at unravelling the impact of lead poisoning on malaria episodes among Beninese infants. We have followed 630 infants up to 12 months and we have assessed their health status with regard to malaria,

other parasites, as well as their haematological profile. In addition their blood lead levels have been analyzed at 12 months of age showing a high prevalence of lead poisoning (17% for blood lead level (BLL)≥10 µg/dL). Multivariate models show significant increased *Plasmodium falciparum* parasitemia associated with increased BLL irrespective of iron status. In addition there is a positive significant association of total body iron with *P. falciparum* parasitemia controlling for clinical, demographic and environmental malaria risk factors. The interaction of BLL and total body iron is also significantly associated with *P. falciparum* parasitemia. In conclusion the significant association impact of lead levels on *P. falciparum* parasitemia and the synergistic interaction of iron and lead on *P. falciparum* parasitemia bring up important research and public health questions in a region where malaria and lead poisoning overlap. To our knowledge this effect had not been shown so far and complementary cohort studies are required to confirm its significance. In any case, the synergistic interaction between lead and iron rises up the importance of analyzing further the convenience of iron supplementation for anaemia treatment and prophylaxis, especially in malaria endemic regions with high exposure to lead. The considerable prevalence of lead poisoning addresses the necessity of investigating the sources of lead pollution and analyzing its impact on the neuro-cognitive development of Beninese infants, as well as its impact on anaemia.

1258

QUALITATIVE AND QUANTITATIVE EVALUATION OF IMPROVED STOVE ACCEPTABILITY AND MULTIPLE STOVE USE IN RURAL WESTERN KENYA

Jennifer D. Loo[1], Tamara Pilishvili[1], Bryan Christensen[1], Ibrahim Sadumah[2], Ronald Nyagol[2], Mike Sage[3], Fuyuen Yip[1], Stephanie Schrag[1], Nigel Bruce[4], Debbi Stanistreet[4]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Safe Water and AIDs Project, Kisumu, Kenya, [3]Global Alliance for Clean Cookstoves, Washington, DC, United States, [4]University of Liverpool, Liverpool, United Kingdom

The majority of households in rural Western Kenya cook indoors using open biomass fires, increasing exposure to household air pollution (HAP), which has the potential to negatively impact health. Our study aimed to evaluate the impact of improved cookstove technologies (ICT), compared to the traditional 3-stone open fire, on effectiveness in reducing HAP and acceptability of use in the community. Preliminary findings showed that many households were using additional stoves, in conjunction with the ICT evaluated during the study. This analysis explores factors associated with multiple stove use among study households. We employed mixed methods in a cross-over study design to evaluate 6 different ICT (2 rocket, 1 rocket with chimney, 3 fan-assisted) in households. One ICT was placed in each household for a 2 week period and was intended to be the sole stove used for daily household tasks. Stoves were rotated until each house had used at least 5 different ICT. Households were monitored for 48-hour periods at the end of each 2 week round and quantitative questionnaires, a cooking activity log, qualitative interviews and focus groups were completed. Multiple stove use was defined as any use of additional stoves other than the ICT under evaluation during the 48-hour monitoring period. Of 43 households, 67% (n=29) indicated use of multiple stoves during the study [8 households (19%) at least 25% of the time, 11 (26%) half of the time, 7 (16%) 75% of the time and 3 (7%) all of the study]; 14 (33%) households reported single stove use. Multiple stove use occurred most often (17/38 households, 45%) when the chimney stove was present and least often (9/35, 26%) when rocket stove B was present. Qualitative findings indicate that stove type, ease of stove use, number of people cooked for and type of meal cooked are likely factors associated with multiple stove use. These findings demonstrate the difficulties of conducting field evaluations for ICT and highlight key factors to consider in developing ICT that are acceptable in meeting the daily needs of users.

1259

TRACHOMA CONTROL THROUGH IMPROVED ACCESS TO WATER AND SANITATION

Mariamo Mbofana[1], Sharone Backers[2], **Philip Downs**[3], Brian Chu[4], Rebecca Mann Flueckiger[5], Rebecca Willis[4], Alex Pavluck[4], Anthony Solomon[6]

[1]Ministry of Health Mozambique, Maputo, Mozambique, [2]RTI International, Maputo, Mozambique, [3]RTI International, Washington, DC, United States, [4]Task Force for Global Health, Atlanta, GA, United States, [5]International Trachoma Initiative, Atlanta, GA, United States, [6]World Health Organization, Department of Control of Neglected Tropical Diseases, Geneva, Switzerland

Facial cleanliness is a key activity to prevent blinding trachoma; a disease which has impaired the vision of more than 2 million people worldwide. The SAFE strategy (Surgery, Antibiotic, Facial cleanliness, Environmental improvement) for trachoma elimination is substantially weakened without access to water and sanitation facilities. According to leading water, sanitation, and hygiene (WASH) specialists, 12.7 million people in Mozambique (over half the population) don't have access to safe water or proper sanitation facilities. The relationship between water, sanitation, and trachoma is complex; those who have easy access to water and latrines may or may not have less active trachoma. To determine whether access to water and latrines at the household level is associated with greater access to functioning hand-washing facilities and soap, the national trachoma control program in Mozambique reviewed results of recent trachoma baseline prevalence mapping, which includes data on basic WASH indicators. Households with water close to the home (water source in yard) were more likely to have access to a hand-washing facility (P<.001). Households with access to a private latrine were more likely to have access to a hand-washing facility, P<.001, RR=1.20 (1.19-1.22), have water available at the hand-washing facility, P<.001, RR=1.15 (1.14-1.16), and soap available at the hand washing facility, P<.001, RR=1.06 (1.05-1.06) compared to households with only access to a public latrine. In households that have access to safe water and latrines, but lack access to hand-washing facilities and soap, programs should identify the barriers to adopting hand and face washing behavior and modify their Behavior Change Communication (BCC) strategies as appropriate. Promotion of private latrine construction may complement BCC strategies and should be targeted in areas with high prevalence of trachoma.

1260

HYDROLOGICAL DRIVERS OF TYPHOID TRANSMISSION IN KIBERA, AN URBAN SLUM IN NAIROBI, KENYA

Adam N. Akullian[1], Eric Ng'eno[2], Alastair I. Matheson[1], Daniel Macharia[2], Leornard Cosmas[2], Judd L. Walson[1], Joel M. Montgomery[2]

[1]University of Washington, Seattle, WA, United States, [2]Centers for Disease Control and Prevention - Kenya, Nairobi, Kenya

Typhoid fever is a systemic enteric disease caused by *Salmonella* vars *typhi* and *paratyphi*. Typhoid fever occurs primarily in densely populated urban areas with poor sanitation infrastructure. The primary route of transmission is thought to be via direct contact with individuals who shed the bacteria in their stool. However, recent evidence from Asia suggests that indirect transmission via contaminated surface water may also play a role in the spread of disease. Using data from a large population-based infectious disease surveillance system operated by the Kenya Medical Research Institute/US Centers for Disease Control (KEMRI/CDC), we mapped the spatial pattern of typhoid fever risk in Kibera, an urban slum in Nairobi Kenya, with an extremely high population density (70,000 individuals/sq km). Cases were defined as individuals with fever and positive blood culture for *S. typhi*. Controls were selected randomly from the population-based cohort to estimate the spatial distribution of the underlying population at risk, and were matched to cases on age, gender, and date of diagnosis. We used a spatial modeling framework to map the

geographic distribution of typhoid fever cases and to test whether any significant spatial patterns could be explained by variations in topography and surface-water-accumulation. The greatest risk of typhoid fever was among those living in the lowest-elevation areas where surface-water flow accumulates (p = 0.01). Our results support indirect environmental transmission of typhoid fever in resource-limited settings. Interventions targeted at reducing typhoid fever transmission (e.g., improvements in sanitation and hygiene, and typhoid vaccination) in upstream areas of typhoid-endemic regions may indirectly benefit residents in downstream areas, who are at increased risk of exposure to *S. typhi* from both immediate and upstream sources of contamination.

1261

CERAMIC WATER FILTERS AND REDUCING THE BURDEN OF DIARRHEAL DISEASE IN INFANTS - WESTERN KENYA, 2013

Jamae Morris[1], Chandra Schneeberger[1], Peter Jaron[2], Fenny Moke[2], Jane Juma[2], John Ochieng[2], Richard Omore[2], Tracy Ayers[1], Jennifer Murphy[1], Jeffery Priest[1], Lihua Xiao[1], Barry Fields[3], Joel Montgomery[3], John Vulule[4], Eric Mintz[1], Vincent Hill[1], Ciara O'Reilly[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Kenya Medical Research Institute/Centers for Disease Control and Prevention, Kisumu, Kenya, [3]Centers for Disease Control and Prevention-Kenya, Nairobi, Kenya, [4]Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya

Unsafe drinking water consumption is a risk factor for diarrhea, a leading cause of death in sub-Saharan African children. Ceramic water filters (CWFs) remove or inactivate waterborne diarrheal pathogens in drinking water through size exclusion and silver exposure. We examined the effectiveness of CWFs to improve drinking water quality and prevent infantile diarrhea in rural western Kenya. A randomized**,** controlled intervention trial was conducted among 240 households with infants 4-10 months old. Each household was randomized into an intervention or control group with or without CWFs, respectively. Trained interviewers performed a baseline survey and visited households weekly for 26 rounds to document recent onset of diarrhea, respiratory infection, and febrile illness in infants. Source and filtered water samples were tested to monitor *Escherichia coli* concentrations, measured as most probable number (MPN). Person-time incidence rates were calculated per 100 person-weeks of observation. Households reported using surface water (36.3%), public taps (29.2%), or rainwater (17.1%) as their primary drinking water sources. Self-reported filter use among intervention households was 99.6% across weeks observed. Compared with the control group, intervention households reported fewer diarrheal episodes (7.6 vs. 8.9, p=0.1) and fewer health facility visits for diarrhea (1.2 vs. 2.2, p<0.01). The incidence of respiratory infection (1.3 vs. 1.1, p=0.61) and febrile illness (4.1 vs. 4.1, p=0.9) remained similar. *E. coli* were detected in 93% of source water samples (median concentration 512 MPN/100mL; range 10 - $1.4x10^4$ MPN/100mL) and in only 29% of filtered water samples (median concentration 7.4 MPN/100mL; range <1.0 - >2420 MPN/100mL). Households using CWFs had improved water quality and reported lower incidence and significantly fewer health facility visits for diarrhea in infants.

1262

WATER ACCESS, QUALITY AND USE IN TEN HEALTHCARE FACILITIES IN HONDURAS AND GHANA

Katharine Robb, Marisa Gallegos, Ledor Igboh, Samantha Lie-Tjauw, Christine Moe

Emory University, Atlanta, GA, United States

In the developing world, it is estimated that 46% of health facilities have access to an improved water source. The 2015 Sustainable Development Goals include a provision to "provide universal access to safe drinking water in health centers." In order to meet this goal, the majority of health facilities in the developing world will need to gain access to an improved water source. However, once access to an improved source is obtained, there remain significant barriers to ensure sustainable water access, quality, and use. From 2012-2014 the Center for Global Safe Water at Emory conducted an assessment of water access, quality and use in 10 district-level hospitals in Honduras and Ghana. Water quality testing, observations and interviews were conducted at each site. All hospitals evaluated had access to improved water sources and on-site treatment systems. Despite this, barriers such as intermittent water and power supplies, wards without piped connections, broken taps, and limited water access points for patients and visitors reduced water access in the hospital. Hospitals increased water access through the use of cisterns and bucket taps. Hospitals spent significant funds to increase water access by purchasing water from tanker trucks and buying bottled water. Methods to improve access often resulted in decreased water quality and increased costs. In over 300 samples tested for E. coli, 77% of samples in Honduras and 61% in Ghana met international drinking water standards. Samples from piped taps were 4 times more likely to meet drinking water standards compared to samples from bucket taps (p=0.0256). Despite variable quality, tap water was used for a variety of drinking, hygiene and medical purposes. In Honduras, 24% of staff reported using tap water for drinking versus 5% of staff in Ghana. Tap water is used for reconstituting and giving medications by 23% of clinical staff in Honduras and 14% in Ghana. While 19% of staff in Ghana use tap water for wound care, no staff in Honduras reported using tap water for wound care. A common barrier to the use of safe water is lack of knowledge about the quality of water from various sources within the hospital. In conclusion, despite improved water sources at healthcare facilities, there exist persistent challenges to consistent safe water access and use. Attaining universal and sustained safe water access and use will require assessment of barriers and the development of mitigation strategies.

1263

EOSINOPHILS DRIVE CELLULAR INNATE IMMUNITY ACTIVATION TO CONTROL HELMINTH LARVAE MIGRATION AND PROMOTE LUNG TISSUE REMODELING BY A TNF-DEPENDENT PATHWAY DURING *ASCARIS* INFECTION

Pedro H. Gazzinelli-Guimaraes[1], Ana Clara Gazzinelli-Guimaraes[1], Vitor L. Mati[1], Fernando S. Barbosa[1], Emilia Araujo[1], Paula Vieira[2], Deborah Negrao-Correa[1], Claudia M. Carneiro[2], Peter Hotez[3], Daniella C. Bartholomeu[1], Lilian Bueno[1], Ricardo T. Fujiwara[1]

[1]Federal University of Minas Gerais, Belo Horizonte, Brazil, [2]Federal University of Ouro Preto, Ouro Preto, Brazil, [3]Sabin Vaccine Institute and Texas Children's Hospital Center for Vaccine Development, National School of Tropical Medicine, Baylor College of Medicine, Houston, TX, United States

While eosinophils have been associated with resistance to helminthic infections, the most important findings related to these cells are based on the peripheral eosinophilia observed in nematode-infected individuals and in the host intestinal mucosal response. In contrast, the role of eosinophils during hepatic-tracheal migrations of nematodes larvae (as frequently occurs in many human nematode infections) is not well understood. In this study eosinophils were evaluated during early *Ascaris* larval infections in wild-type (WT) BALB/c mice compared with an eosinophil-deficient mice model (ΔdblGATA). The absence of host eosinophils resulted in: 1) an increase in the number of *Ascaris* larvae migrating through the liver and lung; 2) a parallel reductions in the pulmonary inflammatory response, with reduced inflammatory infiltrated cells in the parenchymal lung tissue and bronchoalveolar lavage fluid; 3) a decrease in the levels of IL-6 and myeloperoxidase (MPO) produced by related-innate immunity cells during the peak of larvae migration; and 4) a decrease in the production of eosinophil-dependent TNF and eosinophil peroxidase (EPO) in the lungs, with impairments in pulmonary tissue remodeling. Taken together, this study suggest that eosinophils have key roles in both controlling the number of tissue-migrating *Ascaris* larvae and promoting associated airway inflammation through activation of host innate immunity pathways.

Simultaneously, eosinophils promote pulmonary tissue remodeling by EPO and TNF-dependent pathways during larval *Ascaris* sp. infections. These findings suggest an innovative hypothesis on the evolution of eosinophils in the mammalian host-parasite relationship.

1264

HUMANIZED NOD-SCID IL-2RΓNULL (NSG) MICE: RESPONSE TO INFECTION WITH *STRONGYLOIDES STERCORALIS*

Sandra Bonne-Annee[1], John Patton[2], Jessica Hess[2], Tim Manser[2], James B. Lok[3], Thomas J. Nolan[3], Raja Vuyyuru[2], David Abraham[2]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Thomas Jefferson University, Philadelphia, PA, United States, [3]University of Pennsylvania, Philadelphia, PA, United States

Strongyloides stercoralis is a parasitic nematode that infects humans, non-human primates and dogs. Immunocompetent mice will allow infections to persist for up to two weeks with little development beyond the larval stages. Both innate and adaptive immune responses control the infection in mice, with roles for complement component C3, eosinophils, neutrophils and macrophages in the protective innate immune response. Furthermore, CD4+ T cells, TH2 cytokines and the production of parasite-specific IgM and IgG are central to the adaptive immune response in mice. The goal of this study was to characterize *S. stercoralis* infection in the immunodeficient NOD-scid IL-2Rγnull (NSG) and humanized NSG (HIS) mice. Following exposure to the infective stage larvae of *S. stercoralis*, NSG mice supported larval and adult stages of the parasite. Naïve NSG mice had similar levels of C3 when compared to naïve C57BL/6J mice, which retained its ability to collaborate with C57BL/6J effector cells in killing the parasites *in vitro*. However, NSG mice demonstrated an absence of eosinophils and similar neutrophil numbers when compared to C57BL/6J mice. To determine if HIS mice were also susceptible to *S. stercoralis* infection, HIS mice were generated by engrafting NSG mice with human hematopoietic stem cells. HIS mice were susceptible to the infection, although these mice harbored 40% fewer adult parasites than NSG mice. Analysis of HIS mice following infection revealed the presence of human IgM and IgG demonstrating that HIS mice can establish a parasite-specific humoral response. We conclude from these studies that NSG mice are susceptible to the complete *S. stercoralis* life cycle. This may be explained by an absence of eosinophils or a deficit in the function of other effector cells. HIS mice had reduced parasite levels, which suggests that the human adaptive response is playing a role in the control of the parasite. These studies demonstrate that NSG and HIS mice are useful tools for dissecting the immune response of both mice and humans to *S. stercoralis*.

1265

EFFECTS OF MATERNAL GEOHELMINTH INFECTIONS ON THE DEVELOPMENT OF ATOPY, ECZEMA AND WHEEZE DURING THE FIRST THREE YEARS OF LIFE: FINDINGS FROM THE ECUAVIDA BIRTH COHORT

Philip J. Cooper[1], Martha E. Chico[2], Maritza Vaca[2], Leila Amorim[3], Carlos A. Sandoval[2], David P. Strachan[4], Laura C. Rodrigues[5], Mauricio L. Barreto[3]

[1]St. George's University of London, London, United Kingdom, [2]Laboratorio de Investigaciones FEPIS, Quininde, Ecuador, [3]Universidade Federal da Bahia, Salvador, Brazil, [4]St George's University of London, London, United Kingdom, [5]London School of Hygiene & Tropical Medicine, London, United Kingdom

To investigate the effects of maternal geohelminth infections on the development of atopy and allergic disease in early childhood, we analysed data from a birth cohort in a rural District of Esmeraldas Province. Ecuador. A total of 2,404 newborns were recruited in a public hospital serving the District of Quininde and were evaluated at 13, 24, and 36 months of age for eczema and wheeze. Skin prick test (SPT) reactivity to aero and food allergens was measured at 36 months. A single stool sample was collected from the mother in the third trimester of pregnancy and examined for the presence of geohelminth infections using a combination of microscopic methods including Kato-Katz and formol-ether concentration methods. Data was analyzed by multivariate logistic regression. We had complete follow-up data for 2,082 (86.6%) children through to 3 years of age. Geohelminth infections were detected in 46.1% of mothers and were predominantly infections with *Ascaris lumbricoides* (28.0% of mothers) and *Trichuris trichiura* (28.7%). The prevalence of outcomes in children by 3 years was: any episode of eczema (17.5%) and wheeze (26.0%), and SPT (17.2%). Maternal geohelminth infections were associated with an increased risk of eczema by 3 years of age (OR 1.28, 95% CI 1.02-1.61) but with a decreased prevalence of SPT (OR 0.82, 95% CI 0.61-1.00). No association was observed with wheeze (OR 1.01, 95% CI 0.82-1.25). Our data show that maternal geohelminths, in an Ecuadorian population where *Ascaris* and *Trichuris* are the predominant infections, are associated with an increased risk of eczema but with a reduced prevalence of SPT in early childhood.

1266

SYSTEMIC CYTOKINE PRODUCTION, GEOHELMINTH INFECTION AND NEURODEVELOPMENTAL OUTCOMES IN BANGLADESHI INFANTS

Nona M. Jiang[1], Fahmida Tofail[2], Shannon N. Moonah[1], Rebecca J. Scharf[1], Mami Taniuchi[1], Jennie Z. Ma[1], Priya Duggal[3], Genevieve L. Wojcik[3], Eric R. Houpt[1], Shihab U. Sobuz[2], Jena D. Hamadani[2], Eduardo Azziz-Baumgartner[4], Rashidul Haque[2], William A. Petri, Jr.[1]

[1]University of Virginia, Charlottesville, VA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States

An estimated one-third of children under 5 in low- and middle-income countries do not reach their full developmental potential. We recently published that systemic inflammation is linked to the neurodevelopment of children from a slum community in Dhaka, Bangladesh. An interesting finding from our study was that elevated levels of the Th2 cytokine IL-4 in 6-month sera were associated with higher cognitive scores. This finding raises the question of what was driving higher levels of IL-4 in children. Here we tested in the same cohort of Bangladeshi children for factors that could explain why some children had elevated levels of IL-4. Since environmental factors such as helminth infections drive a Th2 response in the host, we tested monthly surveillance stools for the first 6 months of life for the presence of intestinal helminths using multiplex PCR assays. We found that nearly 40% of children were infected with at least one intestinal helminth, with *Ascaris lumbricoides* and *Trichuris trichiura* being the most prevalent at 25% and 16%, respectively. *Ascaris lumbricoides* infection was associated with elevated levels of IL-4 in 6-month sera (p=0.02). Additionally, *Trichuris trichiura* infection was associated with higher cognitive, language, and motor scores on the Bayley Scales of Infant and Toddler Development III at 30 months of age (all p<0.05). We are validating our findings on systemic inflammation and neurodevelopment in a second cohort of children in Dhaka, and are testing for the impact of a SNP in the promoter region of IL-4 (C-589T) that has been shown to influence IL-4 production. The results from these additional studies will be presented. In conclusion, IL-4 and *Trichuris trichiura* infection were associated with better developmental test scores. In addition, elevated levels of IL-4 can partly be explained by helminth infection in this cohort of infants from a low-income setting. Elucidating the cause of elevated IL-4 would greatly enhance our ability to modulate levels of systemic IL-4, which may promote healthy cognitive development in at-risk children.

1267

THE GENOME AND TRANSCRIPTOME OF THE ZOONOTIC HOOKWORM *ANCYLOSTOMA CEYLANICUM* REVEAL INFECTION-SPECIFIC GENE FAMILIES

Erich M. Schwarz[1], Yan Hu[2], Igor Antoshechkin[3], Melanie M. Miller[4], Paul W. Sternberg[3], Raffi V. Aroian[2]

[1]Cornell University, Ithaca, NY, United States, [2]University of Massachusetts Medical School, Worcester, MA, United States, [3]California Institute of Technology, Pasadena, CA, United States, [4]University of California San Diego, La Jolla, CA, United States

Hookworms infect over 500 million people, stunting and impoverishing them. Sequencing hookworm genomes, and finding which genes they express during infection, should help devise new drugs or vaccines against hookworm. Unlike other hookworms, *Ancylostoma ceylanicum* infects both humans and other mammals, providing a laboratory model for hookworm disease. We determined an *A. ceylanicum* genome sequence of 313 Mb, with transcriptomic data throughout infection showing expression of 30,738 genes. ~900 genes were upregulated during early infection *in vivo*, including ASPRs, a cryptic subfamily of Activation-associated Secreted Proteins (ASPs). ASPR genes are also present in the related intestinal parasites *Necator americanus*, *Oesophagostomum dentatum*, and *Heligmosomoides bakeri*, but not the trichostrongylid parasite *Haemonchus contortus*. Genes downregulated during early infection include ion channels and G protein coupled receptors; this downregulation is observed in both parasitic and free-living nematodes. Another novel family of genes are upregulated as larvae develop to the L4 stage and migrate into the intestine; this family has homologs in *N. americanus*, *H. contortus*, and *Angiostrongylus cantonensis*, and its products are predicted to be nonclassically secreted. Still later in infection, as *A. ceylanicum* matures to young adulthood and begins drinking host blood, C-lectin genes are strongly upregulated, some of whose products resemble vertebrate more than nematode lectins. These findings provide new drug and vaccine targets, and should elucidate hookworm pathogenesis.

1268

DETECTION OF GASTROINTESTINAL PARASITES BY MULTI-PARALLEL QUANTITATIVE REAL-TIME PCR AND ASSOCIATIONS WITH GROWTH DELAY IN EARLY CHILDHOOD: FINDINGS FROM A BIRTH COHORT IN RURAL ECUADOR

Rojelio Mejia[1], Andrea Arévalo[2], Yosselin Vicuña[2], Pablo Espinosa[2], Martha E. Chico[2], Maritza Vaca[2], Carlos Sandoval[2], Peter J. Hotez[1], Philip J. Cooper[2]

[1]National School of Tropical Medicine, Houston, TX, United States, [2]Fundacion Ecuatoriana para la Investigacion en Salud, Quito, Ecuador

Gastrointestinal (GI) parasites may have important influences on growth and nutrition in childhood. Previous studies investigating the effects of parasite infections on growth have tended to use poorly sensitive microscopic-based assays. To investigate the effects of single and multiple parasite infections on growth in young children we analyzed data from a birth cohort study in Ecuador, correlating GI parasite affects on anthropometric measures. Stool samples from a random sample of 400 children in the cohort were collected at 13, 24, and 36 months of age and analyzed using our rapid, high throughput multi-parallel quantitative real-time PCR (qPCR) for the 8 most common gastrointestinal parasite pathogens including the helminths, *Ascaris lumbricoides, Ancylostoma duodenale, Necator americanus, Strongyloides stercoralis, Trichuris trichiura* and protozoa, *Cryptosporidium parvum, Entamoeba histolytica* and *Giardia lamblia.* Each child had anthropometric data collected at the same time points including height, weight, head and abdominal circumference. The qPCR detected increased prevalence of infections for *Ascaris* at 13, 24, and 36 months (6.8%, 12.9%, and 15.5%, respectively). Similar results were seen for *Giardia* (31.5%, 44.5%, and 51.6%, respectively) and other parasites. Furthermore, children that were infected at a previous time point tended to be infected at subsequent observation times with higher concentrations of parasite DNA for *Ascaris* and *Giardia* (fg/μL, $p < 0.05$) For all parasites, qPCR was more sensitive than standard microscopic methods. GI parasite infections were associated with growth delays for all anthropometric parameters by comparison with WHO growth curves; growth of abdominal circumference was less in the infected group (1.5 cm) compared to the non-infected group (4 cm)($p = 0.0054$). In conclusion, we have deployed a high throughput, rapid, quantitative molecular based system that has improved diagnostic accuracy compared to stool microscopy. Our data also indicate that GI parasite infections may affect growth during the first years of life.

1269

THE EFFECT OF DEWORMING TIMING AND FREQUENCY ON GROWTH IN EARLY PRESCHOOL-AGE CHILDREN: RESULTS OF A RANDOMIZED-CONTROLLED TRIAL OF MEBENDAZOLE IN ONE TO TWO-YEAR OLD CHILDREN IN THE PERUVIAN AMAZON

Serene A. Joseph[1], Martin Casapia[2], Theresa W. Gyorkos[1]

[1]McGill University, Montreal, QC, Canada, [2]Asociacion Civil Selva Amazonica, Iquitos, Peru

Children under two years of age are in the most critical window for growth and development. As mobility increases, this time also coincides with first exposure to soil-transmitted helminth (STH) infections in tropical environments. WHO recommends deworming as of 12 months in endemic areas; however, the optimal timing and frequency have been understudied in this age group. Many countries still exclude children 12-23 months in deworming programs. We conducted a randomized-controlled trial of deworming (500mg single-dose mebendazole) in 12 and 13 month-old children in Iquitos, an STH-endemic area of the Peruvian Amazon. A total of 1760 children were enrolled from September 2011 to June 2012 at 12 participating health centres. Children were randomly allocated to one of four groups: 1) deworming at 12 months of age and placebo at 18 months of age; 2) placebo at 12 months of age and deworming at 18 months of age; 3) deworming at 12 and 18 months of age; or 4) placebo at 12 and 18 months of age (i.e. control group). Participants were followed up to 24 months of age to assess the benefit of deworming on the main outcome of weight gain. Results were analyzed with an intention-to-treat approach. A total of 1563 children (88.8%) attended their 24 month visit. STH prevalence rose from 12.2% at 12 months to over 40% at 24 months. Mean weight gain (kg) between 12 and 24 months was: Group 1): 2.05 (±0.7); Group 2): 1.94 (±0.8); Group 3): 2.04 (±0.7); and Group 4): 2.00 (±0.7). There was a statistically significant improvement in weight gain in those receiving deworming once at 12 months, compared to those receiving deworming once at 18 months (p=0.028). No difference was detected between those receiving deworming once at 12 months vs. twice at 12 and 18 months (p=0.88). Results remained significant when adjusting for baseline characteristics. Additional analyses were performed to take into account clustering, multiple testing, missing data and compliance. Overall, our results indicate that deworming, provided once-yearly at 12 months of age, has important benefits on growth in early preschool-age children. These results contribute to the evidence-base on deworming policy in over 120 STH-endemic countries worldwide. Emphasis should be placed on translating results into practice, such that children in this vulnerable age group are targeted with the most cost-effective, integrated interventions to reduce health and nutritional burdens.

1270

RE-EMERGENCE OF DENGUE IN SOUTH TEXAS, 2013

Dana L. Thomas

Centers for Disease Control and Prevention, San Juan, PR, United States

Sporadic dengue outbreaks have occurred in south Texas for over 30 years. In 2013, during a dengue epidemic in northern Mexico, 17 suspected dengue cases were identified in two border counties in south Texas during July-October. To characterize the outbreak, Texas Department of State Health Services and CDC implemented enhanced surveillance by: 1) reviewing medical records at eight hospitals for dengue-like illness; 2) performing RT-PCR on serum specimens from suspected dengue cases previously tested by anti-dengue virus (DENV) IgM ELISA at commercial laboratories during October-December; and 3) conducting interviews with laboratory-positive dengue case-patients and offering household members dengue diagnostic testing by RT-PCR and IgM ELISA. During 2013, clinicians in south Texas requested dengue diagnostic testing for 246 patients. Of these, 54 (22%) were laboratory-positive: 32 (59%) by IgM ELISA, 15 (28%) by RT-PCR, and 7 (13%) by both. Of 84 specimens that were negative by IgM ELISA at commercial laboratories and further tested by RT-PCR, 15 (18%) were positive. Of 22 cases positive by RT-PCR, DENV-1 was detected in 19 (86%) and DENV-3 was detected in 3 (14%). Of all laboratory-positive dengue case-patients, 26 (48%) had not left Texas in the 14 days before illness onset, and 20 (38%) reported recent travel to Mexico. Of 22 dengue case-patients' households investigated, 5 (23%) had at least one additional household member with evidence of recent DENV infection without travel history. During a dengue outbreak associated with an epidemic in northern Mexico, enhanced surveillance in south Texas identified the largest number of locally acquired dengue cases ever detected. Dengue diagnostic testing should include both IgM ELISA and RT-PCR as evidenced by the high rate of false negatives with anti-DENV IgM testing alone, due to the number of patients who submit specimens during the acute phase of their infection. Since the burden of dengue is expected to continue in south Texas, dengue surveillance and laboratory capacity should continue to be improved.

1271

LARGE BURDEN OF DENGUE AND WEST NILE VIRUS TRANSMISSION IN COASTAL KENYA

A. Desiree LaBeaud[1], Tamara Banda[1], Crystal Teng[1], Chelsea Heimbaugh[1], Eric Muchiri[2], Peter Mungai[2], Francis M. Mutuku[3], Julie Brichard[1], Ginny Gildengorin[1], Erin M. Borland[4], Ann Powers[4], Uriel Kitron[5], Charles H. King[6]

[1]Children's Hospital Oakland Research Institute, Oakland, CA, United States, [2]Division of Vector Borne and Neglected Tropical Diseases, Msambweni, Kenya, [3]Technical University of Mombasa, Mombasa, Kenya, [4]Centers for Disease Control and Prevention, Division of Vector-Borne Diseases, Fort Collins, CO, United States, [5]Emory University, Atlanta, GA, United States, [6]Case Western Reserve University, Cleveland, OH, United States

Dengue virus (DENV) and West Nile virus (WNV) are endemic to most regions of the world, but accurate prevalence data are lacking in Sub-Saharan Africa. The objective of this study was to measure the burden of DENV and WNV exposure in coastal Kenya and link it to demographics and other risk factors. Demographic and exposure questionnaires were administered to 1,013 participants recruited from two coastal villages, Milalani (55% of total) and Nganja (45%), in 2009. Sera were screened for flavivirus exposure using a commercial DENV IgG ELISA and then confirmed with plaque reduction neutralization tests (PRNT). Chi square, Fisher exact test, t tests and logistic models were used to determine variables that were associated with seropositivity. 343 (35%; 95% CI 32-38%) participants were seropositive (aged 1-87 years, mean 37 years). Ten percent (95% CI 8-14%) of children were seropositive vs. 53% (95% CI 49%-58%) of adults. Of 297 PRNT confirmed positives, 203 samples (68% of positives, 20% of total) were DENV, 49 samples (16% of positives, 5% of total) were WNV, and 45 samples (15% of positives, 5% of total) had high PRNT titers for both DENV and WNV. Age was significantly associated with seropositivity (OR 1.07 per year, 95% C.I. 1.06-1.08). Males, adults who owned a radio or television, and those with schistosomiasis, malaria, or *Trichuris* were less likely to be seropositive ($p<0.05$). A greater proportion of DENV- and WNV-confirmed participants resided in Milalani, though the association with Village was not significant. Flavivirus exposure, particularly DENV, is very common in coastal Kenya, with more than half of adults exposed. Adults and females are more likely to be seropositive, whereas those with parasitic infections are less likely. Interepidemic transmission is suggested by many DENV and WNV seropositive children. The high flavivirus burden documented suggests that DENV and WNV are important causes of disease in coastal Kenya, but limited surveillance, clinical overlap with malaria and other viruses, and limited diagnostics contribute to under-reporting.

1272

VARIABILITY IN DENGUE TITER ESTIMATES FROM PLAQUE REDUCTION NEUTRALIZATION TESTS POSES A CHALLENGE TO EPIDEMIOLOGICAL STUDIES AND VACCINE DEVELOPMENT

Henrik Salje[1], Isabel Rodríguez-Barraquer[1], Kaitlin Rainwater-Lovett[1], Ananda Nisalak[2], Butsaya Thaisomboonsuk[2], Stephen J. Thomas[3], Stefan Fernandez[2], Richard G. Jarman[3], In-Kyu Yoon[2], Derek A. Cummings[1]

[1]Johns Hopkins School of Public Health, Baltimore, MD, United States, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]Walter Reed, Silver Spring, MD, United States

Accurate determination of neutralization antibody titers supports epidemiological studies of dengue virus transmission and vaccine trials. Neutralization titers measured using the plaque reduction neutralization test (PRNT) are believed to provide a key measure of immunity to dengue viruses, however, the assay's variability is poorly understood, making it difficult to interpret the significance of any assay reading. In addition, there is limited standardization of the PRNT cut-point or statistical model used to estimate titers across laboratories, with little understanding of the optimum approach. We used repeated assays on the same two pools of serum using five different viruses (2,319 assays) to characterize the variability in the technique under identical experimental conditions. We also assessed the performance of multiple statistical models to interpolate continuous values of neutralization titer from discrete measurements from serial dilutions and identified the optimal PRNT cut-point for the assay. We found that the variance in plaque reductions for individual dilutions was 0.016, equivalent to a 95% confidence interval of 0.45 - 0.95 for an observed plaque reduction of 0.7. We identified PRNT75 as the optimum cut-point with a variance of 0.025 (log10 scale), indicating a titer reading of 1:500 had 95% confidence intervals of 1:240 - 1:1000 (2.70±0.31 on a log10 scale). The choice of statistical model was not important for the calculation of relative titers, however, cloglog regression out-performed alternatives where absolute titers are of interest. Finally, we estimated that only 0.7% of assays would falsely detect a four-fold difference in titers between acute and convalescent sera where no true difference exists. Estimating and reporting assay uncertainty will aid the interpretation of individual titers. Laboratories should perform a small number of repeat assays to generate their own variability estimates. These could be used to calculate confidence intervals for all reported titers and allow benchmarking of assay performance.

1273

CROSS-REACTIVITY TO HETEROLOGOUS DENV TYPES INCREASES IN THE YEARS FOLLOWING PRIMARY INFECTION AND IS MAINTAINED FOLLOWING SECOND INFECTION IN A PEDIATRIC DENGUE COHORT STUDY IN NICARAGUA

Leah Katzelnick[1], Magelda Montoya[2], Lionel Gresh[3], Angel Balmaseda[4], Eva Harris[2]

[1]Centre for Pathogen Evolution, University of Cambridge, Cambridge, United Kingdom, [2]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States, [3]Sustainable Sciences Institute, Managua, Nicaragua, [4]Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua

The four dengue virus (DENV) serotypes infect an estimated 390 million individuals each year. Following a first DENV infection, the neutralizing antibody response is thought to become increasingly specific to the infecting serotype, and over time, is expected to remain only to the infecting serotype. After secondary infection, neutralization of all serotypes is thought to be relatively balanced and persist over time. However, these hypotheses have not been tested rigorously in a longitudinal cohort. We used reporter viral particles (RVPs) to measure changes in the type-specificity of neutralizing antibody responses to all four DENV serotypes in healthy annual samples from a cohort of Nicaraguan children for 1-6 years after their natural first, second, and, in some cases, third infections. Post-infection neutralizing titers were ranked from highest to lowest in the year after infection, and fold-difference in neutralization between the best-neutralized serotype and each heterologous serotype was measured every year until subsequent infection. As expected, the trajectories of neutralizing responses after infection varied by individual in magnitude and degree of cross-reactivity between serotypes. However, when first-infection neutralizing antibody responses were analyzed as a group, increasing cross-reactivity was observed over time. Specifically, the difference between the best and second-best neutralized serotypes decreased over time, with a mean decline of 1.3-fold/year ($p<0.01$), from an average difference of 8.5-fold in the first year after infection ($p<0.001$). This effect remained significant when serotype, year, and infection outcome were taken into account. Indeed, while the titer to the infecting type did not change significantly over time, the second-best neutralized serotype and third-best neutralized serotype increased by 1.3 fold/year ($p<0.01$) and 1.2 fold/year ($p<0.05$), respectively. When neutralizing antibody responses were analyzed in the same subjects following subsequent second infections, cross-reactivity between serotypes did not change significantly over time, although the magnitude of titers to all four serotypes decreased over time. We find that children living in Nicaragua become more cross-reactive to heterologous DENV serotypes over time following first infection and maintain cross-reactive responses following second infection.

1274

RELATIVE INCIDENCE OF ADULT AND PEDIATRIC DENGUE VIRUS INFECTION IN A PROSPECTIVE LONGITUDINAL COHORT IN THE PHILIPPINES

Maria T. Alera[1], Anon Srikiatkhachorn[2], Jens Levy[1], Stefan Fernandez[1], Catherine Lago[1], Chonticha Klungthong[1], Butsaya Thaisomboonsuk[1], Ananda Nisalak[1], In-Kyu Yoon[1]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]University of Massachusetts Medical School, Worcester, MA, United States

Dengue is the most common mosquito-borne viral infection globally. In Asia where dengue has been hyperendemic for many years, the average age of dengue has been increasing. However, dengue incidence and clinical spectrum have not been as well characterized in adults as in children. In order to determine the incidence of dengue virus (DENV) infection in adults compared to children and the relative proportion of subclinical versus symptomatic infections, a longitudinal prospective cohort of approximately 1000 subjects aged ≥6 months was initiated in Cebu, Philippines in March 2012 and underwent community-based active surveillance for febrile episodes. Acute and 3-week convalescent blood samples were obtained and tested by DENV RT-PCR/nested PCR (acute samples) and DENV IgM/IgG EIA (acute/convalescent samples). Enrollment and 12-month follow up samples were tested by DENV hemagglutination inhibition (HAI) to identify subclinical seroconversion. During one year of surveillance, the annual incidence of total and symptomatic DENV infection in the cohort was 8.5% and 1.5%, respectively. The total and symptomatic incidence in the 6 month-5 year old age group was 11.0% and 2.5%; 6-15 years was 15.3% and 4.4%; 16-30 years was 7.4% and 0.5%; 31-50 years was 4.2% and 0%; >50 years was 4.4% and 0%. DENV-1 was the predominant serotype. The total and symptomatic incidence among 139 subjects with negative DENV HAI at enrollment was 10.1% and 2.9%; among 32 subjects with one positive DENV HAI serotype was 31% and 9.4%; among 682 subjects with ≥2 positive HAI serotypes was 7.3% and 0.7%. Fifty-five percent of subjects ≤15 years old had multitypic HAI whereas 96% of those >15 years old were multitypic. Our results indicate that DENV infection is less frequent and less likely to be symptomatic in adults than children in a hyperendemic area, but much of this effect is due to preceding immune status. DENV infection in adults may become more symptomatic if force of infection decreases due, for example, to future pediatric vaccination programs.

1275

DENGUE VIRUS-SPECIFIC T CELL RESPONSES IN THE GENERAL POPULATION OF NICARAGUA VARY AS A FUNCTION OF THE INFECTING SEROTYPE AND ARE DOMINATED BY HLAB35-RESTRICTED EPITOPES

Daniela Weiskopf[1], Cristhiam Cerpas[2], Rukie de Alwis[1], Michael A. Angelo[1], Derek J. Bangs[1], Ravi V. Kolla[1], John Sidney[1], Bjoern Peters[1], Lionel Gresh[3], Angel Balmaseda[2], Eva Harris[4], Alessandro Sette[1]

[1]La Jolla Institute for Allergy and Immunology, La Jolla, CA, United States, [2]Laboratorio Nacional de Virología, Managua, Nicaragua, [3]Sustainable Sciences Institute, Managua, Nicaragua, [4]Division of Infectious Diseases and Vaccinology, Berkeley, CA, United States

Infections with any of the four dengue virus serotypes (DENV1-4) occur with high incidence in more than 100 countries around the world, accounting for as many as 390 million infections each year. All four DENV serotypes have circulated extensively in Nicaragua in recent years, and as a result, the adult population has generally been exposed to all four serotypes. To assess the T cell response against all four DENV serotypes, we tested predicted motifs in an *ex vivo* ELISPOT assay for their ability to induce an IFNγ response in HLA-matched peripheral blood mononuclear cells (PBMC) of 124 Nicaraguan Red Cross blood donors from the general adult population of Managua, Nicaragua. This proteome-wide screen identified a total of 314 CD8+ T cell epitopes across all 10 DENV proteins (C, M, E, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). Interestingly, we observed different immunodominance patterns of targeted DENV antigens depending on the serotype. DENV3-specific responses equally targeted structural and nonstructural (NS) proteins while DENV1-, DENV2- and DENV4-specific responding epitopes were found to disproportionately (>90%) target NS proteins, especially NS3, NS4B and NS5. We found 30% of the epitopes identified were novel, while 70% were also found in a previous study of Sri Lankan blood donors. Additionally, we observed a striking dominance of HLA B-restricted responses in general and of HLA B*35 in particular, both in terms of breadth as well as magnitude of the DENV-specific CD8+ T cell responses. Interestingly, this allele has been associated with protection from disease in a different population study in Malaysia. We found that the majority of responses were produced by T cells displaying an effector memory phenotype (TEMRA and TEM). In terms of cytokine expression patterns, the majority of cells were double-positive for IFNγ and TNFα, indicating a multifunctional phenotype. Our results provide new insights into HLA-restricted T cell responses against all four DENV serotypes, which are of relevance for both vaccine design and the identification of robust correlates of protection in natural immunity.

1276

EXPLORING THE CELLULAR METABOLOME AS A GATEWAY TO TARGET DENGUE VIRUS REPLICATION IN THE MOSQUITO VECTOR

Nunya Chotiwan[1], Irma Sanchez-Vargus[1], Jeffrey M. Grabowski[2], Amber Hopf-jannasch[3], Victoria Hedrick[3], Erik Gough[3], Ernesto Nakayasu[3], Devika Sirohi[4], Catherine A. Hill[5], Richard J. Kuhn[6], Rushika Perera[1]

[1]Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, United States, [2]Department of Biological Sciences, Department of Entomology, Purdue University, West Lafayette, IN, United States, [3]Bindley Bioscience Center, Purdue University, West Lafayette, IN, United States, [4]Department of Biological Sciences, Purdue University, West Lafayette, IN, United States, [5]Department of Entomology and Bindley Bioscience Center, Purdue University, West Lafayette, IN, United States, [6]Department of Biological Sciences and Bindley Bioscience Center, Purdue University, West Lafayette, IN, United States

The cellular metabolome plays a significant role in the life cycle of enveloped arthropod-borne viruses. For instance, in both the human host and mosquito vector, flaviviruses significantly modify lipid metabolism to enter and exit from cells, as well as to assemble membrane targeted replication factories for efficient viral RNA replication. Additionally, cellular metabolic changes assist in diverting or evading host antiviral defenses. Using technical advances in high-resolution mass spectrometry we have profiled the metabolome of dengue virus (DENV) infected mosquitoes and analyzed the metabolic changes that occur in the salivary glands and midgut tissues during the time course of infection. These studies were carried out in parallel to analysis of the human metabolome, also during infection with DENV. Our results indicated that DENV infection altered the expression of lipids that had the capacity to change the physical properties of the membrane bilayer such as curvature, permeability, and the recruitment and assembly of protein complexes in the membrane. Several of the identified molecules also functioned as bioactive messengers that controlled signaling and membrane trafficking pathways in the cells. Through these efforts we have generated a metabolomic fingerprint of DENV infection within the human host and its mosquito vector, *Aedes aegypti*. We are now exploring the mechanism of how DENV exploits these metabolic pathways for its replication and are evaluating these pathways as novel avenues for the development of antivirals that could target virus replication in both the human and vector hosts. Through these efforts we have also facilitated data linkage between two NIAID Biological Resource Centers, (the virus pathogen resource (ViPR) and VectorBase (VB) to provide these data to the greater scientific community.

1277

TPL2-DEPENDENT INDUCTION OF IL-10 IN HUMAN ALTERNATIVELY ACTIVATED MACROPHAGES FOLLOWING MYCOBACTERIAL INFECTION: INSIGHTS INTO THE HELMINTH/MYCOBACTERIAL INTERFACE

Soumya Chatterjee, Emily E. van Seventer, Joseph Kubofcik, Roshanak Tolouei Semnani, Thomas B. Nutman

National Institutes of Health/National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States

Filarial and other tissue invasive helminth infections are associated with an early IL-4 driven expansion of Th2 cells that in turn drive the expansion of alternatively activated macrophages (AAMφs). In light of the geographic superimposition of tuberculosis and filarial infection we sought to understand how mycobacteria are handled in the context of helminth-induced AAMφ. Using human AAMφs generated *in vitro* from human monocytes by IL-4 and comparing them to LPS and IFN-γ generated classically macrophages (CAMφs) both infected with mycobacteria (BCG), we were first able to demonstrate that AAMφs were more susceptible to infection with BCG than were CAMφs (p=0.02) and this susceptibility was associated with increased IL-10 production, a cytokine known to enhance immune evasion of mycobacteria by impairing macrophage phagolysozome killing and antigen presentation. Not only did mycobacteria increase the production of IL-10 in AAMφs and not in CAMφs; (p=0.017) but we were also able to show that tumor progression locus 2 (TPL2), an upstream activator of extracellular signal related kinases (ERKs) acting through STAT3, itself induces IL-10 production. To explore the relationship between TPL2 and IL-10 in our *in vitro* BCG AAMφ model, we generated both CAMφs and AAMφs, exposed them to BCG at an MOI of 5 and examined TPL2 and its effects. Using qRT-PCR as well as by Western blot, we found increased baseline induction of TPL2 in CAMφs but not AAMφs (p=0.04). Post BCG infection, however, TPL2 levels were increased in AAMφs only (p=0.03). AAMφs (but not CAMφs) showed significantly diminished IL-10 production following the addition of the TPL2 kinase inhibitor ($C_{21}H_{14}ClFN_6$) (IC_{50}= $500x10^{-9}$ M) (p=0.001) BCG infection. These data show that AAMφs (commonly generated in human helminth infection) but not CAMφs activate the positive feedback loop for IL-10 regulation by induction of TPL2, suggesting a mechanism by which IL-10 production is increased in response to mycobacterial infection.

1278

CREATING AN EVIDENCE-BASED AND CLINICALLY-RELEVANT THRESHOLD FOR TB-ASSOCIATED CATASTROPHIC COSTS: A COHORT STUDY, PERU

Tom Wingfield[1], Delia Boccia[2], Marco Tovar[3], Arquímedes Gavino[4], Karine Zevallos[4], Rosario Montoya[4], Knut Lonnroth[5], Carlton A. Evans[1]

[1]Imperial College London, United Kingdom and Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Department of Infectious Disease Epidemiology, London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Universidad Peruana Cayetano Heredia and IPSYD, Lima, Peru, [4]IPSYD, Lima, Peru, [5]Policy Strategy and Innovations, Stop TB Department, World Health Organization, Geneva, Switzerland

Even when tuberculosis (TB) treatment is free, hidden costs incurred by TB-affected households may worsen poverty and health. Extreme TB-associated costs are termed 'catastrophic' but are poorly defined. We studied TB-affected households' hidden costs and their association with adverse TB outcome to create a clinically-relevant definition of catastrophic costs, against which we compared existing thresholds. From 2002-2009, TB patients (n=876, 11% with multi-drug resistant TB) were recruited to a prospective cohort study in shantytowns in Lima, Peru. Patients were interviewed prior to and every 2-4 weeks throughout treatment recording TB-related costs. Costs were expressed as a proportion of that household's annual income. Adverse TB outcome was defined as: death, abandonment or treatment failure, or TB recurrence. 23% (166/725) of patients had adverse TB outcomes. Total costs ≥20% of household annual income were defined as catastrophic because this threshold was most strongly associated with adverse TB outcome. Catastrophic costs were incurred by 345 households (39%). Adverse TB outcome was independently associated with multi-drug resistant TB (OR=8.4, p<0.001), previous TB (OR=2.1, p=0.005), and catastrophic costs (OR=1.7, p=0.01). Adjusted population attributable fraction of adverse outcomes explained by catastrophic costs was 18% (95%CI=6.9-28), similar to MDR TB (20%, 95%CI=14-25). Sensitivity analyses demonstrated that existing catastrophic costs thresholds (greater or equal to 10% or 15% of household annual income) were not associated with adverse TB outcome in our setting. In conclusion, despite free TB care, having TB disease was expensive for impoverished TB patients in Peru to afford. Incurring higher relative costs was associated with adverse TB outcome. Population attributable fractions implied that MDR TB and catastrophic costs had a similar association with adverse TB outcome. As opposed to existing catastrophic costs thresholds, our novel threshold was found to be clinically-relevant in our setting. Our results show that TB is a socioeconomic as well as infectious problem. Tuberculosis control interventions should address both the economic and clinical aspects of TB and policy makers should consider this new evidence-based and clinically-relevant catastrophic costs definition.

1279

INCREASED TUBERCULOSIS INFECTION IN MEN IN PERUVIAN SHANTYTOWNS DESPITE A DECADE OF DECREASING TUBERCULOSIS DISEASE

Leonardo Martinez[1], Sinead C. Isaacson[1], Carlton A. Evans[2], Richard A. Oberhelman[1], Lilia Cabrera[3], Mayuko Saito[4], Robert H. Gilman[5]

[1]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Wellcome Trust Centre for Clinical Tropical Medicine, Department of Infectious Diseases and Immunity, Imperial College London Hammersmith Hospital Campus, London, United Kingdom, [3]Asociación Benéfica Proyectos en Informática, Salud, Medicina y Agricultura, Lima, Peru, [4]Tohoku University Graduate School of Medicine, Sendai, Japan, [5]Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Changes in tuberculosis (TB) disease incidence rates do not reliably indicate TB control because they are prone to ascertainment bias. In contrast, tuberculin skin test (TST) surveys determine the prevalence of TB infection and allow changes in the community annual risk of infection (ARI) to be estimated objectively. We aimed to analyze changes in ARI from 2000 to 2011 in Pampas de San Juan de Miraflores, a Peruvian shantytown. We conducted 3 tuberculin surveys in different years: in 2000 (n=1056), 2005 (n=103), and lastly in 2011 (n=428). Randomly selected shantytown residents were included and had a TST if not pregnant or never received TB treatment. In 2000 and 2011, participants age ≥5 years were included but in 2005 only those age ≥15 years were available. Participants were stratified into youths (5-14 years) or adults (≥15 years). In 2000, the mean age was 18 (IQR 10-32) years and increased in 2005 and 2011 to 29 (IQR 22-38) and 31 (IQR 15-48) years ($p<0.0001$). To account for age differences we standardized the 2000 ARI rate to the 2011 study age distribution when comparing overall rates. Age-standardized ARI in 2000 was 1.9% (95% CI: 1.8, 2.2), similar to actual rates in 2005 and 2011: 2.4% (95% CI: 1.9, 3.0) and 2.2% (95% CI: 1.9, 2.9). Over time, ARI increased among adult males (2.0% [95% CI: 1.7, 2.4] in 2000; 3.1% [95% CI: 2.0, 4.7] in 2005; 3.0% [95% CI: 2.3, 3.6] in 2011) but was similar for adult females (1.5% [95% CI: 1.3, 1.8] in 2000; 2.0% [95% CI: 1.4, 2.8] in 2005; 2.1% [95% CI: 1.7, 2.6] in 2011). Among youths, there were no differences for males (1.2% [95% CI: 0.8, 1.6] in 2000; 1.4% [95% CI: 0.6, 2.3] in 2011) or females (1.4% [95% CI: 1.0, 1.8] in 2000; 1.8% [95% CI: 0.9, 2.8] in 2011) over time. Thus, despite decreasing rates of diagnosed TB disease in this shantytown, transmission causing TB infection was frequent and, from 2000 to 2011, increased significantly in adult men. Consequently, by 2011, adult males were at significantly greater risk of infection than the rest of the population and should be targeted by TB control interventions.

1280

HIGH PREVALENCE OF *PNEUMOCYSTIS JIROVECII* INFECTIONS AMONG MOZAMBICAN CHILDREN <5 YEARS OF AGE ADMITTED TO HOSPITAL WITH SUSPECTED PNEUMONIA

Quique Bassat[1], Miguel Lanaspa[1], Lola Madrid[1], Sónia Machevo[1], Enrique J. Calderón[2], Anna Roca[1], Pedro L. Alonso[1]

[1]Centro de Investigação em Saúde da Manhiça, Manhiça, Mozambique, [2]Instituto de Biomedicina de Sevilla and CIBER de Epidemiología y Salud Pública, Virgen del Rocío University Hospital, Spain

Pneumonia remains the main cause of pediatric mortality in the world. We aimed to assess the specific prevalence of *Pneumocystis jirovecii* (PJ) infections among children <5 years of age admitted to a rural Mozambican hospital with pneumonia, in an area of high HIV prevalence. Methods: As part of an etiology of pediatric pneumonia study, we recruited during 12 months 835 pediatric patients. Collection of standardized clinical data, chest X-rays and screening of nasopharyngeal aspirate (NPA) samples with PCR for 12 respiratory viruses were routinely performed, together with tests for invasive bacterial infection (IBI), malaria, and HIV. Investigations on PJ infection were performed on all NPA samples using a tri-sequential PCR strategy, assessing two multicopy mitochondrial genes (mtLSU y mtSSU) and a third unicopy one, linked to sulfa drug resistance (DHPS). 77/835 (9.2%) of the patients tested positive for at least one of the PJ genes. 32.5% (25/77) patients showed triple (mtLSU, mtSSU and DHPS) gene positivity, while further 41.6% (32/77) showed double (any combination of the three markers) positivity. Twenty (26.0%) further cases tested solely positive for mtLSU. Median age of PCP patients was 3.9 months (IQR 3.1-12.4). Only 30/77 (39.0%) of the confirmed PCP cases had a clinical picture of probable Pneumocystis jiroveci pneumonia (PCP). HIV co-infection was confirmed in 47.8% of the patients with PCP (22/46) for whom HIV results were available. Surprisingly, 16.7% (11/66) of those patients with a valid blood culture result had a concomitant IBI (6 cases of S. pneumoniae, and 5 other bacteria). Viral co-infection was frequent (36/76; 47.4%), being rhinovirus, adenovirus and human metapneumovirus the three commonest viruses found. 5 patients (6.7%) showed also positive *P. falciparum* parasitemias. 15 PCP-infected patients died during admission, yielding a case fatality rate (CFR) of 19.5%, significantly superior to that for non-PCP infections (8.8%; p=0.003). Further 5 PCP patients died at home within the first 21 days post discharge. PCP is a highly prevalent infection among Mozambican infants admitted with severe pneumonia and carries an unacceptably high risk of death, coexisting with other common pediatric infections. The true burden of pediatric PCP in Sub-Saharan Africa needs to be recognized, particularly in the context of the HIV pandemic, and measures to prevent and adequately manage it put in place urgently.

1281

MYCOBACTERIUM TUBERCULOSIS INFECTION INDUCES PERSISTENT NON-RESOLVING INFLAMMATION

Christine A. King[1], Serene John[2], John Kenneth[2], Saurabh Mehta[3]

[1]SUNY Upstate Medical University, Syracuse, NY, United States, [2]St. John's Research Institute, Bangalore, India, [3]Cornell University, Ithaca, NY, United States

Infection with *Mycobacterium tuberculosis* (MTB) is accompanied by an intense inflammatory response thought to be directly related to pathogenesis. Currently, we have little understanding of this inflammatory reaction in individuals and whether it resolves in response to curative treatment. To determine the systemic inflammatory status of individuals we compared the peripheral blood inflammatory gene expression profile of 10 patients with active MTB, latent MTB infection, and cured (for more than six months) MTB, to healthy controls by quantitative PCR in India. Patients with active MTB had dramatically different inflammatory profiles as compared to latent MTB. Patients with active MTB demonstrated greater mRNA levels of potent pro-inflammatory IL-1β and neutrophil chemotactic factors CXCL1 and IL-8, while patients with latent MTB infections had higher levels of monocyte/macrophage activation and chemotactic mediators including IP30, CD14, CXCL3, CCL2 and CCL8 suggesting a switch from a neutrophil centered response to a monocyte/macrophage tailored response. Furthermore, several of these key factors including CD14, IP30, CCL2 and CCL8 remained elevated in cured patients. Our results suggest that MTB infection induces long-term persistent inflammation in the human host in the absence of active infection. Chronic inflammation is widely recognized as a potent driver of many diseases and our data suggests that a significant portion of the population, particularly in high-burden settings such as India, may remain at risk for inflammation-mediated complications even after successful treatment for MTB infection.

1282

LACK OF ASSOCIATION BETWEEN PARASITIC INFECTIONS AND TUBERCULIN SKIN TEST POSITIVITY IN REFUGEES

Mucho M. Mizinduko[1], Elizabeth D. Barnett[2], Anita M. Loughlin[2], Laura F. White[1], Natasha S. Hochberg[1]

[1]Boston University School of Public Health, Boston, MA, United States, [2]Boston University School of Medicine, Boston, MA, United States

Mycobacterium tuberculosis (Mtb) and parasites often co-infect the same person. Immunologic responses to helminth infections may blunt control of mycobacteria, and epidemiologic data suggest an association between helminth infections and tuberculosis disease. Our objective was to determine whether there is an association between parasite infections and positive tuberculin skin tests (TSTs). We reviewed records of patients seen at the Boston Medical Center Refugee Health Assessment Program from 1999-2012. We evaluated demographic characteristics and results of TSTs, stool ova and parasite examinations, complete blood counts with differential, and serologic testing for helminths (done if eosinophilia was found) and looked for an association between TST results and parasite infections. We used multivariate logistic regression models to control for possible confounders (gender, age, WHO region of birth compared to Africa, and protozoal or helminth infection depending on the model). Among 7230 participants, 3843 (53%) were male, mean age was 25 years (range 1-88), and 3355 (46.4%) had positive TSTs. Individuals with positive TSTs were older (mean age 29.9 vs. 21.7 years; p< 0.0001) and more likely to be male (OR = 1.35; 95%CI, 1.23, 1.49). Helminth infections were found in 393 (5.4%) including *Trichuris trichiura* (132/393; 33.6%), *Strongyloides stercoralis* (89; 22.6%), and *Schistosoma* infections (79; 20%). Among 2473 (34%) with protozoal infections, *Blastocystis* spp was found in 1986 (80%). TST positivity was not associated with helminth infections (adjusted OR [aOR] = 1.14; 95%CI, 0.92, 1.41) or protozoal infection (aOR = 1.08; 95%CI, 0.97, 1.20). We found no association between parasitic infections and TST positivity. Unmeasured confounders (HIV infection, poverty, malnutrition, etc.), undetected parasites, pre-departure parasite treatment and other reasons for false negative TSTs may have obscured an effect. More sensitive methods for Mtb detection and a study of the effect of individual parasite species on TSTs are needed to confirm this lack of association.

1283

CHALLENGES OF DETECTING RESISTANCE TO FIRST AND SECOND LINE ANTI-TUBERCULOSIS DRUGS IN SOUTHWESTERN UGANDA

Patrick Orikiriza[1], Becky Tibenderana[2], Mark J. Siedner[3], Frederick Byarugaba[2], Christopher C. Moore[4], Emily E. Evans[4], Maryline Bonnet[5], Anne-Laure Page[5], Joel Bazira[2], Yap Boum, II[1]

[1]Epicentre, Mbarara; Mbarara University of Science and Technology, Mbarara, Uganda, [2]Mbarara University of Science and Technology, Mbarara, Uganda, [3]Harvard Medical School/Massachusetts General Hospital, Boston, MA, United States, [4]University of Virginia, Charlottesville, VA, United States, [5]Epicentre, Paris, France

There are limited data on the drug susceptibility of tuberculosis (TB) in southwestern Uganda. We assessed the proportion of first- and second-line drug resistance in culture-positive tuberculosis specimens from treatment-naïve suspects in southwestern Uganda to guide regional empiric recommendations on multidrug-resistant tuberculosis (MDR-TB). We collected sputum samples for smear microscopy and culture on mycobacterium growth indicator tubes (MGIT) and Lowenstein Jensen (LJ) media from tuberculosis suspects with no prior TB treatment at Mbarara Regional Referral Hospital from February 2009 to February 2013. We tested archived specimens for isoniazid and rifampicin resistance using the MTBDR*plus* assay and GeneXpert. A subsample of isolates selected randomly for geographic variability was also tested with the MTBDR*sl* assay. The resistant isolates were tested further using sequencing and MGIT. Specimens were collected from 190 TB suspects residing within 23 districts of southwestern Uganda, of whom 69% were male, the median age was 33 years (26-43), and the HIV prevalence was 80/190 (42%). No isolates (0%) were rifampicin-resistant and only 1/190 (0.5%) was isoniazid-resistant (0% overall proportion of MDR-TB). Among 92 isolates tested for second-line drug resistance, 71 (77%) had interpretable results, of which 7/71 (10%), 3/71 (4.2%) and 0 (0%) were resistant to fluoroquinolone using MTBDR*sl,* sequencing, and MGIT respectively. None of the isolates were resistant to aminoglycosides, cyclic peptides, or ethambutol. We found no MDR-TB and no resistance to ethambutol or injectables among treatment naïve TB suspects in southwestern Uganda. However, the discrepancy in the fluoroquinolone resistance results of by the three approved methods makes diagnosis difficult and requires establishment of an optimum global second line testing strategy.

1284

ASTMH PERU: THINKING GLOBALLY AND ACTING LOCALLY TO DISSEMINATE GLOBAL HEALTH RESEARCH RESULTS AND TRAIN YOUNG SCIENTISTS

A. Roxana Lescano[1], Daniel G. Bausch[1], Cesar Cabezas[2], Kyle Petersen[1], Karen Goraleski[3], Claudio F. Lanata[1], Alan J. Magill[4], Eduardo Gotuzzo[5], Vilma Bejar[6], Sofia Gonzalez[7], Andres G. Lescano[1]

[1]U.S. Naval Medical Research Unit - 6, Callao, Peru, [2]Instituto Nacional de Salud, Lima, Peru, [3]American Society of Tropical Medicine and Hygiene, Deerfield, IL, United States, [4]Bill & Melinda Gates Foundation, Seattle, WA, United States, [5]Instituto de Medicina Tropical Alexander von Humboldt, Lima, Peru, [6]Instituto de Medicina Tropical Daniel A. Carrion, Callao, Peru, [7]Sociedad Peruana de Enfermedades Infecciosas y Tropicales, Lima, Peru

The Annual Meeting of the American Society of Tropical Medicine and Hygiene (ASTMH) convenes thousands of scientists from around the world to the United States to exchange advances in global health research. Although Peru has had a major presence at the meeting with over 30 abstracts per year in the last decade, the findings are paradoxically less available to many Peruvian scientists, especially scientists in training, for whom financial and logistical barriers often prevent travel to the United States. Thus, an annual local satellite meeting, ASTMH Peru, was established in Lima, as an avenue for dissemination of scientific information and implementation of research results into health policies. Six local and international established leaders in infectious disease research launched ASTMH Peru in 2011. Only abstracts presented at the previous Annual Meeting are included in ASTMH Peru, no call for new abstracts. In addition to the oral and poster sessions, there are keynote presentations by ASTMH leaders on subjects particularly relevant to Peru, including identifying local funding sources, writing scientific manuscripts and grant proposals, and strategies to implement research findings. Primary topics covered are malaria, cysticercosis, dengue fever, leishmania and diarrhea. ASTMH Peru is 100% funded by Peruvian collaborations with registration fees of $28 for professionals and $18 for students. Remaining funds support partial scholarships for young Peruvian scientists to attend the next U.S, meeting if they have an accepted oral presentation. Between 2011 and 2014, the number of posters increased from 45 to 74; oral presentations from 11 to 17, focusing on malaria, cysticercosis, dengue, leishmania and diarrhea. The number of attendees grew from 205 to 388. An important new segment is the support to informed decision making by local authorities. All sessions were heavily attended. Peruvian members in ASTMH also increased from 68 to 101. Six Peruvian scientists have attended the US meeting supported by ASTMH Peru in the last four years. ASTMH Peru constitutes a timely, low cost, and sustainable mechanism for the exchange of high quality scientific knowledge led by local research leaders bridging the Northern and Southern hemispheres together, with a special focus on the training of young scientists.

1285

MECHANICAL, AUTOMATIC INTRAVENOUS VOLUME REGULATOR FOR RESOURCE-LIMITED SETTINGS

Kamal Shah, Erica Skerrett, Matthew Nojoomi, Thor Walker, Kelley Maynard, Michael Pan, Bailey Flynn, Melissa Yuan, Elizabeth Molyneux, Ann Saterbak, Maria Oden, Rebecca Richards-Kortum

Rice University, Houston, TX, United States

We have developed an automatic mechanical volume regulator for IV therapy in the developing world, where 1.4 million children die annually to dehydration caused by diarrhea, malaria, and dengue hemorrhagic fever. These deaths are preventable with IV therapy; however, children who are treated with IV therapy are at risk of overhydration as due to chronic understaffing and high cost of standard volume regulators such as infusion pumps, which require consumables and electricity. Because the risks of overhydration include death, clinicians use oral rehydration for treatment; however, severe cases of dehydration require IV therapy. The IV volume regulator we designed costs less than $80 and does not require electricity. It employs a lever arm with a movable counterweight (similar to a physician's scale) to incrementally dispense IV fluid. A volume indicator slides along the lever arm and allows selection of target volumes in increments of 50 mL. The change in angle of the lever arm as the IV bag drains activates a spring clamp to kink the IV tubing, stopping fluid flow. Performance was assessed in the lab by delivering target volumes of 50 to 850 mL in increments of 50 mL, five flow rates of 20 to 4000 mL/hr, and four initial IV bag volumes between 200 and 1000 mL (n=5 each; n=170 overall). Usability was quantified with a system usability survey (SUS) by 33 nurses, doctors, and medical students at Queen Elizabeth Central Hospital in Blantyre, Malawi. For all parameters, the mean and median residuals were significantly less than 25 mL, and the maximum residual was 30 mL. After training for less than 15 min, Malawian clinicians set up the device within 79.5±31.5 sec. Participants reported a SUS score of 84.4±11.1, which is greater than the 70 threshold for an acceptable product. These promising results will guide the design of a clinical trial evaluating the field accuracy of this device in summer 2014. By enabling clinicians to provide children life-saving IV fluids, this device may potentially prevent over-hydration in resource-limited settings.

1286

GENOME-WIDE ANALYSIS REVEALED THAT *PLASMODIUM FALCIPARUM*-DRIVEN SELECTIVE FORCES MAY HAVE INDUCED HIGH FREQUENCY OF HLA ALLELES ASSOCIATED WITH PODOCONIOSIS

Fasil Tekola-Ayele[1], Adebowale Adeyemo[1], Elena Hailu[2], Chris Finan[3], Abraham Aseffa[2], Gail Davey[4], Melanie Newport[4], Charles Rotimi[5]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Armauer Hansen Research Institute, Addis Ababa, Ethiopia, [3]Wellcome Trust Sanger Institute, Cambridge, United Kingdom, [4]Brighton and Sussex Medical School, Brighton, United Kingdom, [5]National Institutes of Health, Bethesda, MD, United States

Podoconiosis is geochemical elephantiasis of the lower legs among barefoot individuals with long-term exposure to red clay soils. Globally, more than 4 million people are affected. A genome-wide association study conducted by our group in Ethiopia showed that genetic variants in the HLA class II loci confer susceptibility to podoconiosis. Subsequent HLA typing confirmed that among others, HLA-DQB1*02 is a risk allele and HLA-DRB1*13 is a protective allele to podoconiosis. Interestingly, these alleles have been shown to be associated with protection against P.falciparum malaria. It is estimated that three-quarters of the landmass of Ethiopia is malarious predisposing over two-thirds of the population to malaria. In the present study we aimed to investigate possible selective forces that induced high frequency of the HLA alleles associated with susceptibility to podoconiosis. First, we conducted genome-wide analysis of 464,642 single nucleotide polymorphisms (SNPs) comparing ethnic Wolaita Ethiopians (n=120) with 11 population groups from Africa, Europe, and Asia with aim to identify signatures of recent positive selection. We found that HLA loci showing the strongest genome-wide association with podoconiosis were under strong selective pressure in the Ethiopian population, but not in the others. Next, using data from our own cohort and publicly available HLA database, we compared the distribution of DRB1*13 and DQB1*02 alleles in three Ethiopian population groups (Wolaita, Amhara and Oromo) that form 64% of the total population of the country with that of other Sub-Saharan African population groups. We found that the Ethiopian ethnic groups had the highest frequencies of DRB1*13 compared with other populations in Sub-Saharan Africa. Previous studies showed that the DRB1*13-DQB1*05 haplotype was protective against severe malaria in the Gambian population and DRB1*13 was protective against persistent hepatitis B infection. We also found that the Ethiopian population groups had the third highest frequency of DQB1*02 following Burkina Faso's Fulani and Central African Republic's Aka Pygmy. The Fulani closely share the distribution of HLA alleles with the Amhara and Oromo of Ethiopia, and mount stronger humoral immune response against malaria. Together, these data suggest that strong pathogen-driven selective forces induced the high frequency of the risk variants for podoconiosis.

1287

IMPROVING MALARIA SURVEILLANCE THROUGH USE OF MOBILE TECHNOLOGY IN MAINLAND TANZANIA: FINDINGS FROM A PILOT STUDY

Zainab Alidina[1], Oresto Munishi[1], Anold Mutafungwa[1], Elibariki Mwakapeje[2], Ismail Ismail Koleleni[3], Renata Mandike[4], Frank Chacky[4], Winfred Mwafongo[4], Mwendwa Mwenesi[2], Naomi Kaspar[5], Lynn Paxton[6], Mahdi Ramsan[1], Jeremiah Ngondi[1]

[1]Research Triangle Institute, Dar es Salaam, United Republic of Tanzania, [2]Ministry of Health and Social Welfare, Dar es Salaam, United Republic of Tanzania, [3]University of Dar es Salaam, Dar es Salaam, United Republic of Tanzania, [4]National Malaria Control Program, Dar es Salaam, United Republic of Tanzania, [5]President's Malaria Initiative/United States Agency for International Development, Dar es Salaam, United Republic of Tanzania, [6]Malaria Branch and President's Malaria Initiative, Centers for Disease Control and Prevention, Dar es Salaam, United Republic of Tanzania

The Integrated Disease Surveillance and Response (IDSR) system, a paper based system that reports public health surveillance and response data from the health facility level in Tanzania was implemented nationwide in 2002. A mobile phone based electronic system (eIDSR) was created in 2012 to replace the paper based system (paper-IDSR) to increase efficiency and completeness of reporting. A four-week pilot of the eIDSR system took place in Temeke District in Dar es Salaam in November 2013. This study aimed to explore the change in reporting and completeness of surveillance data reporting following the change from the paper-IDSR to the eIDSR system. A total of 67 (64% out of 104 eligible) health facilities participated in the eIDSR pilot following training. For the duration of the pilot, the paper-IDSR and eIDSR system worked concurrently. Data were collected at the district level for the paper-IDSR and through an internet-based database for eIDSR. A data quality assessment was conducted in January 2014 to compare timeliness and quality of data between the two systems. Preliminary findings indicate that 70% of weekly reports were submitted on time through the eIDSR compared to 78% of timely reports via the paper-IDSR system; this is due to a discrepancy in how the paper-IDSR and eIDSR define timeliness (defined by paper-IDSR as Wednesday and eIDSR as Monday 3 pm for the previous week data). Initial analysis indicates that when the same cut off time (Monday) is used for both systems, timeliness in eIDSR is substantially faster than paper-IDSR. All health facilities reported complete data through the eIDSR system while 84% reported complete data through the paper-IDSR system. The paper-IDSR system required dedicated staff to travel from the health facility to the district medical officer to deliver weekly reports. The cost of travel and person hours lost to deliver reports is completely eliminated in the eIDSR system. This

pilot shows that eIDSR improved timeliness of weekly reporting and data completeness. Implementation of eIDSR should be considered in all regions in the Mainland Tanzania to improve surveillance of infectious diseases.

1288

HYBRID STUDY DESIGN FOR COMPLEX INTERNATIONAL FIELD TRIALS: CONDUCTING OBSERVATIONAL RESEARCH ON A RANDOMIZED CLINICAL TRIAL PLATFORM TO ENSURE HIGHEST QUALITY STANDARDS IN SCIENTIFIC DISCOVERY FOR GLOBAL HEALTH

E. Ross Colgate[1], Beth Kirkpatrick[1], Josyf Mychaleckyj[2], Uma Nayak[2], Rashidul Haque[3], Dorothy Dickson[1], Marya Carmolli[1], Jennie Ma[2], Masud Alam[3], Sean Diehl[1], Fardusi Qadri[3], William Petri[2]

[1]University of Vermont College of Medicine, Burlington, VT, United States, [2]University of Virginia, Charlottesville, VA, United States, [3]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Performing interventional Randomized Clinical Trials (RCT) according to strict standards is time consuming, expensive, and often designed to answer a single question about the impact of an intervention. Infrastructure development for this work is extensive to ensure subject protection, preserve trial endpoints, minimize bias, and promote generalizability of results. Most sponsors or funding agencies are increasingly interested in enhancing the value of this time, cost and infrastructure, as well as investing in innovative research around challenging problems in global health. In large and complex field settings, innovation, the rapid integration of new data and exploratory analyses may be impeded by overly rigid research designs, including standard RCT models. A new Hybrid Study Design (HSD) that combines the strengths and ameliorates the weaknesses of RCT and observational study designs may be a more robust model for maximum translational impact. The Hybrid Study Design ensures the rigor of clinical trials to safeguard data quality and subject protection, while allowing for discovery, particularly in rapidly changing fields where new knowledge needs to be tested, confirmed and applied quickly to improve health outcomes. We report on our experience using a HSD in an urban slum setting in Bangladesh to examine the role of Environmental Enteropathy, a poorly characterized disorder of the small intestine, in oral vaccine underperformance through the PROVIDE Study. With a 2x2 factorial design and two vaccine interventions, the PROVIDE study combined the ethical, regulatory, and analytic structure of a RCT with the flexibility required to successfully undertake cutting-edge research in the developing world. The rationale for the HSD will be presented and lessons learned from applying this new model in a birth cohort of 700 infants with two years of follow-up. We propose the HSD as a useful model for research in which an interventional component or non-inferiority question is added to exploratory or descriptive work, particularly in developing world settings.

1289

INCREASED EQUITY IN MALARIA CONTROL INTERVENTIONS IN MALAWI FROM 2000 TO 2012

Jui Shah, Lia Florey, Cameron Taylor, Rebecca Winter, Ana Claudia Franca-Koh, Samantha Herrera, Yazoume Ye

ICF International, Rockville, MD, United States

Sustained resources since 2000 have supported considerable gains in malaria control strategies, including insecticide-treated nets (ITNs) and intermittent preventive treatment in pregnancy (IPTp). Malawi has been a pioneer in adopting effective interventions, offering subsidized ITNs nationally by 2003 and encouraging IPTp by 1993. These programs contributed to a substantial increase in household ownership of at least one ITN from 27.4% in 2004 to 55.0% in 2012. Use of ITNs also increased during this time period among the traditional target groups of children under five years of age (2.8% in 2000 to 56.0% in 2012) and pregnant women (2.6% in 2000 to 50.7% in 2012). While utilization of antenatal services is high, IPTp coverage remains low, with only 53.8% of women receiving two or more doses in 2012. To assess equity of these interventions, Lorenz concentration curves and corresponding concentration index (C-Index) values were derived from 2000, 2004, and 2010 Demographic and Health Surveys and a 2012 Malaria Indicator Survey. Values approaching perfect equity across wealth quintiles (C-Index=0) show that equity has improved for all interventions studied. Increasing ITN ownership corresponded to improved equity over time (C-Index=0.29 in 2004 to C-Index=0.06 in 2012). Even larger gains were seen in ITN use by children under five (C-Index=0.47 in 2000 to C-Index=0.03 in 2012) and pregnant women (C-Index=0.45 in 2000 to C-Index=-0.01 in 2012). Equity in IPTp was stronger from an earlier date (C-Index=0.03 in 2000) and remained similar through 2012 (C-Index=0.06), suggesting that antenatal services are accessible and used equally across all wealth quintiles. The differences between ITN ownership and use and IPTp uptake show how equity may differ for various malaria control interventions. In order to achieve greater returns as Malawi moves toward universal coverage of all interventions and malaria transmission decreases, it will be important to acknowledge equity and focus resources on economic groups with outstanding needs.

1290

EXPLORATORY GEOSPATIAL MODELLING OF ENVIRONMENTAL FACTORS CORRELATED WITH PODOCONIOSIS IN ETHIOPIA

Yordanos B. Molla[1], Nicola Wardrop[2], JeJennifer Le Blond[1], Peter Baxter[3], Melanie J. Newport[1], Peter M. Atkinson[2], Gail Davey[1]

[1]Brighton and Sussex Medical School, Brighton, United Kingdom, [2]Geography and Environment, University of Southampton, Southampton, United Kingdom, [3]Institute of Public Health, University of Cambridge, Cambridge, United Kingdom

Ecological studies have linked podoconiosis (endemic, non-filarial elephantiasis) to red clay soils of volcanic origin, but the precise trigger of the disease is unknown. Histopathology investigations have demonstrated phyllosilicates, aluminium, magnesium and iron in the lower limb lymph node macrophages of both patients and non-patients living barefoot on these clays. We studied local spatial variation in disease prevalence and environmental factors with the aim of increasing understanding of disease pathogenesis. Fieldwork was conducted from June 2011 to February 2013 in 12 kebeles (administrative units) in northern Ethiopia. Geo-located prevalence data and soil samples were collected and analysed along with secondary geological, topographic, meteorological and elevation data. Soil data were analysed for chemical composition, mineralogy and particle size; and interpolated using regression kriging. Exploratory, univariate and multivariate regression analysis of podoconiosis prevalence in relation to primary (soil) and secondary (elevation, precipitation and geology) covariates was conducted. Following appropriate transformation to predict soil covariates, exploratory analysis indicated that podoconiosis prevalence was associated with clay minerals (smectite, kaolinite and mica), quartz (crystalline silica), iron oxide, and zirconium. The final multivariate model included smectite (OR = 2.76, 95% CI: 1.35, 5.73; p = 0.007), quartz (OR = 1.16, 95% CI: 1.06, 1.26; p = 0.001) and mica (OR = 1.09, 95% CI: 1.05, 1.13; p < 0.001). The association between podoconiosis prevalence and smectite, quartz and mica content of the soil suggests that further environmental, biomedical and toxicology studies on podoconiosis should focus on these soil characteristics.

1291

IS EARLY COMPLEMENTARY FOOD INTRODUCTION RELATED TO LOW INFANT WEIGHT-FOR-HEIGHT?

John D. McLennan

University of Calgary, Calgary, AB, Canada

Early complementary food introduction, by definition, undermines exclusive breastfeeding, as well as increases health risks for infants. A

broad array of liquids and solids are reported as introduced early in the diets of infants in many countries despite the promotion of exclusive breastfeeding for the first six months of life as optimal. Despite this pattern, there has been comparably little investigation into factors that may underlie early complementary feeding. Mothers' interpretation that their children's poor growth is a function of inadequacy of their breast-milk alone may be one factor, particularly in low resource settings of low- and middle-income countries. This study investigated whether more extensive early complementary feeding is related to lower child weight-for-height using data from the 2007 Demographic and Health Survey of the Dominican Republic. Of the 763 children under six-months of age with complete complementary feeding data, only 10.7% were classified as exclusive breast-feeders, although 79.6% were breastfeeding at the time of the survey. Among breast feeders who were non-exclusive, plain water and other milk types were the most common complementary products used. Baby cereal and items from the bread/noodles/grain group were the most common foods consumed. A summation of responses to 22 complementary food/liquid items consumed the day prior to the survey was used to index the extent of complementary food use. Inconsistent with the study hypothesis, this measure was not related to the Z scores of the children's weigh- for-height in either bivariate analysis or a multivariate model controlling for child age. Study findings are limited given the cross-sectional nature of the dataset and lack of variables on maternal perception of thinness, the latter which may provide a better index of maternal concern about child growth than the direct anthropometric measure used in this study. Nevertheless, other variables should be examined to identify key factors driving early complementary food introduction.

1292

QUALITY OF EMERGENCY NEWBORN CARE IN RURAL BANGLADESH, 2013

Wit Wichaidit[1], Mahbub Ul Alam[2], Davidson H. Hamer[3], Amal K. Halder[2], Leanne Unicomb[2], Pavani K. Ram[1]

[1]University at Buffalo, Buffalo, NY, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]Boston University, Boston, MA, United States

The neonatal mortality rate in Bangladesh is 26 deaths per 1000 live births; 14% of births are preterm. Signal functions, a list of critical interventions, were recently developed for emergency newborn care (EmNC) but their availability at national level has never been assessed in any country. Our objective was to assess the availability of EmNC signal functions at hospitals predominantly serving rural populations of Bangladesh. Directors of hospitals providing inpatient care to 50 villages, selected to be representative of rural Bangladesh, were interviewed about hospital infrastructure, staffing, and provision of four EmNC signal functions within the past 3 months (neonatal resuscitation, administration of antibiotics, administration of corticosteroids and provision of kangaroo mother care [KMC] for preterm births). Hospitals providing all 4 signal functions, with ≥3 staff on call (24-hour coverage), and an ambulance and phone for referring patients, were considered to provide high quality EmNC. Hospitals with ≥3 signal functions, ≥2 staff (or no 24-hour coverage), and a phone but no ambulance were considered to provide moderate quality EmNC. Hospitals with ≥2 signal functions and ≥1 staff and a phone provided low quality EmNC; the rest were considered sub-standard. Directors of 432 hospitals in 46 of 64 districts in Bangladesh took part; 383 hospitals (89%) were located in urban areas. Newborn care was available in 98% of hospitals. The most commonly available signal function was neonatal resuscitation (90%), followed by administration of antibiotics (86%) and corticosteroids (40%), and KMC (only 8% of facilities). KMC was more common in public hospitals (16% vs. 5%, P = 0.005). Quality of EmNC care was high in 11(3%) hospitals, moderate in 30%, low in 45%, and sub-standard in 22%. Inadequate availability of KMC and corticosteroids represent substantial barriers to providing high quality of care for particularly vulnerable preterm neonates. Efforts to motivate delivery at health facilities should be matched by strengthening EmNC at those facilities.

1293

INTEGRATED SURVEILLANCE FOR DISEASE CONTROL: A NEW ERA IN GLOBAL HEALTH?

Kate Zinszer[1], Sonia Menon[1], Lauren Caroll[2], Neil Abernethy[2], David Buckeridge[1]

[1]McGill University, Montreal, QC, Canada, [2]University of Washington, Department of Biomedical Informatics and Medical Education, Seattle, WA, United States

Donors, ministries of health, and other global health partners share the responsibility of ensuring the availability of valid and timely health indicators. There is growing recognition for the critical role these indicators have in the strategic development of effective responses to global health issues. Unfortunately, data fragmentation across diseases, countries, funding sources, and a wide range of clinical and laboratory data sources pose a barrier to timely estimation of accurate health metrics. Such fragmentation frustrates efforts to merge, analyze, and interpret data across multiple geographical scales, and hinders attempts to use these data to develop and share transparent, scalable tools for decision-making. The purpose of this project is to develop and evaluate 'proof-of-concept' tools and technologies to support the integration and use of global health data collected across a range of diverse sources. We intend to demonstrate the value of these tools and technologies for improving decision-making related to malaria control in Uganda and The Gambia. The first phase of our work entails developing a catalog of data sources for malaria control and describing how malaria control programs, funding agencies, and partners analyze and use these data to make programmatic decisions. The second and third parts of the project involve developing and evaluating the technology and software tools that will interact with the data sources to calculate and analyze valuable health metrics. At the completion of our project, which is anticipated to be November 2014, we will have developed an open-access prototype system that will support sharing of comparable data within and across countries. The system will include tools for supporting the effective use of data, and it will provide a mechanism for facilitating convergence towards common data standards to support the control of malaria and other priority diseases.

1294

MONITORING THE QUALITY OF ANTIMALARIAL DRUGS IN SENEGAL: A STAKEHOLDER PERSPECTIVE

Mirza Lalani

London School of Hygeine & Tropical Medicine, London, United Kingdom

Poor quality antimalarials may have a deleterious effect on public health in malaria endemic countries. A national drug quality surveillance system may minimise the risk of such drugs appearing in the public and private sector, but little is known about these systems in developing countries. The aim of this study was to explore the perceptions of stakeholders on their institutional roles and responsibilities for assuring the quality of drugs, and strengths and weaknesses of the drug quality surveillance system in Senegal. In-depth qualitative interviews were conducted with 27 key stakeholders including representatives of the surveillance system authorities and treatment providers in the regulated public and private health sectors. Interviews focussed on two aspects of drug quality surveillance: 1) understanding the system context including its background, challenges faced and institutional roles and responsibilities of national authorities, and 2) identifying vulnerable components of the system that, if compromised, may increase the risk of poor quality antimalarials in Senegal. A health systems viewpoint was applied, allowing for inductive expansion of emergent themes in relation to the six building blocks of health systems. Preliminary analysis indicates that all stakeholders perceived the system to operate effectively and they had confidence in

the quality of antimalarials available in Senegal. Nonetheless, coordination amongst the different national authorities involved in assuring and monitoring drug quality, and between authorities and treatment providers, was recognised as a challenge. Differences in perceived quality and efficacy of antimalarials were often assumed to be related to their cost and country of manufacture. Insufficient drug storage conditions and the existence of an informal drug sector were seen as the two main risk factors for poor quality antimalarials in Senegal.Stringent drug regulation and a secure drug supply chain were perceived to contribute to the confidence in the quality of antimalarials available Senegal. However a lack of funding, issues of governance, inadequate human resources and an absence of monitoring of the informal sector (due to concerns that acknowledgment would legitimise its existence) all threaten to impair the progress that has been made by national authorities and external partners in assuring drug quality in Senegal.

1295

PERCEPTIONS OF HEALTH AND VULNERABILITIES ALONG THE INTER-OCEANIC HIGHWAY IN MADRE DE DIOS, PERU: RESULTS FROM QUALITATIVE RESEARCH

Valerie A. Paz Soldan[1], Valerie Baquerizo[2], Lara Schwarz[3], Gabriela Salmon-Mulanovich[4], Stella M. Hartinger[2], Daniel G. Bausch[1]

[1]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]McGill University, Montreal, QC, Canada, [4]U.S. Naval Medical Research Unit - 6, Lima, Peru

The Madre de Dios Region in the Peruvian Amazon is a biodiversity hotspot that has been highly affected by human settlement and land-use change since the construction of the inter-oceanic highway (IOH) that crosses the region. We explored societal impacts, quality of life issues, and vulnerabilities associated to the IOH, as part of a larger study focusing on transmission of rodent-borne diseases. Twelve focus group discussions with 83 community members and 21 key informant interviews with local leaders and health personnel were conducted in February 2014 in 8 communities along the IOH. Although people believe the IOH brought positive changes to their communities, they had an overall negative perception of the IOH. They attributed the increase in road accidents, crime and alcohol/drug consumption in recent years to the increase in human migration brought by the IOH. Lack of electricity and clean drinking water were common concerns. Findings suggest migrants tended to settle in the community outskirts, closer to intact rainforest, placing them at higher risk for emerging infectious diseases. For some jobs, people worked for long periods of time in remote locations in the rainforest, with its inherent risks. Several communities mentioned anemia as a significant health problem, but most communities now considered themselves healthy - dengue fever, leishmaniasis, malaria and gastrointestinal parasites were all cited as past problems. People recognized various types of rodents, and some complained about their role as pests, but did not express particular concern about diseases these may transmit. There are distinct differences between the communities north and southwest of the capital city Puerto Maldonado. While the main economic activities in all communities are logging, agriculture and Brazil nut collection, the southwest communities are often surrounded by illegal mining camps. As a result, they have much more support than northern communities from governmental and non-governmental organizations promoting diverse projects to improve the situation. Determining local peoples' perceptions of key issues and vulnerabilities in relation to health and the IOH will enable a greater understanding of how to approach current and future public health problems occurring in this region.

1296

INNOVATION TO ADDRESS DIAGNOSTIC NEEDS FOR RURAL POPULATIONS: INSIGHTS OF JUNIOR MEDICAL DOCTORS AT THE FRONTLINES OF RURAL CARE IN PERU

Cynthia F. Anticona Huaynate[1], Valerie A. Paz Soldan[1], Monica J. Pajuelo[1], Malena Correa[1], Holger Mayta[1], Richard A. Oberhelman[1], Mirko Zimic[2], Robert H. Gilman[3], Laura L. Murphy[1]

[1]Tulane University, New Orleans, LA, United States, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Johns Hopkins School of Public Health, Baltimore, MD, United States

Diagnosis of common diseases can require specific technologies and depends on a skilled workforce, which are common limitations in rural settings. Few studies have assessed health providers' perceived needs for diagnostic tools and barriers they face in diagnosis - information that can guide innovation development and implementation. The aims of this study were to examine, from the perspective of medical doctors (MDs) in rural Peru, the needs and barriers associated with the diagnostic process, and solicit ideas for innovative solutions. Three focus group discussions (12 participants) and 18 individual semi-structured interviews were conducted with recent MD graduates who had completed their medical service in rural areas of the Highlands and Amazon basin in Peru in the last two years. Data were analyzed manually to explore trends in the main themes. The main diagnostic needs for infectious diseases included point of care (POC) tests for: i) the differential diagnosis of malaria vs pneumonia, ii) dengue vs leptospirosis, iii) tuberculosis, iv) vaginal infections and cervical cancer. Ultrasound was a perceived need for obstetric and intra-abdominal conditions. In specific locations, diagnostic tools for neurocysticercosis and for heavy metals toxicity were needed. Barriers impeding the diagnostic process included: distance to and high cost of referral facilities; cultural and linguistic issues; inefficient referral and laboratory systems; and inadequate telecommunication. Innovative ideas proposed by participants included: POC equipment such as a "rural ultrasound" and telemedicine services. Our findings show there is a high demand for improved diagnostic testing in rural communities, so a system based fundamentally on referrals is inadequate: rural doctors need more tools that are technologically and socially viable in context. National strategies supporting the development and implementation of diagnostic innovations are crucial for improving health services. This process should be informed by the perspectives of health providers in the underserved areas.

1297

AN ESTIMATION OF THE ECONOMIC BURDEN OF TYPHOID FEVER ILLNESS IN LOW AND MIDDLE-INCOME COUNTRIES

Vittal Mogasale[1], Brian Maskery[1], R. Leon Ochiai[2], Jung Seok Lee[1], Thomas F. Wierzba[1]

[1]International Vaccine Institute, Seoul, Republic of Korea, [2]International Vaccine Institute, Seoul, Republic of Korea

Background Estimation of typhoid cost of illness in low and middle income countries is important in cost-effectiveness analysis and priority setting for typhoid prevention and control activities such as vaccine introduction. In this paper, we present an estimate of total costs and cost per episode of typhoid fever illness in low and middle income countries by United Nations sub-regions. Methods A decision tree model was developed to represent the clinical outcomes of typhoid illness. The percentage of typhoid cases corresponding to each arm were estimated from a literature review. The indirect costs were measured based on a five country typhoid cost of illness study. The costs related to laboratory diagnosis, service delivery, and medicines were assessed based on literature and World Health Organization data base. Direct and indirect costs were estimated in 2010 United States dollars ($), and segregated by outpatient and inpatient status. Findings The estimated total annual typhoid fever cost of illness in low and middle income countries was $519 million (95% CI=300 million to $836 million) of which 46% came from South Asia. The average cost of typhoid illness per episode was $44 (95% CI= $25 to $69), which

comprised 28% direct costs and 78% of indirect costs. Average cost per outpatient was $31(95% CI=$16 to $52) while the cost per inpatient was about $191(95% CI=129 to 276). The predominant cost drivers were indirect costs, number of episodes in the region and hospitalization rates. Interpretation The main challenge was obtaining the probabilities of health events for the decision tree model due to a lack of data. Our cost estimates were conservative, as typhoid relapse, typhoid death, development of long-term carrier state, and gall bladder cancer were not included. South Asia and East Africa should be the priority for typhoid prevention and control activities due to their high economic burden. There is a need for collecting improved and geographical representative epidemiological and cost data for typhoid fever.

1298

CURRENT EFFORTS OF PHARMACEUTICAL COMPANIES TO ADDRESS THE NEED FOR PEDIATRIC PRODUCT DEVELOPMENT: AN ANALYSIS OF THE R&D PIPELINE

Delphi G. Coppens, Anna V. Massey, Jayasree K. Iyer

Access to Medicine Foundation, Haarlem, Netherlands

The majority of medicines have primarily been developed for use by adults. Treating children with these medicines can be problematic. Children of different ages and weights need different dosages and dosing forms (such as oral liquids) to ensure efficacy, safety, and compliance. Certain age groups also metabolise medicines differently, which needs careful evaluation. In 2007, the World Health Organization (WHO) published its first Essential Medicines List for Children (EMLc). It revealed the need for more evidence on the efficacy and safety of many essential medicines for use in children (including systemic reviews and product development). The WHO repeated this call in 2013 in the update of its report 'Priority Medicines for Europe and the World'. To address such unmet needs, pharmaceutical companies can play a key role, as they have deep expertise on formulation and manufacturing, as well as intellectual property rights on adult medicines. To assess their response, we present a unique analysis of the paediatric R&D activities currently being undertaken by 21 research-based pharmaceutical companies with the highest global market capitalization (as measured by the 2014 Access to Medicine Index). The Index measures the extent to which these companies address the issue of access to medicine for 47 high-burden diseases, with significant overlap of the EMLc. As such, this analysis maps the priority paediatric R&D needs that these companies are addressing, and where gaps remain. Preliminary results show that more than 50% of these companies are involved in paediatric R&D for EMLc diseases, including developing adapted formulations and vaccines specifically for children. The vast majority are medicines target communicable diseases, including tuberculosis and malaria. There are also examples of adapted formulations for non-infectious diseases, including diabetes mellitus. Although limited, adapted formulations for neglected tropical diseases and neonatal health indicates progress in these disease areas as well. This study will be completed in September 2014.

1299

THE PROCESS OF CLINICAL TRIAL IMPLEMENTATION: PARTNERSHIP BETWEEN THE TRIAL RESEARCH TEAM AND LOCAL HEALTH STAFF IN BURKINA FASO, GHANA AND ZAMBIA

Léa Paré Toé[1], Frank Baiden[2], Sebastian Hachizovu[3], James Akizili[2], Halidou Tinto[1], Shepherd Khondowe[3], Yacouba Cissao[1], Raffaella M. Ravinetto[4], Umberto D'Alessandro[5], Koen Peeters Grietens[6], Maxime K. Drabo[1]

[1]Institut de Recherche en Sciences de la Santé, Bobo Dioulasso, Burkina Faso, [2]Navrongo Health Research Center, Navrongo, Ghana, [3]Tropical Diseases Research Centre, N'dola, Zambia, [4]Department of Clinical Sciences, ITM, Antwerp, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Antwerp, Belgium, [5]Department of Clinical Sciences, ITM, Antwerp, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Medical Research Council, Fajara, The Gambia, Antwerp, Belgium, [6]Department of Clinical Sciences, ITM, Antwerp, Belgium

Clinical trials are essential for health research, particularly for the assessment of new health interventions. In order to ensure patients' protection and research quality, these trials follow strict procedures that may not be available for routine practice in poor settings. We researched the partnership between trial teams and local health providers, more specifically whether the technical knowledge gained from the trial is beneficial for local health services in trial settings in Burkina Faso, Zambia and Ghana. We used a quantitative survey and qualitative research methods to collect data from professionals of the Ministries of Health, local health providers and clinical researchers in settings in the respective countries where trials were on-going or had recently ended and compared them to control sites where no trial research had been done. Local health services benefit from the presence of research teams but there is room for improvement. Improved communication with local health staff and their regular involvement in training opportunities provided to the research team, among other factors, could bring long-term improvements to quality of care also in local routine health care, which would be a positive added value of the research in local settings. Clinical trials are beneficial for resource-poor communities not only because of their primary objective of investigating better treatments, but also for the improved quality of care provided during the trial. Nevertheless, local health services could additionally benefit from the trial implementation process, extend its positive impact on routine health care provided to the local populations.

1300

HOSPITALIZATIONS AND DEATHS DUE TO DIARRHEAL AND RESPIRATORY DISEASES AMONG CHILDREN UNDER FIVE YEARS OF AGE-HAITI, 2011-2013

Kavita Vinekar[1], Mentor Ali Ber Lucien[2], Nicholas Schaad[3], Eyal Leshem[3], Ikwo Oboho[3], Gerard Joseph[2], Stanley Juin[4], Fatimah Dawood[3], Umesh Parashar[3], **Rania A. Tohme**[3]

[1]University of Michigan, Ann Arbor, MI, United States, [2]Ministry of Public Health and Population, Port-au-Prince, Haiti, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Centers for Disease Control and Prevention, Port-au-Prince, Haiti

Diarrheal and respiratory diseases are leading causes of morbidity and mortality among children aged <5 years in developing countries; however, data on the burden of these diseases in Haiti are scarce. We conducted a retrospective review of hospital admission registries for children aged <5 years during January 2011 through December 2013 in six major hospitals in Haiti. We recorded numbers of all-cause, diarrheal and respiratory disease admissions by age group, hospital, and epidemiologic week. Diarrheal diseases included diarrhea, gastroenteritis, dehydration, parasitosis, cholera, amoebiasis, dysentery, shigellosis, giardiasis, food poisoning and hypovolemic shock. Respiratory diseases included bronchitis; bronchioloitis; acute sinusitis, epiglottitis, tracheitis, viral rhinitis,

pharyngitis or respiratory illness; bronchopneumonia, asthma, influenza, influenza-like symptoms, bronchiectasis, pneumonitis, laryngotracheitis, croup, pleural effusion, respiratory failure/distress, empyema, pleurisy, apnea, shortness of breath, tachypnea, wheezing, stridor, cough, diphtheria. A total of 31,565 hospital admissions and 1763 deaths were recorded among children aged < 5 years at the six sites. Diarrheal diseases accounted for 8063 (26%) hospitalizations and 224 (13%) deaths. Diarrheal diseases accounted for 39%, and 36% of hospitalizations in children aged 6-11 months and 12-23 months, respectively. While children aged 0-5 months constituted 25% of all diarrheal disease hospitalizations, diarrheal diseases accounted for only 15% of all hospitalizations in this age group. Diarrheal disease admissions peaked in January-April before the rainy season. Respiratory diseases accounted for 9183 (29%) hospitalizations and 301 (17%) deaths. Children aged 0-23 months accounted for 76% of all respiratory disease admissions. Children aged 0-5 months had the lowest proportion of hospitalization due to respiratory diseases (19%) while children aged 6-23 months had the highest (38%). Respiratory disease hospitalizations followed a bimodal seasonal pattern, with peaks during May-June and October-December. Diarrheal and respiratory diseases constitute a significant health burden among children aged < 5 years in Haiti. Having these data before rotavirus and pneumococcal vaccine introduction will be important to monitor the impact of vaccines and other health interventions.

1301

MENTORSHIP FOR GLOBAL HEALTH RESEARCHERS

Hazel E. McCullough[1], Donald C. Cole[2], The Canadian Coalition of Global Health Research International Mentorship Working Group[3]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Dalla Lana School of Public Health, Toronto, ON, Canada, [3]University of Toronto, Toronto, ON, Canada

Mentorship plays a critical role in the development of global health researchers and the strengthening of health research capacity in both high income countries (HIC) and low and middle income countries (LMIC). In 2012 the Canadian Coalition of Global Health Research (CCGHR) brought together colleagues from Canada, Argentina, Chile, Guatemala, Kenya, and the UK/Europe to collate current examples of mentorship practice in global health research (GHR). Using a research story and narrative approach, a set of eleven GHR mentorship research stories were developed on diverse mentorship experiences and programs. Teleconferences and an online project management platform were used to manage global communication, to guide the story development through peer review and reflection, and to promote conjoint analysis. A tabular and curatorial analytic approach highlighted the unique aspects of each of the stories but also provided insights into the challenges, benefits and commonalities that arise in GHR mentorship. The fundamental principles of mentoring were used to develop diverse programs to meet the needs and contexts of the global health researchers for which they were developed. These included mentorship programs for researchers in the field of: global mental health and substance use in Kenya, tobacco control in Argentina, malaria in Sub-Saharan Africa, chronic disease in Guatemala, Chilean led research training in Africa, and Canadian led interdisciplinary programs to create environments for mentorship, bring together mentors and mentees in GHR from HICs and LIMCs in globally hosted summer institutes, and build communities of practice at three Canadian universities. Key outcomes from the project include online access to the mentorship stories in English, French and Spanish, and the creation of an international working group of global health researchers engaged in GHR mentorship within HICs and LIMCs; and who are well situated to contribute to the emergent literature in GHR mentorship.

1302

APPLICATION OF THE UNIFIED THEORY OF ACCEPTANCE AND USE ON COMMUNITY HEALTH VOLUNTEERS' ACCEPTANCE OF MHEALTH

Baaba da-Costa Vroom[1], Richmond Aryeetey[1], Richard Boateng[2], Francis Anto[1], John Gyapong[1]

[1]School of Public Health, University of Ghana, Legon, Accra, Ghana, [2]University of Ghana Business School, Legon, Accra, Ghana

Studies have been conducted to examine the potential benefits of the use of mobile health (mHealth) tools to improve the work of community health volunteers (CHVs). The use of mHealth is expected to enable CHVs take on more challenging tasks and enable them produce more timely and accurate data for their health sectors. However, little is known about CHVs level of readiness and acceptance to use mHealth. This study therefore aims to determine the factors that influence CHVs acceptance and intention to use mHealth applications to collect and report data during mass drug administration (MDA) for lymphatic filariasis control. All CHVs in two districts in Ghana (approx. 300) will complete questionnaires to determine their readiness to adopt and use mHealth for lymphatic filariasis treatment coverage data reporting. The Unified Theory of Acceptance and Use of Technology (UTAUT) model has been used to determine the probability of acceptance of new technology among specific groups to whom new technology tools are being introduced and who are potentially less inclined to accept them. Though this tool has been used to validate technology acceptance among formal health sector workers, little is known about technology acceptance by CHVs. CHVs acceptance and intention to use of mHealth will be measured by four constructs; performance expectation, effort expectation, social influences and other facilitating conditions. The UTAUT model will provide a means to assess the probability of mHealth acceptance and intention to use technology among CHVs. It will also provide theoretical and practical implications for sustaining health sector adoption of mHealth in a low resource setting. This study will be completed by June 2014.

1303

ENVIRONMENTAL PUBLIC HEALTH IMPACTS OF DUST AND SAND STORMS IN CENTRAL AND SOUTHWESTERN REGIONS OF IRAQ

Mohammed A. Kalkhan[1], Sounak Chakraborty[2], Keshab Gangopadhyay[3]

[1]Center for Remote Sensing, University of Kufa, Kufa, Iraq, [2]Department of Statistics, University of Missouri-Columbia, Columbia, MO, United States, [3]Department of Electrical and Computer Engineering, University of Missouri-Columbia, Columbia, MO, United States

Recent severe drought and water shortages in Iraq have led to an increased incidence of devastating dust and sand storms. Drought, degradation of the environment, conflict and climate change are imposing a significant impact on public health with a spatial dimension. Our geospatial approach includes modeling the complex determinants of dust and sand storm development and propagation using remote sensing satellite imagery, geospatial information systems, and geostatistical analysis using modeling techniques with multiscale nested sampling designs. Predictions from this approach are relevant to many environmentally dependent living ecosystems (human, agricultural, water) and can be linked to socio-economic and public health consequences. We account for site specific land ecosystem conditions in Iraq by adapting a geospatial thematic mapping technique that has been successfully used in other types of semi-arid environments at a broad (i.e., "landscape") scale. We use these dynamic geospatial modeling and thematic mapping techniques as a predictive tool to improve the forecasting of dust and sand storms throughout Iraq to better understand the relationship between environment and ecosystem degradation and its impact on public health. A significant outcome of this research is geared towards the analysis of dust and sand dynamics and their effects on sustainable land use decision

making that is important for environmental public health. These geospatial models can inform communities, regional land managers, government policymakers, other constituents and diverse stakeholders regarding the potential impacts of increased dust and sand storms on public health in Iraq.

1304

PRELIMINARY RESULTS OF SENTINEL SURVEILLANCE OF UNDIFFERENTIATED FEBRILE ILLNESSES IN GEORGIA IN 2013

Tinatin Kuchuloria[1], Manana Makhviladze[2], Marine Kanashvili[2], Teona Mikautadze[2], Nora Kokaia[3], Manana Makharadze[3], Nana Gongadze[4], Marina Endeladze[5], Lana Gatserelia[5], Marine Karchava[5], Tengiz Tsertsvadze[5], Maiko Chokheli[6], Nana Mamuchishvili[6], Lile Malania[6], Nino Kiknadze[6], Nazibrola Chitadze[6], Marina Chubinidze[6], Tamar Chikviladze[6], Paata Imnadze[6], Nino Trapaidze[1], Robert G. Rivard[7], Helen Freyberger[8], Mikeljon P. Nikolich[8], Christian T. Bautista[8]

[1]United States Army Medical Research Unit-G/Walter Reed Project Georgia, Tbilisi, Georgia, [2]V. Bochorishvili Sepsis Center, Tbilisi, Georgia, [3]Institute of Medical Parasitology and Tropical Medicine, Tbilisi, Georgia, [4]Medical City Hospital, Kutaisi, Georgia, [5]Infectious Diseases, AIDS and Clinical Immunology Research Center, Tbilisi, Georgia, [6]National Center for Disease Control and Public Health, Tbilisi, Georgia, [7]United States Army Medical Research Institute for Infectious Diseases, Fort Detrick, MD, United States, [8]Walter Reed Army Institute of Research, Silver Spring, MD, United States

This surveillance project seeks to determine the burden of infectious agents of undifferentiated febrile illnesses (UFI) and hemorrhagic fever syndrome (HFS). From June to December of 2013, patients≥4 years of age with a temperature of ≥38°C for ≥48 hours or HFS were enrolled. In addition to blood culture, serologic testing (ELISA) was conducted to detect antibodies against *Leptospira* spp., *Brucella* spp., *Coxiellaburnetii*, CCHF virus, hantavirus, Spotted Fever Group (SFGR), Scrub Typhus group (STG), and Typhus group (TG) *Rickettsiae.* Hantavirus ELISA results were confirmed by IgM/IgG IFA.There were 245 patients enrolled in the study; 30 (12%) returned for the voluntary follow-up visit. Blood culture was positive for only 7 (2.8%). Fourteen (5.7%) patients tested positive by both IgM and IgG against *Brucella* spp. and 29 (11.8%) demonstrated only IgG positivity. *Brucella melitensis* was isolated from one patient. Additionally, *Leptospira* spp. IgM, SFG IgG and *C.burnetii* IgM was positive in 23 (9%), 9 (3.6%) and 7 (2.8%) patients, respectively. Of patients positive for hantavirus, 17 (6.9%) were positive for IgM and 7 (2.8%) were positive for IgG using ELISA. Six of the IgM and 4 of the IgG hantavirus positive samples have been retested using IgM/IgG IFA and were negative. Three (1.2%) patients demonstrated both IgM/IgG and 8 (3%) only IgG positivity against CCHF virus, but none of them had a recent or present history of HFS.These initial results suggest that brucellosis is one of the leading causes of the UFI in Georgia. Additionally our findings suggest that leptospirosis, rickettsiosis and Q-fever are diseases requiring a high index of suspicion by physicians and improved laboratory capacity for correct diagnosis and treatment to take place. Initial ELISA findings on hantavirus and CCHF virus suggest that a more specific test is needed. Surveillance will continue until 2016 to improve the detection and treatment of selected diseases with an emphasis on developing capacity for diagnosis and laboratory confirmation.

1305

A PRELIMINARY ANALYSIS OF THE QUALITY OF PEDIATRIC MEDICINES SUPPLIED BY PRIVATE WHOLESALERS IN KINSHASA, DRC

Benedetta Schiavetti[1], Daniel Ngeleka Mutolo[2], Roland D. Marini[3], Bart De Spiegeleere[4], Epco Hasker[1], Josiane Van Der Elst[5]

[1]Institut of Tropical Medicine Antwerp, Antwerp, Belgium, [2]Direction de la Pharmacie et du Medicament, Kinshasa, Democratic Republic of the Congo, [3]University of Liege, Liège, Belgium, [4]University of Ghent, Ghent, Belgium, [5]Agence Fédérale du Médicament et Produits de Santé, Bruxelles, Belgium

The global pharmaceutical market is characterised by multiple qualitative standards. Low and middle-income countries are particularly permeable to poor quality products: the proportion of substandard medicines in sub-Saharan Africa ranges from 12% to 48%, though accurate figures are not available, especially for paediatric medicines. In the Democratic Republic of Congo, one of the prime objective of the national Health Development Plan 2011-2015 is the reduction of infant mortality and a transversal objective is to ensure that 80% of the medicines available is of good quality. In 2013, the introduction of Minilabs® revealed the presence of substandard products but the actual prevalence of poor-quality medicines in the country is unknown. In the context of a North-South bilateral cooperation program, a cross-sectional survey on the quality of products available in the private market in Kinshasa was carried out with the national medicine regulatory authority (DPM). Paediatric formulations of amoxicillin, artemether/lumefantrine and paracetamol were selected as tracers of medicine quality, based on 8 public health criteria and on the results of informal interviews. Covert shoppers purchased a defined quantity of packs of each brand available in all the licensed wholesalers of the city. To obtain a representative subsample of the most marketed products, the inspectors of the DPM collected the yearly distribution figures from the wholesalers. From all the purchased samples, a weighted subset of 100 for each molecule was randomly selected for analysis. The DPM performed the visual inspection on all the purchased products while the subsample was sent in Belgium and tested according to the United States Pharmacopoeia (USP) analyses. The Medicine Quality Assessment Reporting Guideline was followed for reporting and the information arising from visual inspection was used for identifying lacks in the current legislation. Between 7th and 16th April 2014, 417 samples were collected: 86 paracetamol tablets, 143 amoxicillin and 188 artemether/lumefantrine, both powders for suspension. The visual inspection will be performed in May and pharmacopoeial analyses in August 2014. The overall results are expected by October 2014 and will be presented.

1306

GLOBAL IMMUNIZATION POLICY FORMATION FOR NEW VACCINES

Olwen Jaffe[1], M. Imran Khan[1], Adwoa D. Bentsi-Enchill[2], Ciro de Quadros[1]

[1]Sabin Vaccine Institute, Washington, DC, United States, [2]World Health Organization, Geneva, Switzerland

Stakeholder involvement in the immunization policymaking process is complex and occurs at many different levels. Similarly, the process from vaccine development to implementation and use in an immunization program has many different phases and is typically very lengthy. To ensure that vaccines are having the maximum impact, there is need for vaccine developers to incorporate public health use considerations into these early phases. Implementing vaccine policies can often prove challenging for many countries, yet vaccine developers often overlook these policy challenges In this review paper, international immunization policy is understood to be the immunization policy set by the World Health Organization (WHO) for the purpose of informing regional and national immunization practices and regional immunization policy is the immunization policy set by the six WHO regional offices. To understand

the immunization policymaking process, a review of available documents outlining the various immunization policymaking sub-processes and WHO committees involved in the immunization policymaking process was conducted in concert with a review of available articles on the details of Strategic Advisory Group of Experts (SAGE) and SAGE's working groups, as well as other advisory bodies of the WHO that contribute to the fulfillment of SAGE's mission. Recommendations for immunization policy are made, beginning with at WHO and continuing through the WHO Regional Offices and their respective Immunization Technical Advisory Groups (ITAG), with most finishing with the National Immunization Technical Advisory Groups (NITAG), although others have state or municipal level immunization advisory groups that make even more specific recommendations. Though immunization requirements and laws can only be made at the national, state, or municipal level, the WHO and its Regional Offices play a major role in formulating and influencing national immunization policies. While there is uniformity in the process across national and regional borders, there are stark differences in the actual practice of formulating and/or adopting immunization policy across municipalities, or city or town governments, and countries. This paper attempts to clearly delineate the policymaking process for immunization recommendations from beginning to end, in addition to bringing simplicity and clarity to the intricate process for the average stakeholder.

1307

THE EFFECT OF MASS AZITHROMYCIN DISTRIBUTIONS ON CHILDHOOD MORTALITY: BELIEFS AND ESTIMATES OF EFFICACY

Kieran S. O'Brien, Craig W. See, Jeremy D. Keenan, Nicole E. Stoller, Sun N. Yu, Travis C. Porco, Thomas M. Lietman

F.I. Proctor Foundation, University of California San Francisco, CA, United States

A single cluster-randomized trial conducted in Ethiopia found that mass distribution of azithromycin reduced childhood mortality by 50% in the first year (relative rate, 0.50; 95% confidence interval, 0.29 - 0.86). The magnitude of the observed effect was surprising given that other effective population-level interventions have resulted in more modest benefits. To further investigate, the relative risk of childhood mortality in communities given mass azithromycin distributions was estimated using two different methods: an expert survey and a Bayesian analysis of the cluster-randomized trial. Experts in public health, infectious disease, and demography were asked to estimate the true effect of oral azithromycin distributions on childhood mortality. Separately, an empirical Bayesian estimation of the efficacy was performed. This estimation was determined given the randomized trial's results and prior estimates based on the efficacy of effective non-antibiotic population-level interventions, including vitamin A supplementation and chemoprophylaxis for malaria. The surveyed experts believed mass azithromycin lowers childhood mortality (relative risk, 0.83; 95% credible interval, 0.70 - 1.00). The relative risk from the Bayesian analysis was 0.71 (95% credible interval, 0.39 - 0.93). Both expert opinion and the Bayesian analysis suggest that azithromycin may have a true mortality benefit, but that the most likely effect is smaller than that found in the single available randomized controlled trial. Survey respondents may have used prior information about other beneficial population-level interventions to inform their opinions about the efficacy of mass azithromycin. A large multi-site randomized controlled trial will be necessary to confirm a mortality benefit from mass azithromycin treatments and assess the magnitude of any such benefit.

1308

RECOMMENDATIONS FOR VALID CONSENT FOR RESEARCH WITH ADOLESCENTS IN LOW-INCOME SETTINGS

Phaik Yeong Cheah[1], Michael Parker[2]

[1]*Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand,* [2]*University of Oxford, Oxford, United Kingdom*

Paediatric research is particularly relevant in the tropics where paediatric disease is such an important cause of morbidity and mortality. The current model for consent – where children provide assent (defined as "active agreement") for medical research and their parents must also consent – is not always appropriate, especially in low-income settings. We argue that assuming the research carries minimal risks and meets international ethical guidelines, more emphasis should be placed on the child's wishes. We propose that the default position should be that children who are able to provide valid consent should consent for themselves regardless of age. Many older children (adolescents) in low-income settings have adult responsibilities, may be parents themselves or may be estranged or living independently and not have parents or guardians to look after their interests. The requirements for a valid adolescent's consent should be the same as for adults: (1) the adolescent must be competent, and have the ability to reasonably understand and retain the information, weigh the options and make a decision; (2) the adolescent must be appropriately informed, meaning that the information must be presented in understandable language and illustrated by meaningful examples, and address concerns that are important to adolescents such as stigma and missing school; and (3) the consent must be voluntary and not coerced, taking into consideration that adolescents can easily take to praise and rewards, and may be afraid of adults and those in authority. Apart from these usual requirements, we propose two additional elements: (1) the adolescent must be genuinely mature, meaning he or she has had the life experiences necessary to make such decisions, is able to understand difficult concepts like research, altruism, participant responsibilities and the impact of participation on his or herself and others; and (2) the adolescent should be sufficiently independent: having his or her own accommodation, the ability to travel to attend follow-up visits, and a job or being a parent themselves.

1309

STATISTICAL UNCERTAINTY IMPOSES INHERENT LIMITS ON THE EFFICACY OF TARGETED DISEASE CONTROL

Alex Perkins

University of California Davis, Davis, CA, United States

Contributions of different individuals, groups, or geographic areas to the transmission of infectious diseases are often highly heterogeneous. One of the primary motivations for understanding transmission heterogeneity is the possibility of targeting control measures, such as vaccines, drugs, or insecticides, on individuals, on certain groups, or in areas that make greater contributions to transmission than others. Any effort to target controls must, however, be performed on the basis of some set of measurable factors presumed to be predictive of potential contributions to transmission. The success of efforts to target controls is determined then by the predictive capacity of the factors on which targeting is performed. In light of inherent limits on the capacity of any measurable factor to predict transmission potential, I extend a general and well-known mathematical framework to account for this type of uncertainty. For a given degree of transmission heterogeneity (e.g., 20% of individuals account for 80% of transmission, or the "20/80 rule"), I show how the proportion of variation in transmission potential explained by a set of predictive factors (i.e., R^2) determines the relative benefit from targeting in terms of reducing 1) the critical vaccination proportion, 2) the invasion probability of an emerging pathogen, and 3) the expected size of an outbreak. For the extent of transmission heterogeneity displayed in several well-studied disease outbreaks, I show that significant enhancement of the effectiveness of controls from targeting requires having factors to guide

targeting that explain a substantial proportion of variation in transmission potential. To conclude, I highlight diseases for which factors that could be used to guide targeting appear to be informative, as well as diseases for which predictive factors are unlikely to be found or for which the potential of such factors is not well known. These results highlight the importance of studying factors that underlie transmission heterogeneity and rigorously assessing their predictive capacity.

1310

REAPPRAISING END-OF-LIFE CARE IN THAILAND: A REVIEW OF POLICY AND PRACTICE COMPARED TO THE USA

Rapeephan R. Maude[1], Richard J. Maude[2]

[1]Department of Medicine, Saint Vincent Hospital, Worcester, MA, United States, [2]Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand

In Thailand it is not standard practice to ask patients and family members about code status (do not resuscitate, do not intubate or comfort-measures only) on admission to hospital or to allocate health care proxies. Many Thai patients have not considered these issues prior to the occurrence of critical illness. There are around 3 physicians per 10,000 people in Thailand compared to 24 in the USA, and few work in primary care. The cultural differences are not insurmountable and the US approach to terminal illness may be of benefit to patients in Thailand. A literature review was performed to compare and contrast current practice regarding cardiopulmonary resuscitation and end-of-life care in Thailand with that in the USA highlighting differences in knowledge, attitudes and cultural contexts. We propose that in Thailand early discussion of code status and appointment of a health care proxy should be adopted in hospitals to limit potential unnecessary discomfort and help provide appropriate care for patients with poor prognosis. This will require changes in health policy and training of healthcare providers and education of patients.

1311

MICRONUTRIENT SUPPLEMENTATION DURING PREGNANCY AND ANEMIA IN THE POST-PARTUM PERIOD AMONG WOMEN IN BOLIVIA'S ANDEAN PLATEAU

Adam Lipus[1], Kaitlyn Stanhope[1], Anna Fabiszewski de Aceituno[1], Paulina A. Rebolledo[1], Meghna Srinath[1], Rita Revollo[2], Volga Iñiguez[3], Parminder Suchdev[1], Juan S. Leon[1]

[1]Emory University, Atlanta, GA, United States, [2]The Population Council, La Paz, Plurinational State of Bolivia, [3]Universidad Mayor de San Andrés, La Paz, Plurinational State of Bolivia

Anemia contributes substantially to global morbidity in children and in women of reproductive age and can negatively influence maternal and neonatal outcomes if present during pregnancy. Micronutrient deficiencies (e.g., iron) underlie a substantial portion of the burden of anemia. Thus there is a need to quantify the prevalence of anemia as well as attitudes regarding and acceptability of micronutrient interventions among mothers and pregnant women. In Bolivia, the national universal health plan includes micronutrient supplementation for pregnant and post-partum women (pills containing iron, folic acid, and vitamin C) and for children age 6-24 months (multiple micronutrient powders). Our study assessed anemia status, access to and use of micronutrient supplements, and perceptions regarding the acceptability of supplementation among a predominantly indigenous population of mothers in El Alto, Bolivia, located in the Andean Plateau. Mothers (n=381) of one-month old infants recruited at well-child visits at two hospitals from May 2013 to March 2014 completed interviews on socio-demographic characteristics and prior use of micronutrient supplements. Researchers also performed Hemocue on venous samples and adjusted hemoglobin cutoff values for anemia according to altitude. Promisingly, 89% of mothers reported receiving iron pills during pregnancy, 76% reported taking iron, and only 24% were found to be anemic. However, more than a third of the women who took iron pills reported difficulty in taking these supplements. Similarly, less than a third of women reported having given their age-eligible children multiple micronutrient powder sachets, and only 47% of these women believed that other women would want to use them during pregnancy. These results suggest that coverage of multiple micronutrient supplementation in children lags behind that of iron supplementation of pregnant women. Furthermore, efforts to improve the desirability of these supplements may be necessary in order to maximize adherence among those who receive them.

1312

PREVALENCE OF EARLY-ONSET NEONATAL INFECTION AMONG NEWBORNS OF MOTHERS WITH BACTERIAL INFECTION: A SYSTEMATIC REVIEW AND META-ANALYSIS

Grace J. Chan[1], Abdullah H. Baqui[2], Anne C. Lee[3], Jingwen Tan[2], Robert E. Black[2]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]Brigham and Women's Hospital, Boston, MA, United States

Although neonatal infections cause a significant proportion of deaths in the first week of life, little is known about the burden of neonatal disease originating from maternal infection or colonization globally. We estimate the prevalence of vertical transmission - the burden of neonatal infection among newborns exposed to maternal infection.We searched Pubmed, Embase, Scopus, Web of Science, Cochrane Library, and WHO Regional Databases for studies of maternal infection, vertical transmission, and neonatal infection. Studies that measured prevalence or incidence of bacterial vertical transmission were included. 122 studies met the inclusion criteria. Random effects meta-analyses were used to pool data to calculate prevalence estimates of vertical transmission. The prevalence of early onset neonatal lab-confirmed infection among newborns of mothers with lab-confirmed infection was 17.2% (95%CI 6.5-27.9). The prevalence of neonatal lab-confirmed infection among newborns of colonized mothers was 1.1% (95%CI 0.2-2.0). The prevalence of neonatal surface colonization among newborns of colonized mothers ranged from 30.9-45.5%. The prevalence of neonatal lab-confirmed infection among newborns of mothers with risk factors ranged from 2.9-19.2%. Only seven studies (5.7%) were from high neonatal mortality settings. Considerable heterogeneity existed between studies given the various definitions of infection, colonization, and risk factors of infection. The prevalence of early-onset neonatal infection is high among newborns of mothers with infection or risk factors for infection. More high quality studies are needed particularly in high neonatal mortality settings to accurately estimate the prevalence of early-onset infection among newborns at risk.

1313

TRAINING LAYPERSONS BASIC TRAUMA TECHNIQUES IN LOW-INCOME COUNTRIES

Amina Merchant, Kelly Mcqueen

Vanderbilt University, Nashville, TN, United States

Trauma accounts for over 300 million years of healthy life and 11% of the disability-adjusted life years (DALYs) worldwide. Reduction of DALYs and mortality are linked to adequate prehospital care and decreased transport times to definitive care. Given the financial and resource constraints in low-income countries, simple but systematic prehospital training programs for laypersons have been implemented in rural villages to stabilize patients. Most prehospital deaths are the result of airway compromise, respiratory failure or uncontrolled hemorrhage; all three of these conditions can be addressed by laypersons using basic first aid measures. The hypothesis is that basic prehospital and primary hospital interventions made by layperson first responders and healthcare personnel will decrease mortality and increase the number of capable first responders. In order to test this hypothesis, communities with hospitals that advertise surgical capacity in Mozambique were assessed. Six hospitals and communities served as the intervention group that receives training on four basic resuscitative

and stabilizing efforts in their native language in the Zambesia province of Mozambique. Community members received a four-hour seminar that taught four basic resuscitative and stabilizing interventions prior to transport by ambulance or taxi/bus. These techniques include a modified ABCD (airway, breathing, circulation, disability) noted in developed nations. A is for airway opening that allows victims to receive oxygen by simply opening their mouths and removing any foreign objects if present. B is for bleeding – participants learned how to apply compression or a tourniquet. C represents cervical spine immobilization with simple tools. D is for disability which is reduced by transporting victims with a flat, immobile, safe method. Hospital personnel received the same ABCD training as the community with two additions – vital sign monitoring and IV fluid resuscitation as they are markers of shock and injury. Pre- and post- tests were administered to participants in their native language. Results of the study suggest community members can be trained in basic resuscitative techniques. In conclusion, while laypersons and hospital personnel may receive and feel comfortable administering basic resuscitation techniques, further data must be collected to see if this intervention improves mortality.

1314

PAPER TEST CARDS FOR SCREENING PHARMACEUTICAL QUALITY IN THE FIELD

Nicholas Myers, Abigail Weaver, **Marya Lieberman**

University of Notre Dame, Notre Dame, IN, United States

This presentation will describe a "lab on paper" that can characterize low quality pharmaceutical dosage forms and detect active pharmaceutical ingredients in falsified "herbal" medicines. The test card carries out a dozen color tests in parallel in under 5 minutes, producing a characteristic "fingerprint" of colors in the readout area. Pharmaceutical products which contain little or no active ingredient or which include substitute ingredients give different fingerprints from authentic products, as do "herbal" medicines that are actually spiked with pharmaceutical ingredients. The test cards can be read by eye or with an image analysis program. They are portable, easy to use, and testing of dosage forms can be done in minutes on the corner of a desk. In blinded lab validation studies, the sensitivity and selectivity values for detection of very low quality antibiotics, antimalarials, and tuberculosis medicines are measured as more than 90%. In this presentation, correlations between paper test card results by naive and expert readers and between test card results and HPLC analysis of field samples will be presented. Samples include authentic and falsified drugs as well as "herbal" medications provided by collaborators in Kenya, the US Food and Drug Administration, and the Israeli Ministry of Health Division of Inspection and Enforcement. I will also discuss a new test card for quantitative analysis of beta lactam antibiotics; this card could be used to detect substandard or degraded medications even if there is no giveaway signal from an unapproved excipient. The role of inexpensive screening tests as the top of a "funnel" for monitoring very low quality drugs globally will be discussed.

1315

A COHORT STUDY ON BREASTFEEDING AND EARLY INFANT FEEDING PRACTICES IN THE FIRST SIX MONTHS OF LIFE IN FORTALEZA, CEARÁ, BRAZIL

Bruna L. Maciel[1], Milena L. Moraes[2], Noélia L. Lima[2], Alberto M. Soares[2], Isabelle F. Cruz[2], Cláudia B. Abreu[3], Richard L. Guerrant[4], Aldo A. Lima[3]

[1]Universidade Federal do Rio Grande do Norte, Natal, RGN, Brazil, [2]Federal University of Ceara, Fortaleza, Brazil, [3]Federal University of Ceara and MAL-ED Network, Fortaleza, Brazil, [4]University of Virginia and MAL-ED Network, Charlottesville, VA, United States

Nutritional transition occurs in Brazil, and contrasting trends such as the co-existence of obesity/overweight and anemia are found in our population. The determinants of these trends in children may be associated with early and poor complementary feeding in the first months of life. This study aimed to describe breastfeeding, feeding practices and nutritional status in early childhood in a community from northeast Brazil. A cohort of the 6 first months of life of 242 children was conducted from November, 2010 to February, 2013. Exclusive breastfeeding was received by 64.5% of the children in the first month of life and only 4.8% in the 6th month of life. Complementary feeding was early offered to children: 9.5% received grain derived foods, 15.3% were feed with infant formulas and 13.1% with other milks in the first month of life. We observed increases in z scores for weight-for-age, length-for-age and weight-for-length during the follow up. The prevalence of high weight-for-length and high weight-for-age was 18.9% and 14.9% in the 6th month of life. Nevertheless, at 7 months of age, 42.1% of children had hemoglobin levels under 11mg/dL. The reduction in exclusive breastfeeding during the 6 months of study was associated with the prevalence of high weight-for-length (Chi-squared, $p < 0,0001$). Data is consistent with the nutritional transition phenomena occurring in Brazil and shows the need for public policy focusing on overweight and healthy feeding practices.

1316

ROSIE THE SPRAY TEAM LEADER, EXPANDING OPPORTUNITIES FOR WOMEN IN MALARIA PREVENTION

Jennifer Burnett, Jane Coleman, Nicole de Gier

Abt Associates, Bethesda, MD, United States

The President's Malaria Initiative (PMI) currently conducts indoor residual spraying (IRS) in 12 countries in Africa. Local country teams of 15-20 full time staff members in each country organize the spray campaign and hire hundreds or even thousands of seasonal employees to serve in roles ranging from spray operators to storekeepers and supervisors, all of which are essential to a successful spray campaign. Traditionally, women have been under represented in the IRS workforce and have generally been employed in lower level positions, such as washers. Four gender assessments were conducted in Ethiopia, Rwanda, Ghana, and Senegal in 2013 in order to better understand the number and type of positions that women held in IRS campaigns, identify gender-related challenges and constraints, and suggest areas for improvement. The assessments included focus group discussions, interviews, and an analysis of spray operations. The results indicate that women experience cultural, structural, and social challenges when joining the IRS workforce. Such challenges include women's lack of self-determination in regards to their participation in the spray campaign and perceptions that women are not physically fit enough to be spray operators. PMI has increased women's participation in IRS by specifically targeting them for recruitment through meetings held at the community level and adapting information, education, and communication materials to incentivize women to join the IRS workforce. As a result of these assessments and efforts to increase the number of women employed, and to ensure that they are employed in higher level positions such as spray operators and storekeepers, women currently hold 25% of IRS positions on average. Out of the thousands of workers trained for the 2012 and 2013 Rwanda spray campaigns, 26% and 31% (respectively) were women. This percentage increased to 50% of participation for women in the most recent 2014 spray campaign. This presentation will detail how IRS programs are working toward the proven and achievable goal of equal gender participation in other African countries who conduct IRS.

1317

EXPANDING HEALTH MINISTRY CAPACITY TO DELIVER MALARIA AND OTHER HEALTH COMMODITIES AT THE COMMUNITY LEVEL IN NIGERIAN STATES

William R. Brieger[1], **Bright C. Orji**[2], Emmanuel Otolorin[2], Hajara John Moses[3], John Orok[4]

[1]The Johns Hopkins University, Baltimore, MD, United States, [2]Jhpiego, an Affiliate of The Johns Hopkins University, Abuja, Nigeria, [3]Bauchi State Ministry of Health, Bauchi, Nigeria, [4]Akwa Ibom State Ministry of Health, Uyo, Nigeria

The highly participative process of community directed interventions (CDI) was first pioneered in 1996 by the African Program for Onchocerciasis Control for the delivery of ivermectin. CDI was further tested and found effective in delivering other health commodities. In 2007 Jhpiego began a proof of concept project in Akwa Ibom State, Nigeria and learned that CDI could be a useful vehicle for increasing access to and coverage of malaria in pregnancy interventions. Building on this success, Jhpiego expanded this work to include integrated community case management of malaria, diarrhoea and pneumonia. through community led efforts. The World Bank Malaria Booster Program, observing Jhpiego's efforts in Akwa Ibom State, asked the Nigeria National Malaria Control Program to enlist Jhpiego's help in building the capacity of seven State Ministries of Health (MOH) to organize CDI for what was termed the malaria plus package consisting of community case management and health promotion activities. The scale-up process started with workshops for state CDI implementation teams consisting of staff from malaria control and primary health care in the MOHs. Then these state teams developed their own intervention packages and organized workshops for local government teams, who in turn trained staff from their front line health facilities. These facility staff mobilized communities in their facility catchment areas (wards) to select volunteers for training on the CDI process and intervention package. Although technical assistance was provided to each state, challenges arose including commodity supplies and coordination among different program units within the state MOHs. In conclusion, state teams can train local government teams, ultimately cascading CDI to the community in order to scale up maternal and child health interventions.

1318

INFRASTRUCTURE INDEPENDENT POINT-OF-CARE MOLECULAR DIAGNOSTICS FOR LOW-RESOURCE SETTINGS

Robert A. Burton, Bernhard Weigl, David Boyle, Jered Singleton, Dylan Guelig, Steven Diesburg, Paul LaBarre

PATH, Seattle, WA, United States

In low-resource settings (LRS), limited access to centralized medical facilities presents a critical barrier to timely diagnosis, treatment, and related control and elimination of infectious diseases. Inadequate diagnostic laboratory infrastructure results in increased costs, lost test results, delays, and loss to follow-up associated with specimen transportation to health centers and subsequent response. At the same time, the most accurate diagnostic tests with low limits of detection (LODs) and high clinical sensitivity and specificity are only available through laboratory-based testing and more recently through portable nucleic acid amplification tests (NAATs). Indeed, NAATs are becoming increasingly important to identify and prevent transmission from asymptomatic infections (e.g. malaria) using active infection detection interventions in eliminating countries. NAATs are similarly important for early infection detection scenarios such as early infant or acute case detection (e.g. HIV). Unfortunately, many NAAT approaches are still tied to laboratory-style equipment and instrumentation such as heat blocks, centrifuges, optical detectors, and refrigerated cold-chain logistics, and therefore have limited reach. Additional hurdles must be overcome when considering specimen acquisition and lysis, and nucleic acid extraction and purification sufficient for subsequent amplification and detection in a NAAT. Isothermal amplification NAATs seem to address some low-resource requirements by obviating the need for thermal cycling and improved enzyme tolerance to inhibitors, reducing sample purification requirements. To date, appropriate, portable, rapid, infrastructure-independent, highly accurate NAATs that meet the WHO ASSURED guidelines remain elusive. In this presentation, we will address important considerations for the utilization of molecular diagnostics in LRS and present recent advancements from PATH's product development partnerships toward increasing access to sample-to-results NAATs in remote communities with limited resources, electricity, and infrastructure.

1319

SHARE YOUR FINDINGS: A GUIDE FOR SCIENTISTS AND MEDIA PROFESSIONALS TO GENERATE PUBLIC INTEREST IN RESEARCH

Debora Miranda[1], Douglas Hopper[2]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Bill & Melinda Gates Foundation, Seattle, WA, United States

Global health research aims to address the inequalities in health and improve the lives of populations at risk. But are research outcomes being effectively communicated to those who can put them into practice? An increasing number of funders see the value in allocating resources (budget and staff time) to the dissemination of research and results. Media professionals - from press and communications officers at research institutions to broadcast, print or online journalists - can make an important contribution in bridging the gap between academia and communities affected by global health issues. But many scientists still find themselves feeling frustrated about their work being simplified when it is communicated to wider audiences. On the other hand, the open access movement and the social media revolution are paving the way for scientific knowledge to be broadly publicised. This presentation will share lessons learned by representatives involved in the collaborative process of pitching and disseminating research, with the aim to increase collaboration and best practice. These include communications professionals from research institutions and a research funding organisation, an editorial member of a major peer-reviewed scientific journal, a journalist who has reported on and from an endemic country and a global health scientist with experience in translating research findings into policy.

1320

DE NOVO MICROSATELLITE MARKER MINING FROM SCARCE AMOUNTS OF *CULICOIDES* GENOMIC DNA: PATHWAY TO UNDERSTANDING DISPERSAL AND POPULATION OF THE VECTOR OF OROPOUCHE VIRUS

Maria Gorreti Onyango

Australian Animal Health Laboratory, Geelong, Australia

Oropouche virus is a member of the family Bunyaviridae and the cause of an important arboviral disease in South America. Since its first isolation in 1955, the virus has caused more than 30 epidemics and half a million infections. *Culicoides paraensis* is the major vector of Oropouche virus in urban epidemics of the disease. Dispersal and isolation levels of the different vector populations are key factors for spread of the pathogen and the potential vector control tools. Populations genetic studies of *C. paraensis* will be facilitated by improved genomic resources of the vector. Microsatellites are among the most informative and frequently used genetic markers. Their novel isolation from non-colonizable organisms and with limited quantity of genomic DNA (such as *Culicoides* vectors) can be a major challenge. Identifying effective means of increasing the amount of DNA for de novo microsatellite isolation from *Culicoides* spp. will facilitate study of their genetic variability and adaptation. *C. brevitarsis* is a known vector for bluetongue virus in Australia. Its genomic size overlaps that of *C. paraensis*. DNA from two pools of 15 female *C. brevitarsis* was amplified using the multiple displacement amplification technique. This was subsequently sequenced on ¼ picotitre plate of 454 GS FLX Roche sequencer. A total of 120 005 reads was obtained,

2594 putative microsatellite repeats were isolated from the raw reads using Msatcommander 8.0.2 program. 528 primers were designed to the flanking regions of the microsatellite repeats using primer 3 software. A fraction of these primer pairs were selected for validation. Eight of the primer pairs that amplified 100% of the populations have successfully been used to genotype 96 individuals from two populations of *C. brevitarsis*. This study has been able to overcome huge technical constraint due to the very tiny size of this vector and has developed technical workflow easily translatable to *C. paraensis*, an important vector of Oropouche virus.

1321

COMMUNITY BASED INDOOR RESIDUAL SPRAYING THE TOOL FOR REDUCING COST AND COMPLEXITY OF IRS: A PILOT STUDY IN TANZANIA

Rubona M. Joshua, Rubona M. Joshua

RTI - International, Mwanza, United Republic of Tanzania

Tanzania mainland has implemented indoor residual spraying (IRS) using different operational designs, starting with highly centralized (2007-2009) and medium decentralized (2010-2013). These two approaches were perceived to be complex to manage and expensive. We report a pilot of community based IRS (CBIRS) which is less complex, relatively cheaper and more community owned. CRIRS was organized and implemented at the village level, including: recruitment of spray operators by village governments; use of bicycles by spray operators for transportation; consent by village government to implement IRS; and construction of effluent waste disposal structures using local materials. To evaluate CBIRS pilot, focus group discussion were undertaken with Regional and District IRS technical teams, site managers, sub-site supervisors and Team Leaders, Village mobilizers and Site based mobilizers, Spray operators and community leaders. The evaluation also reviewed IRS implementation guide, IRS performance report, IEC meeting minutes, supervisors report and undertook inspection of constructed sub-sites for compliance to environmental requirements. The evaluation suggests that objectives of CBIRS were attained. CBIRS reduced implementation cost; increased community participation and ownership; reduced organizational complexity of IRS; achieved acceptable quality and quantity of IRS; and maintained compliance to environmental protection requirements. The evaluation revealed aspects that need improvement: training of team leaders was inadequate to cover their important roles in CBIRS; village mobilizer and sub-site supervisor were redundant; effluent disposal sites were unnecessarily large compared to small number of spray teams in CBIRS; and installation of two soak-pits was unnecessary as one pit can accommodate the small amounts of effluent waste generated by a small team.Community based IRS is an ideal approach to reduce cost and complexity of implementing IRS in Tanzania. Some modifications need be considered which include; omitting unnecessary roles like village mobilizer and sub-site supervisor; simplify fabrication of effluent waste disposal structures; and increasing the level of team leaders' training.

1322

NOVEL DETECTION OF *CARDINIUM* ENDOSYMBIONT IN *CULICOIDES* SPECIES

Peter T. Mee[1], Ary A. Hoffmann[2], Andrew R. Weeks[2], Peter J. Walker[1], Jean B. Duchemin[1]

[1]CSIRO Australian Animal Health Laboratory, Geelong, Australia, [2]Pest and Disease Vector Group, Bio21 Institute and the Department of Genetics, University of Melbourne, Melbourne, Australia

Culicoides are blood-sucking midges identified as one of the most significant genera in the family Ceratopogonidae, due to the ability to transmit a diverse range of pathogens. *Culicoides* serve as vectors of medically significant viruses, such Oropouche virus, and transmit a range of nematodes species including *Mansonella perstans* which causes perstans filariasis in Africa and South America. *Culicoides* also transmit a range of viral and filarial pathogens of veterinary importance. Global occurrence, capability of rapid and widespread dispersal and lack of effective control options makes *Culicoides* a major risk factor for the introduction and spread of these pathogens. In recent years, the characterization and use of endosymbiotic bacteria for the prevention of mosquito-transmission of pathogens has proved to have a high success rate in the laboratory. The most predominant example of this being the transinfection of *Wolbachia* into the dengue mosquito vector *Aedes aegypti* and the subsequent blocking of the mosquito's ability to transmit dengue virus. *Wolbachia* and *Cardinium* are both naturally occurring bacterial endosymbionts which infect *Culicoides*. There is currently a lack of information on the distribution and occurrence of these bacterial endosymbionts within these insects and their effects, if any, on pathogen transmission. This study has profiled the distribution of *Culicoides* species in south-eastern Australia and developed a range of screening assays to detect low level infections and explore the distribution of these endosymbionts. We have identified *Cardinium* infection in a range of *Culicoides* species including some of the most significant vector species. Sequence analysis has revealed that this is the same *Cardinium* species which is infecting multiple *Culicoides* species from a range of geographical locations including Japan, Israel, Madagascar, Australia and Africa. Experiments are currently underway to determine the potential influence that *Cardinium* infection may have on the host *Culicoides*. The identification and profiling of the endosymbiont *Cardinium,* could provide the first step towards endosymbiont-based control of these significant vectors of both medically and veterinary important pathogens.

1323

GLUTAMATE-GATED ION RECEPTORS IN THE TSETSE FLY *GLOSSINA MORSITANS MORSITANS*

Paul O. Mireji

KARI-Trypanosomiasis Research Institute, Nairobi, Kenya

Insect glutamate-gated receptors include ionotropic receptors (IRs) that mediate detection of volatiles, ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs) which mediate impulse transmission across synapses. The IRs, are expressed in antennal coeloconic sensillae neurons, while iGluRs and mGluRs are expressed on post-synaptic membranes in central nervous system. In order to identify the *Glossina morsitans morsitans* IRs, iGluRs and mGluRs, known homologs from *Drosophila melanogaster* and ab initio approach based on glutamate-gated channel specific domains were used to search *Glossina* genome assembly and transcriptome Yale strain GMOY1.1. Phylogenetic relationships among *Glossina* IRs, iGluRs and mGluRs and their drosophila and anopheles homologs were determined using Maximum Likelihood estimates, and numerical relationships with selected diverse species gleaned from Phylome database. Relative expression levels among *Glossina* IRs, iGluRs and mGluRs were established using RNA-seq data of adult female fly. Overall, 40 putative glutamate-gated receptor loci comprising 19, 15 and six IRs, iGluRs and mGluRs respectively were recovered in *Glossina*. The *Glossina* iGluRs and mGluRs had higher sequence conservation than IRs relative to drosophila homologs. The *Glossina* IRs lacked at least one glutamate interacting residues except IR8a and IR25a, which showed high sequence similarity to iGluRs. Relative to D. melanogaster, annotation of *Glossina* revealed lower numbers of IRs, but certain loci had multiple related copies. The iGluRs numbers were similar, while mGluR-like loci were more. Suggestively, *Glossina* over-invests specific IR gene lineages for odor detection, but broadens odor-space discrimination in CNS. There was no glutamate-gated homolog of IR93a recoverable in *Glossina*. Apparently, there were three species-specific divergent IRs, perhaps relating to *Glossina*'s stable host range, thus reducing the range of odors to sample unlike other diptera. Because glutamate-gated receptors mediate rapid neuronal communications, they could be perfect targets for manipulation towards improving tsetse control tools.

1324

TARGETING EDUCATIONAL CAMPAIGNS FOR PREVENTION OF VECTOR-BORNE DISEASE: AN ASSESSMENT OF RURAL VS. URBAN SETTINGS IN THAILAND

Macy Brusich[1], John Grieco[2], Theeraphap Chareonviriyaphap[3], Nicole Achee[1]

[1]University of Notre Dame, Notre Dame, IN, United States, [2]Uniformed Services University of Health Sciences, Bethesda, MD, United States, [3]Kasetsart University, Bangkok, Thailand

Vector-borne diseases, such as malaria and dengue fever, are transmitted to humans by mosquitoes. Thailand, an endemic country for both of these diseases, serves as a platform to characterize the relationship between household vector control practices and individual health-seeking behavior. Such studies can guide educational campaigns for the awareness and prevention of disease as this relationship may vary between rural and urban settings. The overall goal of this study was to assess differences between knowledge, attitude, and practice (KAP) in persons presenting to health clinics with malaria and/or dengue fever manifestations in two distinct study sites in Thailand for the purposes of identifying key variables at the individual and household level that influence health behavior related to the prevention of vector-borne disease. Specific methodologies included a survey questionnaire performed at healthcare facilities followed by household mosquito collections and house structure characterization. Analyses will include whether or not the presence of mosquitoes, perception of exposure to mosquitoes and/or current acceptance and uptake of mosquito control practices at the household level differs between rural and urban study sites. Field activities will be completed July 2014 to be presented to the Ministry of Health of Thailand to serve as a guide for enhanced targeting of educational campaigns for the prevention of vector-borne diseases.

1325

EMERGING RESEARCH ON DIPTERA AS MECHANICAL VECTORS: THE CASE OF *BACILLUS ANTHRACIS*

Tatiana Eversley[1], David Adams[2]

[1]AASU, Savannah, GA, United States, [2]AASU/Medical College of Georgia, Savannah, GA, United States

Flies (Order Diptera) are well known for their role as mechanical vectors for enteric pathogens. Recently, however, researchers have found that certain flies, e.g., the Bluebottle Blow Fly (*Calliphora vicina*), the common house fly (*Musca domestica*), and the stable fly (*Stomoxys caltitrans*) are efficient mechanical vectors for *Bacillus anthracis*. Moreover, investigators have concluded that flies helped to trigger *B. anthracis* outbreaks that spanned not only neighboring districts but also international borders. This presentation will examine not only recent developments regarding the role of flies in anthrax outbreaks but also recommend possible prevention strategies.

1326

SCIENTISTS, PUBLICS AND TRANSGENICS: INFORMATION, TRUST, COMMUNICATION AND ENGAGEMENT ON RESEARCH DEALING WITH VECTOR-BORNE DISEASES

Christophe Boete[1], Uli Beisel[2], Luisa Reis Castro[3], Nicolas Cesard[4], Guy Reeves[5]

[1]Institut de recherche pour le développement, Marseille, France, [2]University of Bayreuth, Bayreuth, Germany, [3]Massachusetts Institute of Technology, Cambridge, MA, United States, [4]Kyoto University, Kyoto, Japan, [5]MPG-Plön, Plön, Germany

Infectious diseases transmitted by mosquitoes represent a burden for a variety of countries and especially for the Global South. However research aiming at better understanding them is mainly conducted by institutions from the Global North. Apart from bringing knowledge in biology, this research is obviously associated with the development of methods aiming at reducing the burden of vector-borne diseases and this includes the creation, the use and the release of transgenic mosquitoes. For many in the scientific world, this technological approach offers a promising method against diseases such as malaria or dengue. However the recent field releases of transgenic mosquitoes in The Cayman Islands, in Malaysia and in Brazil have been the source of intense debate in the specialized press as well as in the non-specialized mass media. This lack of transparency, not to say the secrecy, in the way the first trial was conducted is without much doubt the major reason for the controversy that emerged. Brushing aside years of discussion in the scientific world and a shared recognition of the importance to consider ethical, legal and social issues this first trial could be read as a fait-accompli: the cage of transgenic mosquitoes has now been opened. In the complex interactions between science and society around GM technology we cannot avoid questions around the perception of the public by scientists and the related question: How to consult, involve and engage a variety of publics in an effective manner on science and technology? With the will to better estimate the impact of geographic differences (endemic vs non endemic countries), of research topics (work on transgenic approach or not) and of perception of research (applied/ fundamental) we have conducted in 2012/ 2013 a worldwide web-based survey on more than 1800 scientists working on vector-borne diseases. This work reveals several interesting points including the reluctance in involving the public upstream, some lack of confidence in private business as well some level of distrust towards biotechnological progress and the speed at which changes occur because of science and technology. Surprisingly it also highlights a real lack of communication even inside the scientific community.

1327

GENETIC DIVERSITY AND POPULATION STRUCTURE IN THE *LEISHMANIA* VECTOR *LUTZOMYIA (NYSSOMYIA) ANDUZEI* (DIPTERA: PSYCHODIDAE) FROM THE BRAZILIAN AMAZON

Vera M. Scarpassa, Adrya da Silva Figueiredo, Ronildo Baiatone Alencar

Coordenacao de Biodiversidade, Instituto Nacional de Pesquisas da Amazonia, Manaus, Brazil

Lutzomyia anduzei has large geographic distribution in northern South America. This species has been implicated as a secondary vector of *Leishmania guyanensis*, the etiological agent of cutaneous leishmaniasis, in the Brazilian Amazon. In despite of possible involvement of *L. anduzei* in the leishmaniasis transmission, its biology and ecology are poorly known and none population genetics study was performed with this species. We sequenced 74 specimens of six *L. anduzei* localities from the Brazilian Amazon by analyzing 1201 base-pairs of the *COI* gene (mtDNA) to assess genetic diversity and population structure. The genetic diversity was fairly high with 58 haplotypes. Although none haplotype was shared among the localities, all haplotypes were connected in the network. The genetic diversity intra-population was fairly high for all samples (h = 0.859 to 1.000; π = 0.00601 to 0.01008). Values of pair-wise F_{ST} had a large range from 0.042 to 0.413, which were statistically significant (P<0.0001) for the most of comparisons. Similarly, the hierarchical analysis was highly significant among samples (F_{ST} = 0.166; P<0.0001), and the sequence divergence ranged from 0.75 to 1.30%. These results suggest that populations of *L. anduzei* consist of very high genetic variability; however, the gene flow was reduced among populations analyzed that resulted in moderate to large genetic structure. These findings may be implication in the transmission of *Leishmania* and in the control efforts across its range.

1328

DOG SEROLOGY FOR CUTANEOUS LEISHMANIASIS IS ASSOCIATED WITH SAND FLY VECTOR ABUNDANCE AND SUGGESTS ENDEMIC TRANSMISSION IN RURAL PANAMÁ

Luis F. Chaves[1], Azael Saldana[2], Indra Rodriguez[3], Kadir Gonzalez[2], Chystrie Rigg[2], Vanessa Pineda[2], Marcia D. Laurenti[4], Jose E. Calzada[2]

[1]Nagasaki University, Nagasaki, Japan, [2]Instituto Conmemorativo Gorgas de Estudios de la Salud, Panama, Panama, [3]Universidad de Panama, Panama, Panama, [4]Universidade de Sao Paulo, Sao Paulo, Brazil

American Cutaneous Leishmaniasis (ACL), beyond a neglected tropical disease, is a zoonosis, where several vertebrates can be infected by *Leishmania* spp parasites. The body of evidence supporting a reservoir role for dogs (*Canis familiaris*) remains contradictory, and it is unclear whether dogs have become major reservoirs in eco-epidemiological settings that have undergone major ecological transformations. Between April and June of 2010, we studied canine ACL in 52 dogs belonging to 24 households in Trinidad de Las Minas, Western Panamá. We collected information on potential ecological (domestic animal abundance, wildlife animal species diversity among others, vegetation, peridomiciliary structure and housing quality), entomological (sand fly abundance) and epidemiological (human infections) risk factors at the household level, as well as, blood samples and information on the health status of each individual dog. Blood samples were employed for *L.* spp/ *L. panamensis* PCR, ELISA and IFAT diagnostics. Bayesian evaluation of the serodiagnostics in absence of a gold standard, showed ELISA to be the most sensitive (0.79) and specific (0.84) diagnostic. ELISA based canine ACL seroprevalence was 47%. At the household level we found *Lutzomyia trapidoi* was the main risk factor for ELISA seropositive reactions (ESR) in dogs, increasing the odds ratio (OR) 2.28 by each sand fly caught inside the households/trap-night (SFA). At the individual level the OR of dog ESR increased 3.39 and 1.35 times by each SFA and year of age, respectively. Finally, the age specific ELISA based canine ACL seroprevalence curve allowed the estimation of a basic reproductive number (R_0 ± S.E.) of 1.22 ± 0.09 which indicates that canine ACL is endemically established in dogs at our study site. Our data suggest: i) that dogs are likely an incidental *Leishmania panamensis* host where LCA is endemically established and ii) that R_0 estimates from serological surveys should be interpreted cautiously, since they are only a robust indicator for endemic establishment in a focal population.

1329

SLEEPING HABITS AFFECT ACCESS TO HOST BY CHAGAS DISEASE VECTOR *TRIATOMA DIMIDIATA*

Etienne Waleckx, Rafael Pasos-Alquicira, Maria Jesus Ramirez-Sierra, Eric Dumonteil

Universidad Autonoma de Yucatan, Merida, Yucatan, Mexico

In the Yucatan peninsula (Mexico), the causative agent of Chagas disease *Trypanosma cruzi* is transmitted by the bug *Triatoma dimidiata*. While *T. dimidiata* invades and colonizes houses in other regions, this species has an intrusive behavior in Yucatan, probably attracted by artificial light and potential hosts, but has limited ability to establish colonies. Bugs collected inside the homes also have a low nutritional status, suggesting that they cannot efficiently feed inside these houses. We hypothesized here that this low feeding status of *T. dimidiata* may be associated with the local practice in Mayan communities to sleep in hammocks instead of beds, as this sleeping habit could be an obstacle for triatomines to easily reach their host, particularly for nymphal instars which are unable to fly. To test this hypothesis, we used an experimental chamber of 100 cm x 50 cm x 50 cm in which we placed a miniature bed in one side and a miniature hammock on the other side. After placing a mouse enclosed in a small cage in the bed and another one in the hammock, *T. dimidiata* specimens were released in the chamber and their activity was video recorded (7 pm-7 am). Our results show that bugs were similarly attracted to both mice in the bed and in the hammock. However, they were able to reach the mouse located in the bed significantly more frequently than that located in the hammock. Adults reached the bed most frequently by walking, while they reached the hammock most frequently by flying. Interestingly, nymphs were also able, in few occasions, to reach the mouse in hammock by walking. Our conclusion is that sleeping in hammocks as in rural Yucatan makes the host less accessible to triatomines and may explain, at least in part, the low nutritional status and limited colonization of houses by *T. dimidiata* in the region.

1330

ONCHOCERCIASIS TRANSMISSION IN GHANA: EFFECT OF VECTOR SPECIES ON HOST-SEEKING BEHAVIOR AND ONGOING TRANSMISSION

Poppy H. Lamberton[1], Robert A. Cheke[2], Martin Walker[1], Rory J. Post[3], Peter Winskill[1], Daniel A. Boakye[4], Mike Y. Osei-Atweneboana[5], Lee J. Crainey[6], Michael D. Wilson[4], María-Gloria Basáñez[1]

[1]Imperial College London, London, United Kingdom, [2]University of Greenwich of Medway, London, United Kingdom, [3]Liverpool John Moores University, London, United Kingdom, [4]Noguchi Memorial Institute for Medical Research, Legon, Ghana, [5]Water Research Institute, Accra, Ghana, [6]Fiocruz Amazônia, Manaus, Brazil

The World Health Organization has set goals for the control and elimination of human onchocerciasis by 2020 in selected African countries. The feasibility of achieving this depends on the initial level of onchocerciasis endemicity in the communities, the levels of geographical and therapeutic coverage and treatment compliance, and the patterns (intensity and seasonality) of transmission, including the species composition of the simuliid vectors and host-seeking behaviour. Ghana is renowned for its sibling species diversity of the *Simulium damnosum* complex, vectors of *Onchocerca volvulus*. Ghana was originally a country within the umbrella of the Onchocerciasis Control Programme in West Africa (OCP), initially a vector control programme, which operated between 1974 and 2002. We present the spatial and temporal patterns in transmission of *Onchocerca* spp larvae of host-seeking and ovipositing adult parous female flies in communities in Southern Ghana located inside and outside the prior OCP that have been treated with ivermectin for different durations. To date, results include monthly biting rates (MBR) ranging from 714 bites/person/month at Agborle Kame (100% *S. damnosum* s.str./*S. sirbanum* in the savannah region) to 8,586 bites/person/month at Pillar 83/Djodji (98.5% *S. squamosum* in the forest mosaic). MBRs were higher in the wet season. In contrast, parous rates were higher in the dry season (41.8% vs. 18.4%), resulting in higher monthly parous biting rates in the dry season. Monthly infectious biting rates ranged from zero to 79.4 infectious bites/person/month. Monthly transmission potentials ranged from zero to 794.3 infective larvae/person/month. Results will be presented in relation to density of vector and host species and the on-going transmission of *O. volvulus* having been used to parameterise EpiOncho models on the effect of vector species on transmission. Our results show that host choice varies between cytospecies, and may be affected by vector and/or host density with epidemiological relevance for vector-borne disease models.

1331

CIRCULATING ANTIBODY ISOTYPES IN SCABIETIC PATIENT SERA DIRECTED AT MITE ANTIGENS

Marjorie Morgan[1], Larry Arlian[1], Hermann Feldmeier[2]

[1]Wright State University, Dayton, OH, United States, [2]Charité University Medicine Berlin, Berlin, Germany

Scabies, caused by the mite *Sarcoptes scabiei*, is a worldwide neglected disease, particularly in limited-resource settings. The mite has developed resistance to the topical acaricides commonly used to treat this disease. In its early stages, scabies is difficult to diagnose. A focus of our research is to identify molecules that potentially could be used in developing a

diagnostic test for scabies and in a vaccine for its prevention in highly susceptible populations. A confounding problem is that scabies mites are the source of many antigens that cross-react with antigens of the ubiquitous allergy-causing house dust mites *Dermatophagoides farinae, D. pteronyssinus* and *Euroglyphus maynei.* We used an isotype-specific ELISA to screen serum collected from > 100 ordinary scabies patients against extracts of *S. scabiei*, *D. farinae, D. pteronyssinus* and *E. maynei.* At the time of diagnosis, most of these ordinary scabies patients exhibited circulating antibody to scabies antigens with IgG being the predominant isotype. Most patients also had circulating antibodies that bound to antigens from the *Dermatophagoides* mites. The most striking observation was that high scabies antibody titers were paralleled by high levels of antibody that recognized antigens from *E. maynei.* This was especially clear in the case of IgM, the first isotype produced in response to a foreign antigen. The results of this study further demonstrate that the cross-reactivity among mite antigens must be considered as diagnostic tests and vaccines for scabies are developed.

1332

ACCURATE SPECIES IDENTIFICATION AND PHYLOGENETIC RELATIONSHIPS REVEALED BY DNA BARCODING OF PERUVIAN SAND FLIES

Chukwunonso Onyemaechi Nzelu[1], Abraham G. Cáceres[2], Ken Katakura[1], Yoshihisa Hashiguchi[3], Hirotomo Kato[1]

[1]Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan, [2]Departamento Académico de Microbiología Médica, Facultad de Medicina Humana, Universidad Nacional Mayor de San Marcos, Peru, and Laboratorio de Entomología, Instituto Nacional de Salud, Lima, Peru, [3]Department of Parasitology, Kochi Medical School, Kochi University, Japan, Prometeo, Secretaria Nacional de Educacion Superior, Ciencia, Tecnologia e Innovacion, Ecuador, and Centro de Biomedicina, Universidad Central del Ecuador, Quito, Ecuador

Phlebotomine sand flies (Diptera: Psychodidae) are the putative vectors of leishmaniasis worldwide. A reliable species identification of these minute insects constitutes the first step in the surveillance and control of leishmaniasis in endemic areas. Identifying sand fly species based on morphological characteristics is difficult and often complicated by phenotypic plasticity and cryptic species complex as well as demanding considerable taxonomic expertise. The use of DNA barcodes has been proposed recently as a tool for identification of the species in many diverse groups of animal. We assessed the utility of DNA barcoding, based on cytochrome c oxidase subunit I (COI) sequences, for identifying sand fly species from areas where leishmaniasis is endemic in Peru. A total of 89 sand fly specimens belonging to 16 morphological species and 2 genera - Lutzomyia and Warileya, including the major disease vectors were analyzed. We were able to recover and align the target COI fragment from all sand fly species we examined. Phylogenetic analysis of the sequences indicates that the observed species groupings were in confirmation with the morphological identification. The results obtained shows that the barcoding gene was useful in species discrimination in sand flies from Peru.

1333

DEVELOPMENT OF A NOVEL ASSAY TO MEASURE FLIGHT CAPACITY OF *ANOPHELES GAMBIAE* S.L.

Amy R. Krystosik[1], **Diana L. Huestis**[2], Tovi Lehmann[2]

[1]Kent State University, Kent, OH, United States, [2]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Anophelines are important vector species in sub-Saharan Africa and contribute to the continued transmission and burden of malaria worldwide. The dry-season ecology of anophelines, specifically in the arid Sahel conditions, remains unknown, but two hypotheses have been proposed to explain the repopulation phenomenon after the dry season: aestivation and migration. To investigate the migration hypothesis, we developed an activity meter to measure flight by sound accounting for environmental conditions. We found that intensity of sound can predict flight density at frequency of 400-800 Hz; however, this was only achievable at temperatures greater than 63°C in the G3 colony. A second stimulant used to induce flight was patchouli; but, due to background noise in the lab, we could not detect change in intensity by cage density although relative observed flight did increase with cage density. Further work can expand this activity meter to a flight to exhaustion assay which is currently under development. These methods may be field-adaptable, allowing us to study if is it possible that mosquitoes repopulate by migration.

1334

ADULT *AEDES AEGYPTI* SURVEILLANCE USING THE BG SENTINEL TRAP IN PHNOM PENH, CAMBODIA

Michael Prouty[1], Didot B. Prasetyo[1], Rekol Huy[2], Noch Nin[1], Sam Bunleng[2], Ngan Chan Sophal[2], Ngan Chantha[2]

[1]U.S. Naval Medical Research Unit - 2, Phnom Penh, Cambodia, [2]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia

Adult mosquito surveillance was conducted in Phnom Penh, Cambodia using BG sentinel traps (Biogents AG) from October 2012 through October 2013. Traps were set indoors in 18 volunteers' houses around Phnom Penh (two collection sites for every district). Traps were set for a 72 hour collection period per month and mosquitoes were collected every 24 hours from the traps. Habitat variables at each collection site such as premise condition index, presence of paddy fields in the surrounding area, mosquito control effort, and house density were measured. Mosquito specimens were transferred to laboratory and identified to species level. The relationship between weather variables (rainfall, near surface temperature, and specific humidity) and *Aedes aegypti* abundance was measured to determine weather's influence on mosquito population. In total 15,536 mosquitoes, representing 20 species in 9 genera were collected. The predominant species were *Culex quinquefasciatus* (76.57%), *Ae. aegypti* (12.93%), and *Anopheles vagus* (7.03%). *Cx. quinquefasciatuis* the primary vector for *Wuchereria bancrofti* and *Ae. aegypti* for dengue and Chikungunya viruses. Weekly accumulated rainfall (mm) was positively correlated with *Ae. aegypti* abundance at three weeks time lag (P=0.004) while monthly near surface air temperature (°C) was positively correlated at one month time lag (P<0.001). However, no positive correlation was found between specific humidity and *Ae. aegypti* abundance. Monte Carlo permutation test showed that mosquito population was significantly correlated to the presence of paddy fields in the surrounding area (P=0.001), mosquito control effort (P=0.004), and house density (P=0.048). Canonical Correspondence Analysis (CCA) showed that the presence of *Ae. aegypti* was postively associated with house density, and negatively associated with paddy field presence and mosquito control effort at collection sites.

1335

MISUSE AND ABUSE OF ANTIMICROBIALS: COULD WE BE SUPPORTING MALARIA PARASITE DEVELOPMENT IN THE MOSQUITO HOST?

Jewelna Osei-Poku[1], Mathilde Gendrin[2], Dorothy Yeboah-Manu[1], George K. Christophides[2], Michael D. Wilson[1]

[1]Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana, [2]Division of Cell and Molecular Biology, London, United Kingdom

Naturally-occurring bacteria inhabiting the guts of mosquito vectors are important determinants of vector competence; some species can effectively kill ingested parasites, thus reducing disease transmission. Treating mosquitoes with antibiotics/antimicrobials clears the bacteria in the gut allowing for enhanced development and transmission of parasites.

We hypothesize therefore, that the overuse of antibiotics/antimicrobials among human populations may inadvertently impact on the efforts to control malaria transmission. Experiments that have shown the effect of bacteria clearance of *Plasmodium* have used high concentrations of antibiotics/antimicrobials which may not reflect levels that circulate in the human serum. This study seeks to investigate the effect of human serum concentrations of commonly administered antibiotics/antimicrobials on the gut microbiota of *Anopheles gambiae* s.l, and the consequential effect on *Plasmodium falciparum* development. Preliminary results have been obtained from the initial phase of this project which involves determining the core gut microbiome of *Anopheles gambiae* s.l. sampled from Accra, Ghana. DNA from guts of 66 female adults reared from a field collection of larvae and pupae were analyzed using 454-pyrosequencing. Using the Mothur and QIIME software, preliminary results showed the gut microbial community were predominantly *Gammaproteobacteria* (98.5%); *Enterobacter* (24.8%), *Klebsiella* (21.7%), *Serratia* (39.2%) and *Stenotrophomonas* (2.1%) species. This differs from what has been reported in *Anopheles gambiae* from Kenya, which comprised of mainly *Thorsellia* (67.6%) and *Propionibacterium* (9.08%). Further studies will investigate the effects of varying levels of commonly administered antimicrobials on the *An. gambiae* gut microbiota and the consequential effects on *P. falciparum* development. The results from this study are expected to inform on possible negative effect of the unbridled use of antimicrobials on the control of malaria.

1336

YEAST SYMBIONTS IN ARTHROPOD VECTORS: POSSIBLE IMPLICATIONS FOR THE CONTROL OF VECTOR-BORNE DISEASES

Sara Epis[1], Guido Favia[2], Irene Ricci[2]

[1]Universita' Degli Studi di Milano, Milano, Italy, [2]Universita' Degli Studi Di Camerino, Camerino, Italy

The increased awareness for the environmental and the public health problems caused by the use of chemical compounds to combat vector-borne diseases (VBDs) is leading to the development of alternative control strategies. Biological control of arthropod vectors and VBDs is generally based on the use of bacteria, such as *Bacillus thuringiensis*. Although bacterial symbioses in arthropod vectors are the focus of several research programs aimed at developing strategies to control VBDs, such as malaria, dengue, and trypanosomiasis, arthropod-associated yeasts have not yet been deeply investigated. Here we present the first results of a long-term project, aimed at developing strategies for the control of VBDs, exploiting yeasts associated with arthropod vectors. The first disease vectors discovered to harbor yeast symbionts are mosquitoes, from the genera *Anopheles* and *Aedes*. Yeasts isolated from these mosquitoes have been identified as *Wickerhamomyces anomalus*, a typical killer yeast characterized by a wide-spectrum antimicrobial activity, including the production of killer toxins (KTs). Several *W. anomalus* are already used as biopreservation agents in the control of post-harvest diseases of vegetables. The antimicrobial activity of the *W. anomalus* isolated from mosquitoes has been tested *in vitro* against sensitive microbes, showing that these mosquito-associated yeasts actually release an effective antimicrobial KT. Further to mosquitoes, we are currently screening different arthropod vectors such as ticks and send flies for the presence of killer yeasts. Other experiments are aimed at determining whether killer yeasts and their toxins modulate arthropod immunity. Killer yeasts could thus be exploited for a double action, a direct anti-pathogenic effect within the vector and an immune stimulation, with an indirect effect on the reduction of the vectorial capacity. We expect that our project will increase the knowledge on this different type of symbionts, and to the development of novel tools for the biological/integrated control of vector-borne tropical diseases.

1337

HYPOTHESIS TESTING CLARIFIES *TRIATOMA DIMIDIATA* (LATREILLE, 1811) SYSTEMATICS USING NUCLEAR ITS-2 AND MITOCHONDRIAL CYTOCHROME B GENES

Patricia L. Dorn[1], Nicholas de la Rua[1], Heather Axen[2], Nicholas Smith[2], Bethany R. Richards[1], Jirias Charabati[2], Julianne Suarez[1], Dulce M. Bustamante[2], Carlota Monroy[3], Johana Maradiaga[1], C. William Kilpatrick[2], Eric Dumonteil[4], Lori Stevens[2]

[1]Loyola University New Orleans, New Orleans, LA, United States, [2]University of Vermont, Burlington, VT, United States, [3]Universidad de San Carlos, Guatemala City, Guatemala, [4]Universidad Autonoma de Yucatan, Merida, Mexico

The widespread and diverse *Triatoma dimidiata* is the species most important for Chagas disease transmission in Central America and an important vector in Mexico and northern South America. Its diversity may contribute to different Chagas disease prevalence in different localities and has led to conflicting systematic hypotheses describing various populations as subspecies or cryptic species. To resolve these conflicting hypotheses, we sequenced a nuclear (ITS-2) and mitochondrial gene (cytochrome *b*) from an extensive sampling of *T. dimidiata* across its geographic range. We assessed the congruence of ITS-2 and cyt *b* phylogenies and tested the statistical support for constrained topologies representing competing systematic hypotheses. Unconstrained phylogenies inferred from ITS-2 and cyt *b* are congruent. However, hypothesis testing does not support the division of *T. dimidiata* into the previously proposed three sub-species inferred from morphology and ITS-2. Our results identify two cryptic species and indicate *T. dimidiata* sensu stricto is not subdivided into monophyletic clades that might indicate subspecies. Extensive specimen sampling, analysis of both a hypervariable mitochondrial gene and a slower evolving nuclear gene in conjunction with statistical tests of hypotheses has facilitated the clarification of evolutionary relationships among epidemiologically important populations of *T. dimidiata*.

1338

ANTIBACTERIAL ACTIVITY FROM EXTRACTS OF FATTY BODIES AND HEMOLYMPH OF THE BLOWFLY *SARCONESIOPSIS MAGELLANICA* (DIPTERA: CALLIPHORIDAE)

Felio J. Bello[1], Jennifher Gongora[1], María A. Gaona[1], Jesús A. Cortes-Vecino[2]

[1]Universidad del Rosario, Bogotá, Colombia, [2]Universidad Nacional de Colombia, Bogotá, Colombia

Sarconesiopsis magellanica is a necrophagous and hemisynanthropic fly which belongs to the Calliphoridae family. Its importance for human and veterinary medicine lies in its potential for participating as mechanical vector for pathogens such as viruses, bacteria, fungi, protozoa and helminths. Its larvae could cause miasis in some vertebrates, including human beings. Moreover, this fly is used in determining the post-mortem interval. Taking into account its necrophagous habits, this fly could be considered as a potentially useful model in larval therapy. The main goal of this work was to evaluate the antibacterial activity of the extracts of fatty bodies and hemolymph from third-instar larvae of *S. magellanica*. The results were compared with the effects obtained from the same substances derived of the blowfly *Lucilia sericata*, under *in vitro* conditions. The fatty bodies of larvae were removed by dissection technique and the hemolymph via decapitation and centrifugation of larval specimens. The tested bacteria were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The methods used to evaluate the antibacterial activity were agar diffusion and colony forming units. After accurate incubation, the results showed that the antibacterial activity of fatty bodies in both *S. magellanica* and *L. sericata* were effective against *S. aureus* and *P. aeruginosa*, but there was not significant difference between the fly species. However, in the agar diffusion assay the antibacterial activity of the extracts of fatty bodies of both species was found to be more efficient against *P. aeruginosa.*

The obtained results suggest that these substances could have a similar effect against the evaluated microorganisms in the treatment of infected wounds.

1339

NICHE CONSERVATISM AND PHYLOGENETIC STRUCTURE IN BROAD-SCALE SPECIES RICHNESS PATTERNS OF CHAGAS DISEASE VECTORS

Carlos N. Ibarra-Cerdeña[1], Fabricio Villalobos[2], Janine M. Ramsey[3]

[1]Centro de Investigaciones y de Estudios Avanzados del IPN, Unidad Mérida, Mérida, Mexico, [2]Universidade Federal de Goiás, Goiânia, Brazil, [3]Centro Regional de Investigaciones en Salud Publica, Instituto Nacional de Salud Publica, Tapachula, Mexico

A major concern in evolutionary ecology and biogeography is the study of species distribution, especially when species from the same genus have a phylogenetic structure showing non-random spatial association. This pattern can emerge from historical processes as phylogenetic relationships and niche conservatism. In species with public health importance, such as insect vectors, looking into these patterns is of great relevance since processes keeping or avoiding the phylogenetic structure can be key factors in developing control and prevention measures and to anticipate measures to mitigate global change effects. Here, we used simulation models to analyze species richness patterns in the Triatominae (Reduviidae) based on collection data points, species distribution models, climate and phylogenetic information. Patterns of simulated co-distribution and co-diversity under different hypothesis were compared with empirical models. We found that historical processes as phylogenetic relationships and niche conservatism are important causes shaping current patterns of species richness. We consider that our approach has a broad application in quantitative biogeography of vectors of other diseases.

1340

DEPLETION OF TICK THIOREDOXIN REDUCTASE ATTENUATES THE NATIVE TICK MICROBIOTA

Khemraj Budachetri, Shahid Karim

The University of Southern Mississippi, Hattiesburg, MS, United States

The gulf-coast tick (*Amblyomma maculatum*) is a competent vector for a variety of pathogenic microbes, including *Rickettsia parkeri*, a causative agent of Spotted Fever Rickettsiosis. Ticks experience a variety of oxidative stress condition while on and off the vertebrate host. To counter-act the deleterious effects of reactive oxygen species, ticks have numerous antioxidant molecules in their repertoire, such as the Thioredoxin-Thioredoxin Reductase (Trx-TrxR) system, as reported previously. Tick Thioredoxin Reductase has barely been investigated. Our long-term goal is to reduce or block the spreading of vector-borne pathogens by interfering with vector proteins. In this study, we tested our hypothesis that tick TrxR facilitates the colonization of microbes in tick tissues by mitigating the reactive oxygen species. Transcriptional gene expression studies examining the level of TrxR during the prolonged blood-meal in both midguts and salivary glands indicates a potential need of this system during unfed stage. In order to evaluate the functional significance of this highly conserved system, we utilized RNA interference to selectively deplete TrxR transcripts *in vivo*. Both transcriptional gene expression and enzymatic activity studies confirmed the successful depletion of TrxR transcript and activity. However, no significant effect was observed on total tick engorgement likely due to high redundancies or compensatory mechanism in ticks but, the tick salivary glands super oxide dismutase (SOD) was found similarly down regulated with the TrxR depletion. Disruption of TrxR reduces the microbial load in the salivary glands examined by using bacterial universal 16s rRNA gene primers. Our results support the potential role of TrxR in preserving bacterial communities in tick tissues by alleviating the deleterious effect of reactive oxygen species. This work opens new avenues of research in oxidative stress within tick vectors and vector-borne pathogens.

1341

VECTORBASE: A BIOINFORMATICS RESOURCE CENTER FOR INVERTEBRATE VECTORS OF HUMAN PATHOGENS

Gloria I. Giraldo-Calderon[1], Daniel Lawson[2], Scott J. Emrich[1], Frank H. Collins[1]

[1]University of Notre Dame, Notre Dame, IN, United States, [2]EMBL-EBI, Hinxton, United Kingdom

The VectorBase database is updated and expanded every two moths. In the last year we have significantly updated the gene builds, the assemblies or both for *Glossina morsitans, Aedes aegypti*, *Rhodnius prolixus, Anopheles stephensi* and *An. darlingi*. We have also almost tripled the number of hosted genomes from 11 to 30. These new genomes include the two sandflies *Lutzomyia longipalpis* and *Phlebotomus papatasi*, 16 new *Anopheles* species, and the snail *Biomphalaria glabrata*, an intermediate host of *Schistosoma mansoni*. Based on user feedback and internal discussions, all of our tools and resources have had multiple interface and performance improvements, including the possibility to save and reuse job parameters and their results. A new tool called the Population Biology Browser (PopBio), which we had presented initially under a beta version, was also released. This new tool is part of our ongoing efforts to integrate genomic, phenotypic (including insecticide resistance) and population data, as a strategy to integrate basic and applied research. VectorBase also includes new ontologies, mitochondrial sequences, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway information, microarray experiments, single-nucleotide polymorphisms (SNPs) data, RNAseq experiments and other datasets that are available for query and analyses. Also under development is the new VectorBase Galaxy Platform, which will provide our community with a user-friendly interface to perform large scale data analysis on a public site. The data deposited in VectorBase and in the public repositories such as NCBI, are a resource that has been subject to only very limited preliminary analysis. These data are freely available for new analyses, descriptions and hypotheses testing.

1342

SURVEYING THE BACTERIAL COMMUNITY PROFILES IN TICKS FROM THE VILLAGES OF INDIGENOUS PEOPLE IN MALAYSIA

Jing-Jing Khoo, Kai-Ling Kho, Chee-Sieng Khor, Syuhaida Sulaiman, Kim-Kee Tan, Azzy Izyati Ahmad Shanizza, Sun-Tee Tay, Sazaly Abu Bakar

University of Malaya, Kuala Lumpur, Malaysia

Ticks are excellent vectors for disease transmission of a wide variety of zoonotic pathogens, including viruses, bacteria and protozoa. In our laboratory, we are interested to explore the microbiome in ticks collected from the villages within the semi-forested areas in Malaysia. These areas are known as the interfacial zones of inhabitants (IZI), which provide for plenty of opportunities for contact between the indigenous people occupying the villages, tick vectors and wildlife reservoir hosts harbouring an array of zoonotic pathogens. Hence, the indigenous people in the IZI are constantly at threat from tick-borne diseases due to close contact with the tick vectors. We aim to utilize the 16S ribosomal RNA metagenomic sequencing strategy as a means to investigate the bacterial community in ticks collected from IZI, in hope of identifying emerging pathogens in ticks from IZI. Our results indicate that there is prevalence of a number of tick-borne bacterial pathogens harboured by the ticks sampled from the IZI. As the knowledge and data on pathogens harboured by ticks in Malaysia is minimal, studying the bacterial community in ticks, together with clinical surveillance, will provide knowledge that may help in the early detection of emerging pathogens among the indigenous people in Malaysia.

1343

FINE SCALE MAPPING OF QTL ASSOCIATED WITH REPRODUCTIVE DIAPAUSE WITHIN THE *CULEX PIPIENS* COMPLEX USING A RADTAG GENOMIC APPROACH

Roberta Engel, David W. Severson

University of Notre Dame, Notre Dame, IN, United States

Culex pipiens is a broadly distributed species complex that transmits human diseases (e.g., West Nile Virus, Lymphatic Filariasis). *Cx. p. pipiens*, one member of the complex, is found across temperate zones of the world while *Cx. p. quinquefasciatus* is restricted to subtropical and tropical regions. One physiological trait that distinguishes *Cx. p. pipiens* from its sister taxon is its ability to enter reproductive diapause. Photoperiod is the primary trigger of this complex life history trait. Previous work using markers developed with traditional methods inferred four quantitative trait loci (QTL) in an F2 mapping population. The ability to generate informative Single Nucleotide Polymorphic markers (SNPs) and infer QTL has increased dramatically with the advent of massively parallel sequence technology (e.g., Illumina HiSeq2000). In addition, a published reference genome for *Cx. p. quinquefasciatus* is available. An advanced intercross line (*Cx. p. quinquefasciatus* Johannesburg x *Cx. p. pipiens* South Bend) was established. First instar larvae collected from the F6 generation were exposed to diapause inducing conditions (i.e., 8:16 light:dark cycle and 18C). Follicle size in ten-day old adult females was used to score phenotype. Only the extreme phenotypes were sampled to construct a reduced representation paired-end library. Using a RADtag approach, each of the 100 samples had a unique identifier. SNPs were generated *in silico*; a filtered subset of 2000 SNPs was used to infer linkage groups. Linkage groups with at least 15 markers were assigned to chromosomes. Marker density on the linkage map is an order of magnitude greater than on the map used in an earlier study. Presently we are mapping QTL regions on a fine scale. This has positioned us to advance our understanding of what genes and genetic pathways regulate reproductive diapause.

1344

PHYSICAL MAPPING REVEALS CHROMOSOME-SPECIFIC GENOMIC LANDSCAPES IN *ANOPHELES STEPHENSI*

Ashley Peery, Xioafang Jiang, Brantley Hall, Maria Sharakhova, Zhijian Tu, Igor Sharakhov

Virginia Polytechnic Institute and State University, Blacksburg, VA, United States

Anopheles stephensi type form is the key vector of malaria on the Indian subcontinent and the Middle East. Additionally, *An. stephensi* is an emerging model species for genetic and genomic studies of mosquito biology and mosquito-parasite interactions. However, success of genomic analyses will be limited if researchers deal with numerous sequencing scaffolds, rather than with a chromosome-based genome assembly. Here we report the first chromosome-based genome assembly for the Indian wild type strain of *An. stephensi*. Our physical chromosome mapping ordered 62% of the *An. stephensi* sequencing scaffolds and facilitated analysis of chromosome arm-specific genomic landscapes that is seldom feasible in next-gen genome projects. Comparative analysis between *An. stephensi* and *An. gambiae* revealed differences in genome organization and highlighted varying rates of evolution between autosomes and the sex chromosome. The genome landscape of *An. stephensi* is characterized by relatively low repeat content compared with that of *An. gambiae*. Our analysis demonstrated extremely high rate of rearrangements in the X chromosome as compared with autosomes despite the lack of polymorphic inversions in the X chromosomes in both species. Additionally, the difference between the rates of the X chromosome and autosome evolution is much more striking in *Anopheles* than in *Drosophila*. We found that the high rates of evolution in the X chromosome highly positively correlated with the density of simple repeats, suggesting their role in genomic plasticity. While, the rate of autosomal evolution and distribution of common polymorphic inversions positively correlates with the densities of microsatellites and genes, but negatively correlates with the coverage of matrix associated regions and transposable elements. Our data indicate that overall high rates of chromosomal evolution are not restricted to Drosophila, but may be a feature common to Diptera. The chromosome-based genome assembly for *An. stephensi* will provide a valuable tool for the vector biology community as we seek a better understanding of mosquito biology.

1345

SILENCING CASPASE DECREASES DENV-2 INFECTION OF THE MOSQUITO VECTOR *AEDES AEGYPTI*

Matthew W. Eng, David W. Severson

Eck Institute for Global Health, Department of Biological Sciences, Notre Dame, IN, United States

The mosquito *Aedes aegypti* is the primary vector for dengue virus (DENV). An understanding of host-pathogen interaction is important in understanding what factors contribute to vector competence. However, many of the molecular mechanisms for vector competence remain unknown. Our previous global transcriptional analysis has suggested the induction of apoptotic proteins in the involvement of resistance and susceptibility to DENV-2 infection. Here we analyze the possibility that programmed cell death is actively involved in the defense of *A. aegypti* host cells to DENV-2 infection. The initiator caspase, Dronc, has been previously shown to be an essential component of the core apoptotic pathway. This caspase showed higher expression *in vitro* in infected *A. aegypti* cells as well as in resistant mosquitoes following infection. However, TUNEL staining of midguts from DENV-2-resistant and -susceptible mosquitoes revealed that apoptosis is activated at near-basal levels early during infection. Interestingly, dsRNA interference of Dronc decreased virus titer and infection in resistant mosquitoes. This reveals that Dronc may be important for affecting DENV-2 infection in *A. aegypti*. Furthermore, we investigate whether silencing of Dronc effects non-apoptotic processes influencing DENV-2 infection.

1346

POPULATION OF *LUTZOMYIA LONGIPALPIS* (DIPTERA: PSYCHODIDAE: PHLEBOTOMINAE) FROM PANAMA

Anayansi Valderrama Cumbrera[1], Jose Dilermando Andrade Filho[2], Mara Garcia Tavares[3]

[1]Gorgas Conmemorative Institute, Panama, Panama, [2]Instituto René Rachou-Fiocruz, Belo Horizonte, Minas Gerais-Brasil, Brazil, Brazil, [3]Universidade Federal de Viçosa, Brazil, Brazil

Although many studies on vectors of cutaneous leishmiansis have been done in Panama, the perdomestic distribution of *Lutzomyia longipalpis* have been documented recently. With the purpose of estimated the divergence time and difference in genetic population between peridomestic and selvatic species of *Lu. longipalpis*, we performed this study to estimate and compare the intra- and inter-population genetic variability of wild and near-residential populations of *Lu. longipalpis*, obtained in a locality with high incidence of cutaneous leishmaniasis in Panama. Using a mitochondrial DNA sequences of cytochrome B were analyzed of *Lu. longipalpis* populations from Panama. Of the 11 haplotypes obtained, seven were present exclusively in the town of El Limón and three, exclusively in Bona Island. A single haplotype was shared between the two communities. The haplotype and nucleotide diversities were h=0.70 and π=0.0015 for the population of Bona and h=0.95 e π=0.003 for the population of El Limón. The genetic differentiation analyses between the two populations showed significant differences (Fst=0.17; $p<0.05$) between them. Significant differences ($p<0.05$) were also obtained when the Panama sequences were compared to others obtained in Genebank cytochrome B the populations of *Lu. longipalpis* from Colombia (Fst=0,98), Costa Rica (Fst=0,98), and Brazil (Fst=0,72). The existence of unique haplotypes in each community and the significant genetic differentiation reported suggest that the *Lu. longipalpis*

populations in Panama are in the middle of a speciation process due to the isolation of the two populations because of the Pacific Ocean and the events that characterized the emergence of the Isthmus of Panama. The fact that *Lu. longipalpis* was found in near residential areas in Panama is important as a risk factor and to increase epidemiological surveillance. We result indicate the need to constantly and systematically monitor of this vector species in regions with high incidence of leishmaniasis and review the symptoms produced by different cryptic species of *Lu. longipalpis*. Meanwhile, little is currently known about the distribution, occurrence, and implications of this species in the transmission of leishmaniasis in the country.

1347

BIONOMICS AND PHYLOGENETICS OF THE DENGUE VECTOR *AEDES AEGYPTI* FROM THE ARABIAN PENINSULA

Emad I. Khater[1], Farrukh Baig[1], Amgad A. Saleh[1], Kashif Munawar[1], Hany A. Kamal[2]

[1]College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia, [2]Ain Shams University, Research and Training Centre on Vectors of Diseases, Cairo, Egypt and Dallah Corp., Jeddah, Saudi Arabia

Aedes aegypti is the principal vector of dengue in the world, including Saudi Arabia and Yemen in the Arabian Peninsula south-western regions; where disease outbreaks have occurred since 1995. Understanding the ecology and population genetics of *Ae. aegypti* is crucial for understanding dengue virus transmission patterns and for effective disease control. We report here on the ecology and phylogenetics of *Ae. aegypti* collected from western Saudi Arabia, from Jeddah governorate, a major harbour on the Red Sea. Phylogenetics analysis was carried out using the ribosomal DNA-internal transcribed sequence 2 (ITS2) and the mitochondrial cytochrome *c* oxidase I (COI) and NADH dehydrogenase subunit 4 (ND4) genes. *Aedes aegypti* larvae and pupae collected represented 23.9% (n= 772: 712 larvae, 60 pupae) of the total culicines mosquitoes collected. Most of water sites were anthropogenic, of which plastic drinking water tanks were the most productive for larvae (av. 55.5±55.5 larva/site). The most productive sites for pupae (47.5% of total pupae) were large concrete underground tanks or plastic elevated tanks (1000-5000 L capacity). The pupal yield is much lower than those reported from other countries. Single nucleotide polymorphisms (SNPs) and Neighbour-Joining (NJ) phylogenetic trees were built using COI and ITS2 sequences obtained from *Ae. aegypti* from Saudi Arabia or retrieved from the Genbank for other populations from Africa, Asia and the Americas. NJ trees identified ten COI and 21 ITS2 haplotypes, with many haplotypes unique to Arabian *Ae. aegypti* populations. Data on ND4 analysis will be reported when appropriate. We provide novel phylogenetic information of *Ae. aegypti* populations from the Arabian Peninsula and other parts of the Oriental, Afrotropical and Palaearctic zoogeographic zones, which shows the presence of considerable genetic differentiation between them. These studies will give broader insights on the dispersal patterns of *Ae. aegypti* and transmission dynamics of dengue virus, with important implications for disease control under national and regional biogeographic conditions.

1348

MOLECULAR PHYLOGENETICS OF MOSQUITOES FROM THE ORIENTAL AND AFROTROPICAL ZOOGEOGRAPHIC REGIONS

Kashif Munawar, Amgad A. Saleh, Emad I. Khater

College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia

The Arabian Peninsula (PA) has peculiar position bordering the Oriental, Afrotropical and Palaearctic zoogeographic zones with diverse ecology and fauna. In Saudi Arabia (SA) (the largest country in PA) about 35 culicine mosquito species were reported including eight dominant vector species. The most important of these are *Aedes aegypti* and *Culex pipiens* complex, vectors of arboviruses, and two *Anopheles* malaria vectors, *An. stephensi* in Asia and *An. arabiensis*, the only member of *An. gambiae* complex outside Africa. We present here new information on phylogenetics of *An. stephensi* and *An. arabiensis* and other anopheline species collected from different SA regions. Neighbour-Joining (NJ) phylogenetics trees were constructed using DNA sequences of the ribosomal DNA-internal transcribed sequence 2 (ITS2) and the mitochondrial cytochrome *c* oxidase I (COI) gene. These sequences were obtained from mosquitoes field-collected from SA or from lab colonies from other countries in the Oriental or Afrotropical zones and sequences retrieved from the Genbank. Multi-locus phylogenetic analysis of COI & ITS2 sequences of all *An. stephensi* populations identified new haplotypes, including unique haplotypes to SA, and haplotypes broadly-distributed across the Oriental zone including AP. These results confirm that *An. stephensi* is a monophyletic species composed of ecotypes. However, unlike in Iran and India, we could not differentiate between *An. stephensi* type and mysorensis ecotypes, which might be due to inter-population extensive gene exchange. New *An. arabiensis* haplotypes were identified SA and related to field and lab populations from the Afrotropical region. In this report we provided new information on the phylogenetic relationships of anopheline mosquitoes from different zoogeographic regions including malaria vectors and suspected or non-vectors. Such information is important for understanding malaria transmission under broad biogeographic conditions across different zoogeographic zones. The COI or ITS2 sequences could also be used to develop species-specific molecular assays to complement pectorial keys to accurately identify species in AP and their cryptic or ecotypes if exist.

1349

POPULATION STRUCTURE OF THE VECTOR MOSQUITO *AEDES AEGYPTI* AND HUMAN-MEDIATED DISPERSAL IN THE PHILIPPINE ARCHIPELAGO

Eugenio Fonzi[1], Yukiko Higa[1], Arlene G. Bertuso[2], Emiko Kawashima[1], Kogomi Minagawa[1], Kyoko Futami[1], Jinping Hu[1], Noboru Minakawa[1]

[1]Institute of Tropical Medicine - Nagasaki University, Nagasaki, Japan, [2]University of the Philippines, Manila, Philippines

Aedes aegypti is the primary vector of most of the so-called arboviruses (ARthropod-Borne viruses), like dengue fever, yellow fever or chikungunya. Massive employment of insecticides favoured the development of insecticide resistance. *Ae. aegypti* is a highly anthropophilic mosquito and it is believed that human transportation played and still plays an important role in its dispersal. The dispersal ability of a vector is connected to its ability to spread the diseases as well as the insecticide-resistance mutations. Knowledge of the genetic structure of the populations of mosquitos can help to infer its patterns of dispersal. The Philippines are endemic for dengue fever and recently a high level of insecticide resistance was found. With its 7000 islands the philippine archipelago is therefore an appropriate environment to analyze the relationships between mosquito dispersal and both land and marine human transportation. With the objective of determining the distribution and population structure of Ae aegypti, during September-October 2013 a sampling took place in 7 major islands in the northern part of the Philippines (Luzon), in 11 seaports and 7 inland areas. In each area, at least 7 breeding sites were sampled; in order to reduce the presence of sibling individuals, (1) the flight range of *Ae. aegypti* was taken into account and (2) 1 out of 6 larvae collected from each site were randomly selected for the study, yielding between 19 and 67 individuals to be analyzed in each area. All the inland areas had to be discarded because of lack of specimen. Up to now, a preliminary analysis has been conducted with 6 microsatellite markers, but more are planned to be added henceforth. Between 4 and 9 alleles were found at each locus. Generally no significant deviation from HWE was found. The total Fst value was 0.06, quite low, suggesting gene flow between the islands. Interestingly, the pairwise Fst values were, at average, lower for the biggest and busiest seaports while higher for the smallest seaports.

1350

GENOME-WIDE HAPLOTYPE MAP REVEALS INSECTICIDE SELECTIVE SWEEPS IN WILD *ANOPHELES GAMBIAE* POPULATIONS FROM KENYA

Xiaohong Wang, Jun Li

University of Oklahoma, Norman, OK, United States

Nearly a million people die from malaria annually. *Anopheles gambiae* in Africa is the major malaria vector. Examining the molecular basis of mosquito traits of interests needs the information of genetic variations and haplotype map (HapMap) in wild *A. gambiae* populations from malaria endemic areas. We sequenced the genomes of nine wild *A. gambiae* individually, and detected 2,219,918 single nucleotide polymorphisms (SNPs) with 88% novel, and 43,765 nonsynonymous. SNPs are not distributed on *A. gambiae* evenly, and the lower SNP frequency regions overlays with heterochromatin and chromosome inversion. About 785,687 SNPs that were genotyped correctly in all individual mosquitoes with 99.6% confidence were extracted from high throughput sequencing data. Based on these SNP genotypes, we for the first time constructed the genome-wide HapMap of wild *A. gambiae* mosquitoes from malaria endemic areas in Kenya, and made it available through a public web with graphic user interface. Low LD is consistently observed with average linkage disequilibrium (LD) block size less than 40 bp. Meantime, we discovered that several large LD blocks were clustered in *A. gambiae* genome. Interestedly, detailed analysis of the genomic locus of chromosome 2 (2R:57.6-2L:4.0MB) that has fewer SNPs and largest linkage disequilibrium (LD) blocks revealed para gene at the center of this region with homozygous insecticide knock-down resistance (kdr) allele 1014F in all sample mosquitoes, supporting the hypothesis of insecticides DDT and pyrethroids selective sweeps in western Kenya.

1351

MOLECULAR ADAPTATION OF THE OLFACTORY SYSTEM TO HUMAN HOSTS IN *ANOPHELES GAMBIAE*

Giridhar Athrey[1], Theresa Hodges[1], Luciano Cosme[1], Willem Takken[2], Michel A. Slotman[1]

[1]Texas A&M University, College Station, TX, United States, [2]Wageningen University, Wageningen, Netherlands

The dominant African malaria vector *Anopheles gambiae s.s* preferentially takes it blood meals from human hosts, often at rates as high as 90%. This adaptation to human hosts is expected to have a genetic basis in the olfaction system, which includes several key gene families - the olfacation receptors (ORs), ionotropic receptors (IRs), odorant binding proteins (OBPs). We previously identified six narrow QTL for human host preference on chromosomes 2 and 3, that together explain 49% of the phenotypic variance. A total of 34 ORs, 7 IRs and 21 OBPs are located inside these QTL. In addition, a comparison of antennal transcriptomes identified 11 olfaction genes that are located inside QTL and that were significantly higher expressed in *An. gambiae* vs the zoophilic *An. quadriannulatus*. The genes involved in the adaptation of *An. gambiae* to human hosts should show evidence of positive selection. Therefore, we examined the evolution of olfaction genes (spanning all three gene families) from 95 individuals comprising five member species of the *An. gambiae* complex – *An. gambiae* (M + S), *An. arabiensis*, *An. melas*, *An. merus* and *An. quadriannulatus.* We used a phylogenetic framework (PAML) to test if the *An. gambiae* lineage evolved under positive selection– based on the ratio of non-synonymous to synonymous (dN/dS) substitutions, and signatures of selective sweeps. The presence of olfaction genes that evolved under positive selection inside human host preference QTL indicates their importance for human host choice in *An. gambiae.*

1352

UNEXPECTED STRONG REDUCTION OF GENE-FLOW WITHIN *ANOPHELES GAMBIAE* IN AN AREA OF HYBRIDIZATION WITH *AN. COLUZZII* IN THE "FAR-WEST" OF THEIR RANGE

Beniamino Caputo[1], Davis Nwakanma[2], Francesco Paolo Caputo[1], Musa Jawara[2], Cheryll Eniyou Oriero[2], Majidah Hamid-Adiamoh[2], Ibrahima Dia[3], Lassana Konate[4], Vincenzo Petrarca[1], Joao Pinto[5], David Conway[6], **Alessandra della Torre**[1]

[1]University of Rome Sapienza, Rome, Italy, [2]Medical Research Council The Gambia, Fajara, Gambia, [3]Institute Pasteur Dakar, Dakar, Senegal, [4]University of Dakar, Dakar, Senegal, [5]Universidade Nova de Lisboa, Lisbon, Portugal, [6]London School of Tropical Medicine & Hygiene, London, United Kingdom

The *Anopheles gambiae* complex includes mosquito species at different stages of speciation, ranging from clearly defined, although morphologically indistinguishable, bonae species to closely-related sympatric taxa such as *An. gambiae* and *An. coluzzii* (recently raised to formal species), which represent the major vectors of human malaria in sub-Saharan Africa. Extensive genetic studies have trusted these species as models of ecological speciation and highlighted the effect of this process in malaria epidemiology and control. We sampled *An. gambiae* and *An. coluzzii* populations from diverse habitats along the Gambia River (West Africa), an area characterized by higher level of inter-specific hybridization compared to most of the species range. We carried out a comparative analysis of these samples by presumably neutral nuclear microsatellite markers on chromosome-X and -3 and by presumably adaptive chromosomal paracentric inversions on chromosome-2. Both genetic markers reveal unexpected striking genetic differences, compatible with a strong reduction of gene-flow, between *An. gambiae* populations west and east of the central part of the transect, apparently exclusively colonized by *An. coluzzii*. While *An. gambiae* western populations are characterized by low chromosomal inversion diversity, a very high degree of chromosomal variation, based on a higher number of inversion polymorphisms, is observed in eastern populations. Consistent with this chromosomal divergence, high genetic differentiation at the microsatellite level, not explained by geographic distance alone, is observed between western and eastern populations. Notably, this microsatellite differentiation is higher than that observed between *An. gambiae* and *An. coluzzii*, and mostly due to loci in the centromeric region of chromosome-X. This suggests that the two *An. gambiae* populations may be at an advanced stage of reproductive isolation, likely triggered by human-made habitat fragmentation, and provides new evidence of a speciation continuum within the *Anopheles gambiae* complex.

1353

METAGENOMICS OF *AEDES ALBOPICTUS*: IMPACT OF LARVAL HABITAT TYPES AND MOSQUITO AGE ON THE MICROBIOME STRUCTURE OF MOSQUITO GUTS

Xiaoming Wang[1], Daibin Zhong[2], Thomas M. Gilbreath, III[2], Guofa Zhou[2], Tong Liu[1], Xiaoguang Chen[1], Guiyun Yan[2]

[1]Key Laboratory of Prevention and Control of Emerging Infectious Diseases of Guangdong Higher Education Institutes, Department of Pathogen Biology, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou, China, [2]Program in Public Health, College of Health Sciences, University of California Irvine, Irvine, CA, United States

Recent metagenomic studies suggest microbiomes of disease vectors may have profound impacts on vector development, reproduction, immunity against pathogens and vectorial capacity. However, the relationship between vector environments and vector microbiome structure and composition is unknown. Given mosquito larvae are confined to the aquatic habitats, it is hypothesized that microbial community in the larval habitats may largely determine the contents of mosquito larval guts, but larval gut microbials may have little effects on the gut microbial community of adult mosquitoes due to constant acquisition of new

microbials in the process of sugar and blood feeding. The present study tested this hypothesis with the Asian tiger mosquitoes (*Aedes albopictus*), a most invasive species and also an important dengue vector. We examined the dynamics of gut microbial communities of *Ae. albopictus* from three types of larval habitats, mosquito larvae, pupae and adults from these habitat types. Microbial community of the larval habitats and larval and adult mosquito guts was examined by pyrosequencing of bacterial 16S rRNA gene V4 hyper-variable region. A total of 15 million 250bp paired-end sequence reads were obtained. Preliminary analysis found that the composition of the microbiomes varied significantly among larval habitat types, and varied between larvae and adults whereas microbiomes of larvae and pupae were similar and resembled to the microbiomes of the larval habitats. *Proteobacteria*, *Bacterioidetes* and *Firmicutes* were the predominant bacteria across mosquito life stages. Blood feeding showed a significant impact on mosquito gut microbiomes. The present metagenomic study established a metagenomic foundation for better understanding the impact of environmental microbials on vector development and disease transmission.

1354

DISSECTING GENETIC AND MICROBIAL FACTORS OF *AEDES AEGYPTI* FIELD POPULATIONS WITH DISTINCT SUSCEPTIBILITY DO DENGUE VIRUS

Carine Spenassatto, Jaqueline Jarusevicius, Jayme A. Souza-Neto

São Paulo State University, Botucatu, Brazil

Dengue is the arboviral disease of highest public health concern due to its increasing expansion in recent years worldwide. Due to the lack of a licensed anti-dengue therapy, the prevention of dengue virus (DENV) dissemination is still limited to the control of its vector, the mosquito *Aedes aegypti*. DENV propagation and transmission is determined by the mosquito's vector competence, which has been associated to both genetic factors and the gut microbiota. Here we assess both the genetic variation of DVHFs (dengue virus host factors) and the microbial diversity of three field-derived Brazilian *Ae. aegypti* populations displaying distinct susceptibilities to DENV. Mosquitoes were collected in three different locations (Botucatu-SP, Neópolis-SE and Campo Grande-MS). We assessed dengue viral susceptibility of each population through oral infection by DENV-4 and quantified the relative number of viral particles by real-time PCR. Our data suggest that mosquitoes from Botucatu are nearly 3-fold less susceptible to the virus than those from Campo Grande ($p<0.001$). Sequencing analysis of the DVHFs lola and NADH of these two populations revealed a total of 9 SNPs, with 5 of them causing amino acid changes to the predicted polypeptide sequence of such genes. In order to verify a potential association between mosquito's microbial diversity and susceptibility to the virus, we are also performing Illumina 16S rRNA surveys to analyze the gut microbiota of such mosquitoes. Surprisingly, our results revealed that the midguts of the mosquitos from Botucatu are colonized mainly by Gram-positive bacteria from the Lactobacillus genus (34% of the total number of bacteria), even though there was a higher number of Gram-negative genera than Gram-positive ones in these mosquitoes. We are now assessing the Campo Grande population microbiome in order to determine whether the microbial diversity of these highly DENV-susceptible mosquitoes is different from that of the Botucatu population. This work will shed light on our understanding of the molecular interactions of DENV-mosquitoes-microbiota and may ultimately lead to the development of new dengue control strategies.

1355

GENOME-WIDE ISOLATION WITHIN THE WEST-AFRICAN MALARIA VECTOR *ANOPHELES MELAS*

Kevin C. Deitz[1], Giridhar Athrey[1], Musa Jawara[2], Hans J. Overgaard[3], The Anopheles Genomes Cluster Consortium[4], Michel A. Slotman[1]

[1]Texas A&M University, College Station, TX, United States, [2]Medical Research Council, Bakau, Gambia, [3]Norwegian University of Life Sciences, Ås, Norway, [4]University of Notre Dame, Notre Dame, IN, United States

Anopheles melas is a locally important malaria vector along the West-African coast where it breeds in brackish water. A recent population genetic study of this species revealed species-level genetic differentiation between two population clusters on the mainland: *An. melas* West and *An. melas* South. *An. melas* West extends from The Gambia to Tiko, Cameroon (near Mount Cameroon). The other mainland cluster, *An. melas* South, extends from the southern Cameroonian village of Ipono to Angola. Species level differentiation was also found between mainland and Bioko Island, Equatorial Guinea populations. To examine how genetic differentiation between these three forms is distributed across the genome, we pooled samples from a representative population of each of the three genetically isolated *An. melas* clusters. We performed whole genome sequencing on these pools and conducted genome-wide analyses of divergence and selection. Our analyses reveal that these three forms show high levels of genetic differentiation across the genome, including the presence of genome-wide fixed differences. Levels of genetic differentiation are particularly high on the *X* chromosome and low in heterochromatic regions. Additionally, we analyzed genome-wide differentiation between *An. gambiae* and *An. melas* West to put our results in the context of the *An. gambiae* species complex evolution. We also investigated how divergence in specific genes and genomic regions may have led to the genomic isolation of these putative species.

1356

MICRORNAS (MIRS): A VIABLE OPTION FOR TRANSGENIC MOSQUITO CONTROL?

Luciano V. Cosme, Craig J. Coates, Michel A. Slotman

Texas A&M University, College Station, TX, United States

MicroRNAs (miRs) are small non-coding RNAs that can each regulate the expression of up to hundreds of genes. Therefore miRs that are active during changes in host seeking behavior, as mosquitoes age or acquire a blood meal, may be a viable target for the transgenic manipulation of mosquitoes. However, the number of known miRs in the yellow fever mosquito is small compared to other insects and little is known about which genes they regulate. Because olfaction genes are crucial for host seeking, we examined if miRs play a role in olfaction gene regulation as mosquitoes develop and blood feed. We extracted total RNA from the antennae and head+thorax from females of various ages, as well as males (12h non-host seeking females, 4 days old host seeking females, 4 days old males, and 3h, 24h, 48h and 72h after blood feeding). Poly(A)+ RNA, 3' UTR and small RNA were sequenced on the Illumina platform. Global gene expression analyses revealed 52 genes that are highly and uniquely expressed in the antennae of 4 days old females (low or absent expression in 12h old females antenna, male antenna or head+thorax of 4 days old females). Similarly, 1,150 genes are uniquely expressed in the antenna of 12h old females. While 37 olfaction genes are differentially expressed between antenna of 12h old females and 4 day old females, only five of these are significantly different 24h after blood feeding in comparison to the 4 day old unfed females. The most expressed miRs in the antenna of females is aa-miR-236a, which has 82.65 fold higher expression compared to the head+thorax sample. We are particularly interested in miRs that do not kill mosquitoes, but decrease their attraction to humans. To identify miRs important for host seeking, we are injecting its antagomir (synthetic

anti-sense miR) into late larvae, pupa and 12h old adults. Preliminary results from pupa injection are very promising; injected mosquitoes are being subjected to a dual choice assay.

1357

ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN IMMUNE RESPONSE PATHWAYS GENES, AND SUSCEPTIBILITY/RESISTANCE PHENOTYPES OF *ANOPHELES DARLINGI* TO *PLASMODIUM VIVAX*

Nilton B. Rodrigues[1], Rejane C. Simoes[2], Nagila F. Secundino[1], Yoosook Lee[3], Wanderli P. Tadei[2], Paulo F. Pimenta[1], Gregory Lanzaro[3]

[1]Centro de Pesquisas René Rachou - FIOCRUZ, Belo Horizonte - MG, Brazil, [2]Laboratory of Malária and Dengue, Instituto Nacional de Pesquisas da Amazônia, INPA, Manaus, Brazil, [3]Vector Genetics Laboratory, University of California Davis, CA, United States

Populations of *Anopheles* malaria vector in nature are composed of both, *Plasmodium* susceptible and resistant individuals. Susceptibility and resistance to malaria parasites were, and still are, the subject of intense study. With the availability of *Anopheles darlingi* genome sequence, this new knowledge has opened doors for investigating genetic determinants for susceptibility to *P. vivax* infection of this major malaria vector in Americas. This work aims to describe the occurrence and distribution of SNPs (single nucleotide polymorphisms) in genes of the immune signaling pathways using samples taken directly from natural populations of *A. darlingi* (collected in Amazonas and Pará States in Brazil) and to investigate their association to susceptibility / refractoriness to *P. vivax* infections. We identified homologs of 172 immune genes in the *A. darlingi* genome from the data available on VectorBase. We conducted whole genome sequencing on 24 individuals both infected and uninfected groups and identified SNPs on immune genes. A SNP genotyping assay will be developed from a panel of non-synonymous SNPs in immune genes and genotyping assay will be conducted on 400 individuals of both infected and uninfected groups. Our goal is to identify SNPs associated with the susceptibility / resistance to *P. vivax* in *A. darlingi* populations of different genetic backgrounds. We will use the knowledge of the molecular genetics of *A. darlingi*, available on the Vector Base, to create and establish a database to be used in the recognition of genetic markers, which can be used as indicative of the existence of subpopulations of *A. darlingi* with distinct vector competence for transmission of human malaria in different localities of the Amazon. We intend to develop a predictive model of transmission that will point out where are the most competent mosquitoes population for the transmission of the parasite, which may help to establish strategies focused on the monitoring and control of the disease.

1358

IDENTIFICATION OF *ANOPHELES* (DIPTERA: CULICIDAE) FAUNA FROM COLOMBIA THROUGH DNA BARCODES

Giovan F. Gómez, Natalí Álvarez, Juan J. Quintero, Estefani A. Piedrahita, Margarita M. Correa

Universidad de Antioquia, Medellin, Colombia

Taxonomic determination of *Anopheles* species constitutes an essential baseline for targeted malaria vector control. Historically, morphological characters have been used for taxonomic identification; however, the existence of species complexes, closely related species and inter and intra phenotypic variation, makes this task difficult. Therefore, a DNA barcoding strategy based on a fragment of the *COI* gene has been proposed to identify specimens at the species level. In Colombia, approximately 47 *Anopheles* morphospecies have been recorded, however, molecular work has mainly focused on the main malaria vectors. The aim of this work was to provide a sequence reference library that includes DNA barcodes available for the corresponding Colombian species. In total 41 Molecular Operational Taxonomic Units (MOTUs) representing species/lineages were compiled, 30 of them were sequences obtained by our group or from GenBank. The remaining represented specimens from neighbor countries but that have also been recorded in Colombia. Neighbour-joining analysis based on Kimura's two parameter (K2P) showed non-overlapping clusters for all species and lineages with high bootstrap support, whereas similarity methods, Best Match, Best Close Match and All Species Barcode used with the typical 3% threshold proposed for barcode, correctly assigned 95.59% , 91.82% and 67.5% of the sequences respectively, to its original species. These results demonstrate that barcode constitutes an important tool for taxonomy in *Anopheles*; however, being a single-gene method its use constitutes a baseline approach, and other biological, morphological and ecological markers should be implemented for species delimitation. Importantly, the barcode sequence library presented here can be used as a benchmark for molecular confirmation.

1359

ENTOMOLOGICAL INVESTIGATIONS FOR UNDERSTANDING JAPANESE ENCEPHALITIS VIRUS TRANSMISSION DYNAMICS: LESSONS FROM BANGLADESH

Jennifer S. Lord[1], H. M. Al-Amin[2], Sumit Chakma[2], Shafiul Alam[2], Emily S. Gurley[2], Juliet R. Pulliam[1]

[1]University of Florida, Gainesville, FL, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Understanding pathogen transmission dynamics is imperative for identifying and implementing cost effective interventions for optimal impact. One of the first steps toward understanding transmission dynamics of mosquito-borne zoonoses is to identify the host and vector species necessary for maintaining, amplifying and bridging transmission to humans. Such investigations were first undertaken for Japanese encephalitis virus (JEV) in Japan in the 1950's. Since this time, the dominant vector species – *Culex tritaeniorhynchus* – and reservoir hosts – pigs and ardeid birds – that were identified in these studies have generally been assumed to drive JEV transmission across the whole of Asia. This transmission cycle is likely to be responsible for human risk in areas where pigs are dominant within the community of vertebrate hosts and *Cx. tritaeniorhynchus*, confirmed in field and experimental settings to feed predominantly on large mammals, is relatively more abundant than other potential vector species. Such ecological contexts are found in Thailand and Malaysia; however, the presumption that this group of species drives transmission in all regions may impede our understanding of spatiotemporal variation in transmission dynamics of JEV. Countries where transmission drivers may differ from that of Japan include India, Indonesia and Bangladesh, where dead-end hosts (cattle) are found in substantially higher density than pigs. We utilize field data obtained during a preliminary entomological survey in Bangladesh to show that the observed dominance of any mosquito species within a community can be dependent on the sampling method employed. In addition we utilize an equation for the basic reproduction number of a zoonotic mosquito-borne virus, parameterized from field data and literature surveys, to demonstrate that the vector species observed to be most abundant may not necessarily drive transmission. To conclude, we emphasize that multiple, carefully selected mosquito sampling methods should always be considered for estimation of mosquito relative abundance as well as species blood-feeding patterns, when undertaking surveys to implicate vector and host species in new geographic regions.

1360

EPIDEMIOLOGICAL PATTERNS OF ROSS RIVER VIRUS DISEASE IN QUEENSLAND, AUSTRALIA, 2001-2011

Weiwei Yu

Queensland University of Technology, Brisbane, Australia

Ross River virus (RRV) infection is a debilitating disease which has a significant impact on population health, economic productivity and tourism in Australia. This study examined epidemiological patterns of

the RRV disease in Queensland, Australia between January 2001 and December 2011 at a statistical local area level (Figure 1). Spatial-temporal analyses were used to identify the patterns of the disease distribution over time stratified by age, sex and space. The results show that the mean annual incidence was 54 per 100,000 people, with a male: female ratio of 1:1.1. Two space-time clusters were identified: the areas adjacent to Townsville, on the eastern coast of Queensland; and the south east areas (Figure 2). Thus, although public health intervention should be considered across all areas in which RRV occurs, it should specifically focus on these high risk regions, particularly during the summer and autumn to reduce the social and economic impacts of RRV.

1361

RISK EVALUATION OF THE RIFT VALLEY FEVER EMERGENCE IN EUROPE: COMPETENCE OF THE EUROPEAN MOSQUITOES AND ADAPTABILITY OF THE VIRUS

Faustine Louis, Ilaria Castelli, Marie Vazeille, Anna-Bella Failloux

Institut Pasteur Paris, Paris, France

The Rift Valley Fever virus (RVFV) first detected in Kenya in 1930 causes a zoonose with an important impact on livestock. Very recently, it has expanded its natural range of distribution outside the Sub-Saharan Africa, in Saudi Arabia, Yemen, Madagascar, the Comoros and Mayotte islands. Its current expansion questions on the risk of a RVFV emergence in Europe. RVFV is an arbovirus with an enveloped particle composed of 3 negative single-stranded RNA segments which is transmitted by more than 30 different mosquito species. It circulates among wild mammals at a low prevalence but when environmental conditions are favorable for mosquito proliferation, an epidemic can occur causing mass abortions and death of young animals. Humans are mainly contaminated by direct contacts with tissues and blood when manipulating infected animals. Thus, the economic and social impacts of a RVFV epidemic can be dramatic. The aim of our study will be to evaluate the risk of RVFV emergence in Europe and the conditions that could favor its transmission. It will be done by developing two objectives: (i) determine the distribution and the competence to RVFV of potential mosquito vectors in France, and (ii) determine if molecular changes in the viral genome can be associated to an increased transmission by European mosquitoes?

1362

INSECTICIDE RESISTANCE STATUS IN *ANOPHELES GAMBIAE S.L.* FOLLOWING THE SCALE UP OF MALARIA CONTROL INTERVENTIONS IN RWANDA

Emmanuel Hakizimana[1], Gad Iranzi[1], Dunia Munyakanage[1], Rhodaly Muthoni[2], Corine Karema[1]

[1]Ministry of Health, Rwanda, Kigali, Rwanda, [2]Abt Associations Inc., Kigali, Rwanda

The scale up of malaria vector control interventions in particular universal coverage (one net for two persons) with Long Lasting Insecticidal Nets (LLINs) achieved in February 2011 and Indoor Residual Spraying (IRS) have played a major role in reducing by 86 % malaria incidence in Rwanda. The spread of insecticide resistance that has been reported in the African region may reverse the tremendous gains made in malaria control. Since 2010, the Malaria and Other Parasitic Diseases Division (MOPDD) in Rwanda has conducted resistance monitoring of malaria vectors to detect trends and to guide vector control interventions. Since 2010, resistance monitoring of malaria vectors was conducted in eight sentinel sites located in four provinces in Rwanda with varying malaria endemicity. The collection of *Anopheles* larvae in the field was conducted as described by WHO (2002) and reared to adults in controlled field conditions. In 2010, resistance testing was carried out using the CDC bottle assay. From 2011, WHO insecticide susceptibility testing was used (WHO 1998). The susceptibility outcomes were assessd according to WHO standard procedures (WHO, 2013). In 2010, resistance of *Anopheles gambiae s.l.* was only detected to DDT 4% in two (25%) out of eight sites surveyed. In 2011, resistance to DDT 4% was confirmed in four sites (28%) and emerging resistance to Permethrin 0,75% in three (21%) out of 14 sites. In 2012, the resistance was again confirmed to DDT 4% in one site (20%) and to pyrethroids in two sites (40%) out of 5 sites. Likewise, in 2013, the resistance to DDT 4%, Pyrethroids (Lambdacyalothrin 0,05%, Permethrin 0,75%, Deltamethrin 0,05% and Etofenprox 0,5%) and Bendiocarb 0,1% was respectively found out in eight (29%), fifteen (55%) and two (7%) sites out of 27 sites monitored. During this period, all specimen of *Anopheles gambiae s.l.* tested were susceptible to organophosphates (Fenitrothion 1% and Malathion 5%) at all sites. In conclusion, the scale up of malaria vector control interventions has associated with the spread of insecticide resistance of malaria vector mainly to pyrethroids. In response to this threat, an insecticide resistance management strategy was developed by the Ministry of Health of Rwanda and has to be regularly reviewed. Therefore, further investigations have to be undertaken to elucidate the resistance mechanisms.

1363

POPULATION DYNAMICS OF MAJOR MALARIA VECTORS AND THE IMPACT OF INDOOR RESIDUAL SPRAYING ON ENTOMOLOGICAL INOCULATION RATE IN NASARAWA STATE, NORTH CENTRAL NIGERIA

Petrus U. Inyama[1], Lazarus M. Samdi[1], Henry Nsa[1], Nduka O. Iwuchukwu[1], Lena Kolyada[2], Aklilu Seyoum[3], Dereje Dengela[2], Bradford Lucas[2], Funto Adewusi[1], Joseph Ifeanyi Okeke[1], Christen Fornadel[4]

[1]AIRS Project, Abuja, Nigeria, [2]Abt Associates, Bethesda, MD, United States, [3]AIRS Project, Ghana, Accra, Ghana, [4]United States Agency for International Development, Bureau for Global Health/HIDN, Washington, DC, United States

The President's Malaria Initiative | Africa Indoor Residual Spraying project, executed asecond year of spray operations in Nasarawa Eggon and Doma Local Government Areas of Nasarawa State, Nigeria. Molecular tools were used to identify the predominant vectors responsible for malaria transmission in the study area, and entomological inoculation rates (EIRs) were calculated pre and post intervention. Mosquitoes were identified morphologically and by molecular methods using polymerase chain reaction (PCR). The *Plasmodium falciparum* circumsporozoïte indexes were measured by ELISA and the EIRs were calculated for the 3 areas. A total of 2,539 *Anopheles* mosquitoes were caught in the intervention areas and control site. Of these, 1,653 (65.1%) were caught in Doma, 525 (20.7%) in Lafia and 361(14.2%) in N/Eggon respectively. A subsample of 1,265 *Anopheles* mosquitoes were randomly selected for PCR analysis. Morphological analysis indicated that 1,174 (92.8%) were *An. gambiae* s.l., while the remaining were *An. funestus* (3.6%), *An. pharoensis* (2.8%) and *An. squamosus* (0.8%). PCR analysis of the *Anopheles gambiae* s.l. revealed a predominance of *An. gambiae* s.s (68.5%), while 29.9% were *An. arabiensis*. ELISAs showed that *P. falciparum* sporozoite infection rates were 1.7% in *An. gambiae* s.s. and 0.6% in *An. arabiensis*. There was a significant difference between the sporozoite rate of *An. gambiae* s.s. and *An. arabiensis* (χ^2=8.696, p<0.0032, df=1). At baseline (pre-intervention), EIR was found to be 1.31infective bites/person/night (bpn) in Doma, 0.16 in N/Eggon and 0.13 in Lafia, including both indoor and outdoor collections. After the IRS intervention, EIR was reduced to 0.9 in Doma and 0.11 in N/Eggon, while it remained the same at the control area in Lafia at 0.13 bpn. There was a significant difference in EIR reduction (p<0.0001) between the intervention areas and the control site. Although ELISA tests incriminated *An. gambiae* s.s. as the predominant vector responsible for transmission of malaria in the study area, *An. arabiensis* was also found to be sporozoite positive. *An. funestus* group were not incriminated in malaria transmission. Post intervention EIRs were observed to have significantly decreased in the intervention areas. These findings provide information on the relative roles of the main malaria vectors found in the study areas and the impact of indoor residual spraying on malaria transmission.

1364

DYNAMIC RELATIONSHIPS BETWEEN MOSQUITO MICROBIOME AND VECTOR COMPETENCE

Brittany Dodson[1], Laura Kramer[2], Jason Rasgon[1]

[1]Pennsylvania State University, University Park, PA, United States, [2]New York State Department of Health, Wadsworth Center, Slingerlands, NY, United States

The hologenome theory of evolution proposes that natural selection of an organism is also driven by its symbiotic microorganisms. Research on the insect holobiome (the host plus all associated microorganisms) has largely been descriptive and often ignored when studying influences on phenotype. For insect vectors of medical importance, pathogen transmission capability is often variable, possibly due to differences in the internal host environment. Given that, functional knowledge about the holobiome of insect vectors is key to understanding vector-borne disease distribution and anticipating possible consequences of global climate change. Several studies have described mosquito symbionts and have suggested that microbe abundance and diversity can impact malaria parasites. However, the function and utility of those microbes are virtually unknown, especially in mosquitoes that transmit viruses. We compared microbiome data of two phenotypically distinct colonies of *Culex tarsalis* mosquitoes for West Nile virus (WNV) vector competence. Our data suggests that vector competence may be influenced by the mosquito microbiome and specific candidate microbes may be responsible for these phenotypic differences. A dynamic relationship appears to exist between the mosquito holobiont and WNV vector competence in *Cx. tarsalis*.

1365

ANOPHELES ARABIENSIS IS NOT SUCCESSFULLY CONTROLLED BY INDOOR RESIDUAL SPRAYING IN NORTHWEST TANZANIA: IMPLICATION FOR MALARIA VECTOR CONTROL IN THE AREA

Natacha Protopopoff[1], Jovin Kitau[2], Alexandra Wright[1], Philippa West[1], Franklin W. Mosha[2], William Kisinza[3], Immo Kleinschmidt[1], Mark Rowland[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Kilimanjaro Christian Medical University College, Moshi, United Republic of Tanzania, [3]National Institute for Medical Research, Muheza, United Republic of Tanzania

In East Africa scale up of Insecticide Treated Net (ITN) and Indoor Residual Spraying (IRS) has been associated with a drastic reduction in the abundance of *Anopheles gambiae* s.s. the primary vector in the area. This led to an apparent shift in An. gambiae s.l. sibling species ratio toward the more zoophilic and more exophilic *An. arabiensis* which is less likely be killed by IRS and ITN. The impact of IRS with bendiocarb on the relative abundance of *An. gambiae* s.s. and *An. arabiensis* was evaluated in North West part of Tanzania during a community randomised trial. Pre intervention, *An. arabiensis* represented 18.6% (95%CI: 13.9-24.6) and *An. gambiae* s.s. 81.4% (95%CI: 75.5-86.1) of the population of *An. gambiae* s.l. collected with indoor light traps, while *An. arabiensis* accounted for 3.8% and *An. gambiae* s.s. 96.2% of the population found resting indoor. Sporozoite rate was 1.4% (95%CI: 1.1-2.0) and only *An. gambiae* s.s. were found positive. After IRS, density of *An. gambiae* s.s was reduced by 75% (p=0.046) and *An. arabiensis* by 25% (p=0.745). In the IRS villages sporozoite rate in *An. gambiae* s.s. was 1.8% and 0% for An.arabiensis. There was a significant difference in the gambiae s.s./arabiensis species ratio with *An. arabiensis* constituting 11.3% the control arm alone compared to 26.1% in the IRS arm (OR: 2.8 (95%CI: 1.1-6.8) p=0.027). Indoor Residual Spraying was more effective in controlling *An. gambiae* than *An. arabiensis* in North West Tanzania. *An. arabiensis* in this area is a secondary vector and appeared to contribute little to malaria transmission. The focus of control should remain on *An. gambiae* s.s. the main vector in this area while more specific vector control tools for *An. arabiensis* could be investigated.

1366

TRANSCRIPTOMICS OF DIFFERENTIAL VECTOR COMPETENCE: WNV INFECTION IN TWO POPULATIONS OF *CULEX PIPIENS QUINQUEFASCIATUS* LINKED TO OVARY DEVELOPMENT

Chelsea T. Smartt, Dongyoung Shin

University of Florida, Vero Beach, FL, United States

Understanding mechanisms that contribute to viral dissemination in mosquito vectors will contribute to our ability to interfere with the transmission of viral pathogens that impact public health. The expression of genes in two *Culex pipiens quinquefasciatus* populations from Florida with known differences in vector competence to West Nile virus (WNV) were compared using high throughput sequencing. Results A total of 15,176 transcripts were combined for comparison of expression differences between the two populations and 118 transcripts were differentially expressed ($p<0.05$). The fold change in expression of the differentially expressed genes ranged from -7.5 - 6.13. The more competent population for WNV (Gainesville) over expressed 77 genes and down regulated 44 genes, compared with the less competent population for WNV (Vero Beach). Also, splicing analysis identified 3 transcripts with significantly different splice forms between the two populations. The functional analysis showed that the largest proportion of transcripts was included in the catalytic activity and transporter activity groups except for those in the unknown group. Interestingly, the up- regulated gene set contained most of the catalytic activity function and the down- regulated gene set had a notable proportion of transcripts with transporter activity function. Immune response category was shown in only the down regulated gene set, although those represent a relatively small portion of the function. Several different vitellogenin genes were expressed differentially. Based on the RNAseq data analysis, ovary development was compared across the populations and following WNV infection. There were significant differences among the compared groups. In conclusion, this study suggests that ovary development is related to vector competence in two Culex populations in Florida. Both populations control energy allocations to reproduction as a response to WNV. This result provides novel insight into the defense mechanism used by Culex spp. mosquitoes against WNV.

1367

POPULATION SUBDIVISION WITHIN *ANOPHELES GAMBIAE* MAY IMPACT MALARIA TRANSMISSION IN GUINEA BISSAU

José L. Vicente[1], Beniamino Caputo[2], Marco Pombi[2], Carla A. Sousa[3], João Dinis[4], Amabélia Rodrigues[4], David Weetman[5], Alessandra della Torre[2], **João Pinto**[1]

[1]Centro de Malária e outras Doenças Tropicais, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon, Portugal, [2]Istituto Pasteur-Fondazione Cenci-Bolognetti, Dipartimento di Sanità Pubblica e Malattie Infettive, Università di Roma "La Sapienza", Rome, Italy, [3]Unidade de Parasitologia e Microbiologia Médicas, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon, Portugal, [4]Instituto Nacional de Saúde Pública, Ministério da Saúde Pública, Bissau, Guinea-Bissau, [5]Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Anopheles coluzzii and *Anopheles gambiae*, formerly *Anopheles gambiae* s.s. M and S forms, are generally characterized by low levels of hybridization along most of their west African sympatric range. However, high levels of hybridization and genetic introgression have been detected in the western African limit of their distribution, particularly in Guinea Bissau. In this study, we have characterized levels of genetic differentiation within and between *A. coluzzii* (M-form) and *A. gambiae* (S-form) samples collected from 10 localities along an east-west transect in Guinea Bissau, during the rainy season of 2010. Samples were identified to species by IGS-rDNA and SINE200X markers, genotyped for 19 microsatellites and for the insecticide resistance associated ace-1 and kdr loci. In addition, ELISA was used to determine blood meal origin and to assess sporozoite

rates in selected localities. Microsatellite data showed that hybridization between *A. coluzzii* and *A. gambiae* occurs mainly in coastal areas, with hybrid rates up to 19.4%. Moreover, Bayesian clustering analysis revealed a subdivision within *A. gambiae* into east and west/coastal populations. These populations are geographically separated by a central region where *A. coluzzii* prevails. Genetic partitioning within *A. gambiae* was also evident from the distribution of ace-1 and kdr resistance-associated alleles, that reached frequencies of 3% and 83% in east localities but were absent from west/coastal sites. West/coastal *A. gambiae* presented human blood indexes (HBI) between 26.4% and 77.8% whereas in the east population HBI was 99.3%. Sporozoite rates between 3.7% (N= 54) and 7.2% (N= 69) were recorded in east populations of *A. gambiae* but no CSP-positive mosquitoes were detected in west/coastal populations (N= 196). The differences in anthropophily and sporozoite rates found between east and west/coastal populations suggest that the genetic partitioning within *A. gambiae* is likely to have an impact on malaria transmission in the country.

1368

ELIZABETHKINGIA ANOPHELIS: MOLECULAR MANIPULATION AND INTERACTIONS WITH MOSQUITO HOSTS

Shicheng Chen, Michael Bagdasarian, Edward Walker

Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, United States

The microbiota of the mosquito gut can profoundly influence metabolism, fecundity, development and immunity of the mosquito host. Further, this microbiota may through natural processes or by paratransgenesis provide a promising method to constrain malaria parasite development. In this study, we used the bacterial commensal *Elizabethkingia anophelis* from *Anopheles gambiae* as a model to study its interaction with host mosquitoes. A genetic manipulation system involving plasmids, selectable markers, a reporter system, and transposons was newly developed for an *Elizabethkingia* strain isolated from our laboratory colony of *An*. *gambiae*. A replicable plasmid carrying the antibiotic resistance gene *ermF* was efficiently introduced into *Elizabethkingia* by conjugation from *E. coli*, resulting in erythromycin-resistant colonies. Plasmids from *Elizabethkingia* were successfully transferred to *Elizabethkingia* by electroporation, but transformation was at low frequency with the same plasmids and an *E. coli* donor, suggesting the presence of a restriction barrier. The transposon pMiniHimar-Em1 was conjugatively introduced into *Elizabethkingia* from *E. coli*. It transposed randomly, resulting in Em-resistant colonies; transposition efficiency was improved by modifying transposase promoter activity. A strong flavobacterial expression system based on promoter P*ompA* was engineered into pMiniHimar and adapted to *Elizabethkingia*. A GFP- and Nanoluc- tagged *E. anophelis* strain fed to larvae of *Anopheles gambiae* and *Aedes triseriatus* showed transtadial persistence and propagative growth in the *An. gambiae* gut environment but not in *Ae. triseriatus*, indicating that *Elizabethkingia* has a limited host range. Paratransgenesis potential of *Elizabethkingia anophelis* will be discussed.

1369

INFLUENCE OF DENGUE VIRAL TITER ON *AEDES AEGYPTI* BEHAVIORAL RESPONSE TO DEET

Victor A. Sugiharto[1], John P. Grieco[1], Michael J. Turell[2], V. Ann Stewart[1], Edgar D. Rowton[3], Michelle G. Colacicco-Mayhugh[4], Cara H. Olsen[1], Nicole L. Achee[5]

[1]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD, United States, [3]Walter Reed Army Institute of Research, Forest Glen, MD, United States, [4]Medical Education and Training Campus, Ft. Sam Houston, TX, United States, [5]University of Notre Dame, South Bend, IN, United States

Mosquito behaviors are heavily driven by odor cues within the surrounding environment. These cues are recognized by chemosensory receptors. Previous studies have shown that arboviral infections can alter mosquito behavior based on dysfunction of mosquito organs, particularly those of the nervous system.. Previously we have reported on the behavioral responses of DENV-1 infected mosquitoes exposed to DEET in the HITSS contact irritancy (CI) on 1, 4, 7, 10, 14, and 17 days post-injection (DPI). Here, we explore the association between dengue virus-1 (DENV-1) RNA copy in mosquito heads and their corresponding CI response in time series after infection. Viral RNA copy of individual head-preps of each DPI cohort are being used in reverse transcriptase RT-PCR to quantify viral RNA copy in both responders (irritated by DEET) and non-responders (no irritation upon exposure to DEET). Time to viral RNA copy plateau and, more importantly, differences in viral RNA copy between the responders and non-responders on any day post injection will be presented. Data will be used to determine the correlation between viral RNA titer with *Aedes aegypti* response against DEET. Findings will enhance our understanding of the potential attenuation in efficacy of chemical products designed to reduce the probability of human contact with infected vectors - a vital component for prevention of dengue virus transmission. Data collection will be completed by July 2014.

1370

EXPERIMENTAL EVIDENCE AND EMPIRICAL PROOF FOR CONTROL OF *PHLEBOTOMUS PAPATASI* SAND FLIES (DIPTERA: PSYCHODIDAE) USING A FEED-THROUGH LARVICIDAL RODENT BAIT WITH A BUILT-IN VALIDATION SCHEME

Thomas M. Mascari[1], Hanafi H. Hanafi[2], Souad Ouahabi[3], Btissam Ameur[4], Chafika Faraj[3], Peter J. Obenauer[2], Joseph W. Diclaro[2], Lane D. Foil[1]

[1]Louisiana State University, Baton Rouge, LA, United States, [2]U.S. Naval Medical Research Unit - 3, Cairo, Egypt, [3]Medical Entomology Laboratory, Rabat, Morocco, [4]Department of Epidemiology and Disease Control, Rabat, Morocco

Leishmaniasis is a neglected tropical disease for which very little can be done on an operational scale to reduce sand fly vector populations, parasite transmission, or the incidence of disease. Previously, control of sand fly larvae has not been an option because little is known about the larval ecology of most species, and because no reliable larval sampling method exists. Recently, however, stable isotope analysis of adult sand flies demonstrated that half of the sand fly larvae at a site in eastern Morocco developed to the adult stage on rodent feces. Previous lab studies have shown that rodent diets containing insecticides that pass into the feces effectively kill sand fly larvae. We report the results of a small-scale field trial on the use of feed-through larvicidal rodent baits to reduce sand fly populations. Half of the study sites received insecticidal rodent baits and half received untreated baits. Rather than solely measuring changes in the adult sand fly population, both the insecticidal and untreated rodent baits were co-formulated with a fluorescent tracer dye that passes into rodent feces, and marks both the sand fly larvae that feed on the feces and the subsequent adults. This tracer system provided a crucial entomological indicator: the proportion of adult sand flies that had fed as larvae on the feces of baited rodents. We observed significant reductions in the adult sand fly population and, through the use of the tracer, had empirical evidence for a causal link between the lower number of adult sand flies captured and the efficacy of the insecticidal rodent bait.

1371

A QUALITY MANAGEMENT SYSTEM FOR *ANOPHELES* INSECTARIES IN FDA-REGULATED STUDIES IN MALI

Mamadou Coulibaly[1], Joseph P. Shott[2], Brandyce St Laurent[3], Ibrahima Baber[1], Jennifer M. Anderson[3], Sara A. Healy[4], Yimin Wu[4], Issaka Sagara[1], Patrick E. Duffy[4], Robert W. Gwadz[3], Sekou F. Traore[1]

[1]Mali International Center for Excellence in Research, Malaria Research and Training Center, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali, [2]Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [3]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [4]Laboratory of Malaria Vaccinology and Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Human feeding studies using endemic vectors for arthropod-borne diseases have become a cornerstone for evaluating novel interventions such as vaccines, drugs, and repellants. Potential barriers to evaluating these interventions in endemic settings include: ensuring the fitness and safety of the vectors, and competency of regulatory sciences by entomological staff, particularly when involving studies overseen by the U.S. Food and Drug Administration. As part of a Phase 1 trial of a *Plasmodium falciparum* transmission-blocking vaccine (Pfs25), we have created a Quality Management System for our *Anopheles* insectary in Mali. This QMS is based on the tenets of Good Laboratory Practices and Good Manufacturing Practices including: standard operating procedure improvement and harmonization, training and competency assessment, quality control & assurance, and rigorous documentation practices. Our efforts to ensure tightly regulated processes for the continuous production of high-quality, safe vectors for human studies may benefit other institutions involved in the entomological components of interventional studies.

1372

POPULATION DYNAMICS OF *AEDES AEGYPTI* AND *ALBOPICTUS* IN NEW ORLEANS, LA, 2009-2013

Justin K. Davis, Dawn M. Wesson

Tulane University, New Orleans, LA, United States

Given the recent identification of autochthonous cases of dengue virus (DENV) in Texas and Florida and the apparent introduction of chikungunya virus (CHIKV) to the western hemisphere, it is reasonable to suspect that the viruses will eventually reach endemicity in the New Orleans, LA area, where two competent vector species, *Ae. aegypti* and *albopictus*, have established abundant populations. To investigate their population dynamics, oviposition cups were used to solicit eggs, larvae, and pupae in various areas in New Orleans, LA between 2009 and 2013. Samples are related to remotely-sensed vegetation indices and to meteorological covariates by the gamma distributed lag model of Schmidt (1974). We conclude that the two species respond systematically but differently to environmental covariates, namely temperature and precipitation, and that different weather scenarios imply predictable differences in the risk to humans of these two viruses. A paradoxical result is identified, implying that oviposition cups have a methodological bias that must be understood in practice.

1373

OCCURRENCE OF PAROUS FEMALES IN COPULA IN NATURAL SWARMS OF *ANOPHELES GAMBIAE* S.L.: EVIDENCE FOR RE-MATING BETWEEN GONOTROPHIC CYCLES

Simon P. Sawadogo[1], Thierry Lefèvre[2], Abdoulaye Diabaté[1], Carlo Constantini[2], Rosemary S. Lees[3], Gabriella Gibson[4], Roch K. Dabiré[1]

[1]Institut de Recherche en Sciences de la Santé/Centre Muraz, Bobo-Dioulasso, Burkina Faso, [2]Laboratoire MIVEGEC (UMR CNRS 5290, IRD 224, UM1, UM2), Montpellier, France, [3]Polo d'Innovazione Genomica, Genetica e Biologia S.C.a.R.L, Perugia, Italy, [4]Natural Resources Institute/ University of Greenwich, London, United Kingdom

The mating behavior of mosquito disease vectors has important implications for the implementation of novel approaches to vector control, such as the sterile insect technique (SIT) and the release of genetically-modified mosquitoes. From 2006 to 2009, the natural swarming and mating behavior of *Anopheles gambiae* s.l. was investigated in two sites near Bobo-Dioulasso, Burkina Faso. The gonotrophic status, insemination rate and parity rate of indoor resting and swarming (pairing and single) female *An. gambiae* s.l. were determined. We report the presence of parous *An. gambiae* s.l. females mating in natural swarms. The parity rates of mating females, swarming single females and indoor-resting females were, respectively, 5.0% (30/606), 4.1% (21/517) and 16.9% (239/1416). Because females lay eggs only when inseminated, these observations indicate that re-mating can occur between gonotrophic cycles in *An. gambiae* s.l., the major vector of malaria in Sub-Saharan Africa.

1374

COMPARATIVE ABILITIES OF MICROFILAREMIC VERSUS NON-MICROFILAREMIC BIRDS TO INFECT *CULEX PIPIENS* MOSQUITOES WITH WEST NILE VIRUS

Jefferson A. Vaughan[1], Elizabeth S. Andrews[2], Juanita Hinson[2], Michael J. Turell[2]

[1]University of North Dakota, Grand Forks, ND, United States, [2]U.S. Army Medical Institute of Infectious Diseases, Frederick, MD, United States

Vertebrate reservoirs of arboviruses are often infected with microfilariae (MF). Previous laboratory studies have shown that MF can enhance the infectivity of arboviruses to mosquitoes. Soon after being ingested, MF penetrate the mosquito midgut. If the blood meal also contains virus (i.e., the vertebrate reservoir is dually-infected), penetrating MF may introduce virus into the hemocoel. This can transform otherwise virus-incompetent mosquito species into virus-competent species and simultaneously accelerate viral development, allowing mosquitoes to transmit virus sooner than normal. This phenomenon is termed microfilarial enhancement of arboviral transmission. Because the prevalence of MF is very high in many passerine populations in North America, we investigated if microfilarial enhancement by microfilaremic passerines could have facilitated the spread of West Nile virus (WNV) across the USA. To do this, we injected two groups of Common Grackles with WNV; one group possessed naturally-acquired infections of Chanderella quiscali MF (n=6), and one group did not have MF infections (n=4). Different batches of *Culex pipiens* mosquitoes were allowed to feed on these birds during the next two to three days and at various time points thereafter (days 3, 4, 5, 7 and 14), mosquitoes were tested by plaque assay to determine rates of WNV infection (i.e., increased vector competence) and dissemination (i.e., decreased extrinsic incubation period). At the time of this writing (April 2014), final data are still forthcoming and will be presented at the meeting.

1375

REDUCING *AEDES ALBOPICTUS* HUMAN LANDING RATES IN ITALY THROUGH INNOVATIVE MOSQUITO TRAPS

Scott W. Gordon[1], Christian Engelbrecht[2], Claudio Venturelli[3], Andreas Rose[1], Martin Geier[1]

[1]Biogents, Regensburg, Germany, [2]Institut für Zoologie, Regensburg, Germany, [3]Azienda Unità Sanitaria Locale, Cesena, Italy

Since its introduction and establishment in Italy during the early 90s, the Asian tiger mosquito has spread over large parts of Italy and other Mediterranean countries. *Aedes albopictus* is not only a cause of biting nuisance but also a competent vector for various arthropod-borne diseases. Conventional attempts to control *Ae. albopictus* include source reduction, larvicides and adulticides. Although efficient traps for *Ae. albopictus* exist and are used for population monitoring, their use as a control tool has not been extensively studied. In this study, we assessed the ability of BG-Sentinel mosquito traps to control local populations of *Ae. albopictus* over a 15-week period in Cesena, Italy. Six experimental sites were matched and paired for the criteria of urbanization level, surface vegetation and mosquito density. In each pair, one site was selected as an intervention site and treated with 7-8 traps. The other site was designated as a control site and did not receive traps. Trap density ranged from one trap per 150 m^2 to one per 300 m^2. Mosquito populations in both the intervention and in control areas were monitored weekly with human landing collections and ovitraps. Results from human landing collections indicated biting rates were reduced between 60 and 90% in the treatment areas compared to the untreated control sites. These results indicate that the sustained use of efficient mosquito traps can significantly reduce the nuisance caused by *Ae. albopictus* in residential areas.

1376

DATA-DRIVEN MODELING FOR RECEPTIVITY AND SPREAD OF THE HIGHLY INVASIVE MOSQUITO, *AEDES ALBOPICTUS*

Christopher M. Barker[1], Diego Montecino[1], Susanne Kluh[2], Kenn Fujioka[3], Tanya Posey[2], Brian Sorvillo[3], Wakoli Wekesa[3]

[1]University of California Davis, CA, United States, [2]Greater Los Angeles County Vector Control District, Santa Fe Springs, CA, United States, [3]San Gabriel Valley Mosquito and Vector Control District, West Covina, CA, United States

The mosquito, *Aedes albopictus*, is among the world's most invasive species. Its spread has been facilitated by rapid global transport of cargo and potentially by climate warming, and it is now established on every continent except Antarctica. This species represents a "triple threat" to human health, being a day-biting pest, a competent vector of globally important dengue and chikungunya viruses, and a potential bridge vector of several zoonotic arboviruses. As a result of its importance, the biology of *Ae. albopictus* is also well-studied, but the fine-scale processes by which it becomes established in a given location are poorly understood because even intensive surveillance systems yield limited information during the early phase of invasions when densities are low, and detection often occurs after populations are relatively widespread. Fine-scale spatial models for mosquito dynamics and movement offer a way forward, marrying our understanding of *Ae. albopictus* biology with surveillance paradigms and detailed data on the real landscapes where invasions occur. Here, we consider the ongoing invasion and establishment of *Ae. albopictus* in Los Angeles since late 2011. We use hierarchical modeling with remote sensing and surveillance data from the study area to account for heterogeneities in household-level receptivity, then we model the stochastic dynamics of *Ae. albopictus* on this landscape using the suitability surface and a temperature-dependent, dynamical model for reproduction and spread. We found the probability of establishment to be much greater for introductions of eggs in containers compared to single adult females that might arrive by automobile. We also show that the rate of spread was strongly seasonal and greatest during late spring and summer, and the ability to contain the mosquitoes' spread diminished rapidly with increasing delays to detection, regardless of the control methods used.

1377

VILLAGE EDGE ASSOCIATION, DIEL FLIGHT ACTIVITY AND HOST SELECTION PATTERNS OF MALARIA VECTORS IN VILLAGES OF MADANG PROVINCE, PAPUA NEW GUINEA

John B. Keven[1], Edward D. Walker[2], Lisa Reimer[3], Peter M. Siba[1], James W. Kazura[4], Peter A. Zimmerman[4]

[1]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [2]Michigan State University, East Lansing, MI, United States, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [4]Case Western Reserve University, Cleveland, OH, United States

The diel flight and host seeking behavior of females of 5 species of *Anopheles* mosquitoes was studied in 4 malaria-endemic villages of Madang Province, Papua New Guinea, using a vertical barrier screen sampling system, during May to August, 2012. The screen consisted of shade cloth configured to posts and erected vertically to a height of 2.5 meters. It captured both host seeking and blood fed individuals throughout the night. More non-blood fed females were captured on the side of the screen facing the bush, earlier in the evening, whereas more blood fed females were captured on the village side of the screen later in the evening to early morning. These results suggest commuter behavior of host seeking females from outside to inside the village nightly, followed by village exiting behavior back to the surrounding bush. Host identification of blood meals by sequencing of the mitochondrial cytochrome B gene revealed that humans and domestic pigs were the most common and often only hosts, even though other potential vertebrate hosts were present in abundance. *An. punctulatus* and *An. koliensis* were highly anthropophagous, *An. farauti* s.s, *An. longirostris*, and *An. farauti* (species 4) relatively less so, whilst *An. bancrofti* fed mostly on pigs. The implications of these findings for malaria transmission are discussed with reference to the human blood index.

1378

PRE-CLINICAL EVALUATION OF A COMBINED LIVE ATTENUATED (LAV) AND SUBUNIT (DEN-80E) PRIME-BOOST VACCINE APPROACH AGAINST DENGUE

Danilo R. Casimiro[1], Beth-Ann Coller[1], Steve Meschino[1], Elizabeth Thoryk[1], Ramesh Chintala[1], Govindarajan Dhanasekaran[1], Stephen Whitehead[2], Andrew J. Bett[1]

[1]Merck Research Labs, West Point, PA, United States, [2]Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Merck & Co is developing a tetravalent recombinant subunit vaccine for dengue. The current vaccine candidate (referred to as V180) consists of four truncated, soluble dengue envelope protein (DEN-80E from DENV1-4) produced in Drosophila S2 cells and administered with a saponin-based adjuvant, ISCOMATRIX™ adjuvant. In previous non-human primate studies V180 was able to (a) induce a balanced immune response across all 4 types and (b) protect against virus challenge in rhesus macaques. It is currently the subject of a Phase I trial in flavivirus naïve subjects. While the inclusion of a novel adjuvant in V180 appears necessary to induce a rapid robust response, it may also complicate the development path. In contrast, live attenuated viral (LAV) candidates typically have good immunogenicity, memory/durability, and favorable CoGs but may be complicated by interference, under/over-attenuation, and/or extended dosing schedules. The recently reported poor efficacy of the chimeric dengue vaccine against DENV2 in a Phase II trial may also suggest that the induced titers may not be sufficient to provide protective efficacy in the field. For this reason, we have conducted rhesus macaque studies in which the tetravalent DEN-80E vaccine (with or without the use of ISCOMATRIX™) is combined with a tetravalent LAV in a heterologous prime-boost immunization regimen.

The objective is to optimize the neutralizing titers across all 4 types for both magnitude and longevity. Immunological data on the heterologous and homologous prime-boost vaccinated monkeys will be presented. The combined use of live/non-live vaccine immunogens has the potential of being an effective vaccine approach against multiple dengue types.

1379

COMPARISON OF PASSIVE AND SENTINEL-ENHANCED DENGUE SURVEILLANCE SYSTEMS IN PUERTO RICO

David Noyd[1], Olga Lorenzi[1], Aidsa Rivera[1], Janice Perez Padilla[1], Luisa Alvarado[2], Kay M. Tomashek[1], **Tyler M. Sharp**[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]San Lucas Episcopal Hospital, Ponce, PR, United States

Dengue represents an increasingly important global and public health challenge; however, current passive surveillance systems underestimate the true burden of disease. In 2009, the World Health Organization urged countries to implement sentinel-enhanced surveillance to characterize the epidemiology of dengue to better evaluate new prevention methods. To estimate underreporting of dengue via passive surveillance in Puerto Rico, we analyzed the epidemiologic trends of suspected dengue cases reported to the long-running island-wide passive dengue surveillance system (PDSS) and compared them to those obtained from cases identified by a hospital-based Sentinel Enhanced Dengue Surveillance System (SEDSS). Dengue diagnostic testing for both PDSS and SEDSS includes RT-PCR to detect dengue virus (DENV) nucleic acid and ELISA to detect anti-DENV IgM antibody. Analyzed data were collected from PDSS and SEDSS in the Ponce health region between May 7, 2012 and May 6, 2013, the first year of operation of SEDSS. Of 3,483 suspected dengue cases reported to PDSS and 2,027 cases identified by SEDSS, 1,444 (41.5%) and 621 (30.6%) were laboratory-positive dengue cases, respectively. Compared to dengue cases reported to PDSS, those identified by SEDSS were younger (25 years vs. 19 years; $p < 0.0001$), presented for care earlier after illness onset (3 days vs. 4 days; $p < 0.0001$), were hospitalized less frequently (46% vs. 64%; $p < 0.0001$), and demonstrated higher completion of demographic and clinical variables for the case investigation form (61% vs. 27%; $p < 0.0001$). There were no significant differences in other demographic variables or DENV type distribution between SEDSS and PDSS. This evaluation demonstrated that SEDSS provides more robust clinical information and more accurately identifies non-hospitalized patients, though it may bias toward younger individuals. Enhanced dengue surveillance should be implemented in other locations of the world to complement existing passive surveillance systems to better understand the epidemiology and burden of dengue.

1380

WHAT PROPORTION OF DENGUE VIRUS INFECTIONS RESULT IN NO APPARENT DISEASE?

Hannah E. Clapham[1], Derek A. Cummings[1], Michael A. Johansson[2]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Division of Vector Borne Diseases, Centers for Disease Control and Prevention, San Juan, PR, United States

The concept of the dengue iceberg is a well-known one; a large proportion of dengue virus infections result in minimal or no symptoms and are thus never reported to surveillance systems. However, there is no consensus on what this proportion is and estimates in the literature vary substantially. We define infections as either apparent or inapparent. Though the definition of an apparent infection may vary depending on the study methodology, this is generally a dengue virus infection that results in overt illness, symptoms or healthcare seeking. An inapparent infection is one in which individuals have a serological response consistent with infection, but no accompanying illness (as defined above). Estimates in the literature of the proportions of dengue virus-infected people who experience apparent or inapparent infections vary widely. Though some of this variation may be due to differential definitions, there is some evidence that these proportions depend not only on whether the infection is a first, second or post-second infection, but also on infecting serotype (and in some cases genotype) and on the age of the infected individual. Combining published data from dengue cohort studies and from outbreak situations with serological data from multiple settings over multiple years, with consideration of the definitions used in each study, we aim to estimate the proportion of dengue virus infections that are apparent and inapparent. We also aim to estimate the influence of immune history, infecting serotype and age on these proportions. By combining datasets, the similarities and differences between these settings provide increased information about the effect of each of these factors. A Bayesian framework is used, thereby allowing the inclusion of uncertainty in the data and our resulting estimates. Better estimates of the proportions of dengue virus infections that are inapparent will be of use for understanding transmission of dengue viruses in multiple settings. For example, inapparent infections may be contributing to transmission. In addition, inapparent primary infections, though not producing apparent infection and thus not contributing to disease burden, leave individuals primed for secondary infections, so will be important for understanding the future disease burden in a population.

1381

CLINICAL COURSE AND OUTCOME OF DENGUE INFECTION DURING PREGNANCY

Ananda D. Wijewickrama[1], Gaveshika Abeyratne[1], Damayanthi Idampitiya[1], Priyankara Jayawardena[2]

[1]Infectious Diseases Hospital, Angoda, Sri Lanka, [2]Castle Street Hospital for Women, Colombo, Sri Lanka

Dengue, a major mosquito borne infection in the tropics, is hyper-endemic in Sri Lanka since 2009 with an annual incidence of more than 25,000 cases. The maximum rate of infection is seen in the 20-39 age category, making the pregnant population a vulnerable category.A retrospective observational study was conducted on all pregnant patients with Dengue admitted during 2013 to two major hospitals in Colombo, Sri Lanka. Data including clinical & laboratory parameters, interventions made, and complications were documented for analysis.Dengue infection was confirmed in 58 patients admitted. Mean age was 28.45(SD: 5.573) yrs. 55.2% had Dengue fever (DF) while 44.8% had Dengue Haemorrhagic fever (DHF). 19%, 46.6% and 34.5% were in the first, second and third trimester respectively. 82.8% had Rh positive blood groups, with 27.6% B positive, 25.9% O positive, 20.7% A positive and 8.6% AB positive while in 3.8% the blood group was not known. All had fever and 86.2% had myalgia. Hepatic tenderness, persistent vomiting and postural dizziness were seen most commonly with DHF (81.8%, 100% & 70% respectively). Mean day of entry into critical phase was 4.5 (SD: 0.990) day of the illness. The mean lowest platelet count in DF was 90.94 while in DHF was 37.81 ($p<0.000$), which was observed on a mean day of 5.25 in DF & 6.04 in DHF ($p< 0.03$). Most of the DF patients (31.2%) had highest AST levels in the 32-100 range while most of the DHF patients (34.6%) had levels in the 501-1000 range. Most of the DF patients (34.4%) had normal ALT level whereas most of the DHF patients (38.5%) had highest ALT in the 101-300 range. 3.1% of DF and 7.7% of DHF patients had fetal distress while 3.1% of DF and 3.8% of DHF patients had intrauterine death (IUD). 52% of all patients needed HDU/ICU care. All the patients recovered completely. This study shows incidence of DHF is higher in pregnancy than in the normal population. High numbers of patients with Rh positive blood groups were among pregnant Dengue patients. Some parameters like low platelet count, high AST and ALT were significantly high in DHF, indicating these can be used to identify high risk groups for developing DHF. Careful management assures full recovery of mothers, however, adverse outcome on the fetus remains high.

1382

USE OF A HOUSEHOLD SEROSURVEY TO ESTIMATE THE MAGNITUDE OF A DENGUE OUTBREAK IN MOMBASA, KENYA, 2013

Esther M. Ellis[1], John Neatherlin[2], Abdinoor Mohamed[2], Barry Fields[2], Daniel Ondari[2], Shem Patta[3], Mark Delorey[4], Brenda Torres-Velasquez[1], Nina Marano[2], Lilian Waiboci[2], Melvin Ochieng[2], Stella Gikunju[2], Harold Margolis[1], Joel Montgomery[2], Kay M. Tomashek[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]Kenya Medical Research Institute/Centers for Disease Control and Prevention, Nairobi, Kenya, [3]Ministry of Health, Mombasa, Kenya, [4]Centers for Disease Control and Prevention, Fort Collins, CO, United States

Dengue is endemic in Africa where an estimated 64 million dengue virus (DENV) infections occurred in 2010. Few outbreaks have been reported from East Africa since dengue was first detected in the late 1800s, and the geographic distribution of infections is uncertain. In February 2013, several individuals with dengue-like illness and negative malaria blood smears were identified in Mombasa, Kenya. Serum samples from initial cases confirmed dengue, and an investigation was conducted to determine the incidence of DENV infection in Mombasa. A stratified multistage sample of households was selected from the Tudor community of Mombasa where the incidence of reported dengue was high. Household residents provided serum specimens and information on medical and travel history. Serum was tested for DENV nucleic acid by RT-PCR, NS1 ELISA (i.e., current DENV infection), and anti-DENV IgM antibody by ELISA (i.e., recent DENV infection). Design-based estimates incorporated probabilities for selection of households and used a finite population correction factor. Of 1,502 participants living in 701 households, 207 (14%) had evidence of current (n = 103) or recent (n = 104) DENV infection, with a design-based estimate of 13% (95% CI: 10-16). DENV-1 and -2 were detected equally. Of the 207 participants with evidence of DENV infection, 91 (44%) reported fever in the past month; three (1%) were hospitalized; and two (1%) had bleeding manifestations. Reporting a fever in the past 30 days was significantly associated with DENV infection (OR=2.8; CI 1.9-4.2). Reporting open windows at nighttime was a risk factor for infection (OR=2.3; CI 1.1-4.8). Daily use of mosquito repellent daily was protective from infection (OR=9.1; CI 3.7-20.0). This investigation revealed a high burden of dengue in this part of East Africa. Behavioral strategies to avoid mosquito bites should be advocated for individuals to avoid DENV infection. Surveillance for and clinical and public awareness of dengue should be improved in East Africa to reduce the morbidity and mortality due to this disease.

1383

DEVELOPMENT OF A NS1AG-ELISA FOR THE DETECTION OF ALL DENGUE 1 TO 4 TYPES

Katia Souza[1], Gérman Anez[2], Andréa Teixeira - Carvalho[3], Luz Espina Gomes[4], Caren Chancey[2], Andriyan Grinev[2], Daniel Heisey[2], Erna Kroon[5], Maria Rios[2]

[1]Departamento de Bioquímica e Imunologia, Belo Horizonte, Brazil, [2]Food and Drug Administration, Bethesda, MD, United States, [3]Centro de Pesquisa Rene Rachou, Belo Horizonte, Brazil, [4]Seccion de Virologia, Instituto de Investigaciones Clinicas, Maracaibo, Brazil, [5]Laboratório de Vírus Departamento de Microbiologia, Belo Horizonte, Brazil

Dengue is the most important arthropod-borne viral disease in tropical and subtropical areas of the world. It is a public health concern in the Americas, including the US, because it is endemic in Puerto Rico and caused outbreaks in Florida, Texas, Hawaii, and in other US islands. The infection is mainly transmitted by the mosquito *Aedes aegypti*. Dengue diagnosis is commonly made using MAC-ELISA which indirectly detects specific antibodies to the E protein of the Dengue virus (DENV). Direct detection by virus isolation in culture and reverse transcriptase-polymerase chain reaction (RT-PCR) is used less frequently due to time, limited availability and high cost. An attractive alternative to culture and molecular tests is the detection of NS1 in the sera of patients during the acute phase of dengue. This viral protein is produced and secreted by infected human cells in the early stages of infection. Unfortunately, NS1 assays currently available have low sensitivity and specificity, and have been reported to miss DENV-4. In order to address this issue we developed an ELISA-based NS1 antigen assay (NS1Ag-ELISA) that uses two monoclonal antibodies, selected among 10 commercially available, that recognizes all DENV1-4 NS1 proteins. Initially, we were tested: (a) 130 specimens positive for DENV RNA and/or IgM (29 DENV-1, 4 DENV-2, 7 DENV-3, 16 DENV-4 and 54 not typed), all produced positive results on the NS1Ag-ELISA. (b) 75 DENV-negative specimens (19 West Nile Virus (WNV) positive, 6 Yellow Fever Virus (YFV) positive and 50 negative). 66 tested negative while 9 (8 WNV-positive and 1 YFV-positive), tested negative for DENV by RT-PCR but produced false positive results in our assay, possibly due to cross-reactivity between Flavivirus NS1 under the current assay conditions. We are modifying the assay to enhance DENV NS1 specificity and reduce cross reactivity. We expect to have in a few months a robust diagnostic test for the acute phase of dengue infection that may help early identification of serious dengue disease and facilitate critical care.

1384

SPATIAL CLUSTERING OF DENGUE AT THE HOUSEHOLD LEVEL IN A HIGHLY URBAN SETTING

Katherine Anders[1], Tran Van Ngoc[2], Nguyen V. Chau[2], Nguyen Dac Tho[3], Cameron Simmons[1]

[1]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, [2]Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, [3]Preventive Medicine Centre, Ho Chi Minh City, Vietnam

In the absence of a vaccine or therapeutic, the only strategy currently available for dengue prevention is control of the mosquito vector *Aedes aegypti*. In many endemic countries, including Vietnam, this is pursued primarily by responsive insecticide spraying around homes of reported cases. Central to this approach is the assumption that most DENV infections are acquired in the home. Evidence of highly focal DENV transmission at household level has been demonstrated in a rural village setting in Thailand, but it is unclear whether this assumption is valid in highly urban, mobile populations. We conducted a community-based study to investigate clustering of dengue risk around households in Ho Chi Minh City, Vietnam. We enrolled clusters of 25-35 household members and neighbours living within 25 metres of an index case with clinically suspected dengue. Laboratory diagnosis of the index cases allowed us retrospectively to classify them as confirmed dengue cases (n=52) or non-dengue controls (n=19), and to calculate the relative risk of 1) incident DENV infection during a two-week follow up period and 2) recent DENV infection at baseline in case clusters compared to controls (representing background risk). There was no difference in the risk of incident DENV infection between case and control clusters (82 in 1341 participants (6.1%) vs 31/569 (5.4%), respectively). However participants in case clusters were significantly more likely to have had a recent DENV infection at baseline than those in control clusters (OR = 2.3; 95%CI 1.2-4.7). The prevalence of DENV-infected *Ae. aegypti* collected from index houses was low overall (1%), with no difference between cases and controls, however case houses were significantly more likely to have high adult vector densities than controls. Our findings show that although there was clustering of recent DENV infections around households, there was no excess of incident infections in the two weeks following index case detection; this suggests that responsive vector control activities in this window are unlikely to have a large impact on DENV transmission in this setting.

1384

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1385

EPITOPE PRESERVATION, IMMUNOGENICITY AND PROTECTION OF INACTIVATED DENGUE VIRUS ANTIGENS FORMULATED WITH A NOVEL BIOLOGICAL ADJUVANT IN RHESUS MACAQUES

Laura J. White[1], Wahala Wahala[1], John Gibbs[1], Melissa Mattocks[1], Idia Rodrigez[2], Melween Martinez[2], Teresa Santiago[2], Petraleigh Maldonado[2], Robert Johnston[1], Aravinda de Silva[3], Carlos Sariol[2]

[1]Global Vaccines Inc., Research Triangle Park, NC, United States, [2]University of Puerto Rico, San Juan, PR, United States, [3]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Dengue viruses (DENV1-4) are considered the most important emerging, human arboviruses with worldwide distribution in the tropics, yet there are no licensed antiviral therapies or vaccines available. Although there are live attenuated virus vaccine candidates in clinical trials, there is an urgent need to accelerate the development of second-generation vaccine strategies. We developed a dengue vaccine based on a purified, inactivated virion (iDV) mixed with a novel alphavirus adjuvant (GVI3000/3A). The GVI adjuvants are disarmed viruses that derive their activity from the replication of a truncated alphavirus RNA. *In vivo*, the GVI adjuvants target DC in the DLN and mimic the earliest stages of a viral infection. The antigenic integrity of purified dengue virus antigens (iDV) was determined after inactivation by different protocols. A panel of mouse and human MAbs were used as probes to confirm the preservation of conformational epitopes in different domains of E protein, including recently characterized serotype specific, strongly neutralizing human MAbs that map to epitopes only preserved in the quaternary structure of the virion. Safety, immunogenicity and protective efficacy of GVI-adjuvanted iDV were determined in rhesus macaques, comparing 3 adjuvant doses and 2 adjuvant modalities. A tetravalent iDV mixture formulated with a GVI adjuvant demonstrated 1) significant increases in neutralizing antibody titers, 2) protection from viremia, and 3) no adverse events in any of the vaccinated animals. These results support the advancement of this new dengue vaccine candidate toward clinical trials in humans.

1386

THE DETECTION OF ANTI-DENGUE VIRUS IGM IN URINE AS A PUTATIVE MARKER FOR SEVERE DISEASE

Elba V. Caraballo, Katie Poole-Smith, Kay M. Tomashek, Brenda Torres, Elizabeth Hunsperger

Centers for Disease Control and Prevention, Division of Vector-Borne Diseases, Dengue Branch, San Juan, PR, United States

Dengue is globally the most important arbovirus disease with an estimated 300 million dengue virus (DENV) infections however, only 100 million dengue cases are reported per year. It is estimated that 5-10% of cases result in severe dengue, which may include glomerular changes associated with renal dysfunction. We looked for the presence of anti-DENV IgM antibodies in urine as an indicator for severe dengue among patients identified with acute febrile illness in our Sentinel Enhanced Dengue Surveillance System (SEDDS) site in Ponce, Puerto Rico. Between May 2012-March 2013, 1560 patients with fever or history of fever for ≤7 days were enrolled, a past medical history of chronic illnesses was obtained, and they were followed through their febrile illness. Serum and urine specimens were collected during the acute (days post onset of fever (dpo)=0-5) and convalescent phase (dpo=6-14) of their illnessAcute serum was tested for DENV RNA by RT-PCR . All urine specimens were tested for anti-DENV IgM. The results from the urine anti-DENV IgM were compared to the results in serum to determine sensitivity and specificity. The sensitivity of urine anti-IgM was 37% and specificity was 98% compared to serum. When compared to serum RT-PCR results, the sensitivity of IgM in urine was 24% and the specificity was 93%. To determine if IgM in urine might be an early indicator of disease severity, we compared this result to patient hospitalization; hospitalization being used as a surrogate for disease severity. Hospitalized dengue patients were 3.2 times more likely to test positive for IgM in urine than IgM negative (OR = 3.2 95CI 4.9-2.2).There was no correlation between the presence of IgM in urine with sex, age or pre-existing chronic diseases such as diabetes, high blood pressure, or anemia. While detection of anti-DENV IgM in urine lacked adequate diagnostic sensitivity when compared to serum, its presence may be a marker for hospitalization or disease severity.

1387

DEVELOPMENT OF DENGUE VIRUS PRM-REACTIVE ANTIBODIES AS TOOLS FOR MEASURING THE VIRION MATURATION STATE OF INFECTIOUS VIRIONS

Swati Mukherjee[1], Soila Sukupolvi-Petty[2], Aihua Zheng[3], Margaret Kielian[3], Michael S. Diamond[2], Theodore C. Pierson[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Departments of Medicine, Molecular Microbiology, Pathology, Washington University School of Medicine, St. Louis, MO, United States, [3]Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY, United States

Newly formed dengue viruses (DENV) incorporate envelope (E) proteins in complex with the viral structural protein premembrane (prM) as heterotrimeric spikes. During egress from infected cells, prM is cleaved to pr and M protein by host furin-like proteases to produce infectious virions. However, we and others have shown that this process is inefficient and leads to the release of partially mature DENV that retain non-cleaved

prM and have significantly different functional properties. The extent of prM cleavage required for production of an infectious virus is unknown. In this study, we characterized a panel of murine monoclonal antibodies (mAbs) that bind prM produced by immunization with recombinant pr protein. prM-reactive mAbs were extensively cross-reactive and shown to be capable of enhancing DENV infection of Fc-receptor-expressing cells. Several prM-reactive antibodies displayed a significant capacity to neutralize infection, although this pattern was cell type-dependent. Examination of neutralization dose-response curves on Raji cells expressing DC-SIGNR revealed the presence of a fraction of virions resistant to neutralization; the size of this population could be varied by altering the efficiency of the virion maturation process. We conclude that in this context, viruses resistant to neutralization are those that display prM epitopes with a stoichiometry insufficient to satisfy the threshold requirements for neutralization. We demonstrate the potential for prM-reactive antibodies as a sensitive functional probe of the maturation state of DENV released from cells, providing a method to deconstruct the structural heterogeneity of DENV produced under a variety of conditions.

1388

COSTS OF DENGUE FEVER TO THE HEALTH SYSTEM AND INDIVIDUALS IN COLOMBIA IN 2010 TO 2012

Raul Castro Rodriguez[1], Katia Galera[1], Juan Guillermo López Yescas[2], Jorge Rueda[1]

[1]Universidad de los Andes, Bogota, Colombia, [2]Sanofi Pasteur, Mexico City, Mexico

Dengue fever is an important health issue in Colombia but detailed direct information on economic costs is lacking. We estimated the average cost per case of ambulatory dengue fever (aDF), hospitalized dengue fever (hospDF) and dengue hemorrhagic fever (DHF) over the period. Tallied costs included direct and indirect medical costs, as well as non-medical costs to the healthcare system, and indirect costs to patients, using information from official databases and an extensive population-based face-to-face survey of 1,089 households with recent dengue fever patients. In 2010, the mean direct medical cost per case for the healthcare system of aDF, hospDF, and DHF were, respectively $52.8USD, $235.8, and $1,512.2. To the individuals, the mean direct non-medical costs ($29.7, $46.7 and $62.6, respectively) greater than the mean household direct medical costs ($13.3, $348 and $57.3, respectively). The average cost to the healthcare system of a case of ambulatory dengue fever in the epidemic year of 2010 was 57% that in 2011.Our results highlight the high economic burden of the disease and could be useful for assigning limited health resources..

1389

INNATE IMMUNITY AND TRANSCRIPTOME PROFILING AFTER ADMINISTRATION OF TAKEDA'S LIVE ATTENUATED DENGUE VACCINE CANDIDATE IN FLAVIVIRUS-NAÏVE HUMAN VOLUNTEERS: ASSOCIATION OF GENE EXPRESSION WITH DEVELOPMENT OF NEUTRALIZING ANTIBODY RESPONSES TO DENV

Sarah George[1], Jorge Osorio[2], Dan T. Stinchcomb[2], Aurelia Haller[2], Ellis Reinherz[3], Vladimir Brusic[3]

[1]St. Louis University, St. Louis, MO, United States, [2]Takeda Vaccines, Inc., Madison, WI, United States, [3]Dana Farber Cancer Institute, Boston, MA, United States

We conducted a phase I, randomized, double-blind dose-escalation study of two different formulations of Takeda's live attenuated dengue vaccine candidate in 72 healthy flavivirus-naïve adults at the Saint Louis University VTEU (NCT01110551). Volunteers received 2 doses of a live attenuated tetravalent dengue vaccine candidate 90 days apart. Samples of whole blood were collected on days 0, 2, 4, 7, and 11 after vaccination to assess early responses to the vaccine by transcriptome analysis. Total RNA was isolated from whole blood and T7 transcribed-linear RNA was amplified and hybridized to Illumina Human HT12 v 4 microarrays. These microarrays detect all mRNAs expressed from the human genome. RNA expression data was exported and analyzed by genesets using the canonical pathways stored within the MSigDB database. No significant changes in geneset expression were identified that correlated with: 1) route of vaccine administration (SC vs. ID), 2) viremia after vaccination, or 3) neutralizing antibody titer (above or below the group mean on day 120 after vaccination). However, there were significant differences in geneset expression in subjects who developed a tetravalent neutralizing antibody response to DENV vs. subjects who had a mono/bivalent response. Subjects with a tetravalent response had at least a 1.5-fold increase in expression of genesets involved in integrin signaling, the complement pathway, interferon signaling, cytokine expression, and innate immune responses. In summary, increased expression of genesets which mediate the innate immune response and translation to adaptive immunity was significantly correlated with a tetravalent neutralizing antibody response to Takeda's live attenuated dengue vaccine candidate.

1390

IMPORTATION FOLLOWED BY LOCAL TRANSMISSION OF TWO LINEAGES OF DENGUE VIRUS TYPE 1 IN THE UNITED STATES: 2013

Gilberto A. Santiago[1], Dana Thomas[1], Brian Smith[2], Danielle Stanek[3], Ana M. Rivas[4], Carina Blackmore[3], Linda Gaul[2], Kay Tomashek[1], Jorge L. Munoz-Jordan[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]Department of State Health Services, Texas, Harlingen, TX, United States, [3]Florida Department of Health, Tallahassee, FL, United States, [4]Universidad Autonoma de Monterrey, Monterrey, Mexico

Dengue, one of the most important arthropod-borne tropical diseases globally, is a significant public health concern affecting an estimated 100 million people in 2010. Dengue is caused by any of the four genetically related dengue viruses (DENV-1-4) that are maintained by transmission in large urban areas by *Aedes* mosquitoes. The proliferation of urban areas, frequent international travel and climatic changes, have been proposed to contribute to the increased dissemination of DENV-1-4. We have previously reported local transmission of a monophyletic lineage of DENV-1 in Key West, Florida in 2011-2012. In 2013, DENV-1 was identified in febrile patients residing in two Texas counties, Cameron and Hidalgo, adjacent to the border with Mexico. These patients reported no recent travel history. DENV-1 was also identified in febrile patients with no recent travel history residing in two Florida counties, Martin and St. Lucie. Identification of patients with laboratory-confirmed DENV-1 continued for time periods of 4-6 months in both locations. In this study we have conducted an in-depth envelope gene sequence analysis to characterize the emergence of DENV-1 in Texas and Florida and their relatedness with viruses from Key West, Mexico, Central America and the Caribbean. All sequences grouped within the American-African genotype of DENV-1. Bayesian phylogenetic analyses show a strong association of Texas and Northern Mexico DENV-1 with viruses from Central America, with the Texas isolates forming a monophyletic group. In contrast, the Florida isolates formed two independent subgroups: the previously reported Key West virus of Central American origin and the Martin-St. Lucie virus of Caribbean origin. The monophyletic characteristic of these lineages supports local transmission of DENV-1, and that conditions are suitable to sustain transmission with the potential to cause outbreaks.

1391

DENGUE QUASISPECIES COMPLEXITY ANALYSIS FOR MOSQUITO AND HUMAN SAMPLES FROM KAMPHAENG PHET, THAILAND: CLONING AND IMPLICATIONS FOR HIGH THROUGHPUT SEQUENCING METHODS

Melanie C. Melendrez[1], Piyawan Chinnawirotpisan[2], Alongkot Ponlawat[2], Chonticha Klungthong[2], Tao Li[1], Stephen J. Thomas[1], Robert V. Gibbons[2], Alan Rothman[3], Timothy P. Endy[4], In-Kyu Yoon[2], Thomas W. Scott[5], Jason H. Richardson[1], Richard G. Jarman[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]University of Rhode Island, Providence, RI, United States, [4]SUNY Upstate Medical University, Syracuse, NY, United States, [5]University of California Davis, CA, United States

All four serotypes of dengue virus (DENV) exist as quasispecies populations. Quasispecies are described as a spectrum of variants, genetically linked through mutation creating an interactive population where selection acts on the population rather than individual variants. While cloning provides linkage of all population mutations to single genomes; laborious methods are required to adequately sample the quasispecies population. High-throughput sequencing has become the method of choice in reconstructing quasispecies populations; though mutations within the population cannot be linked to single viral genomes. We examined the complexity and mutations discovered in clone-based quasispecies population analysis and compared results with assemblies obtained from the 454 sequencing. The E gene for 4 quasispecies populations from DENV-3 from a 2010 study in Thailand were cloned and sequenced using Sanger sequencing. Full genomes were obtained using 454 sequencing and E gene mutation comparisons with clones were conducted. The cloned populations, explored sequence space in several directions with transmission of dominant and subdominant variants (3-6 subdominant variants) and varying degrees of complexity. The percent of variants within the populations containing variable nt sites ranged from 68.9-77.6% (a.a. 46.7-57.1%) suggesting high population plasticity. Hinge region and non-functional variants were found in cloned populations however not in 454 assemblies due to low coverage. As read depth increased the probability of detecting cloned mutations increased. Full genome assemblies showed other potentially transmissible quasispecies mutations. Investigating dengue quasispecies diversity and behavior has relevance for understanding population responses to selective pressures such as innate and vaccine induced immunity. Work to investigate quasispecies population dynamics and complexity during illness and *in vivo*/vitro cycling using the MiSeq and PacBio systems to achieve high coverage and linkage of mutations to genomes within the population are underway.

1392

REAL-TIME FORECASTING OF THE 2014 DENGUE FEVER SEASON IN THAILAND

Nicholas G. Reich[1], Krzysztof Sakrejda[1], Derek A. Cummings[2], Paphanij Suangtho[3], Soawapak Hinjoy[3], Sopon Lamsirithaworn[3], Hannah Clapham[2], Henrik Salje[2], Justin Lessler[2]

[1]University of Massachusetts, Amherst, MA, United States, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]Thai Ministry of Public Health, Bangkok, Thailand

Dengue is a major cause of morbidity in Thailand. Annual outbreaks of varying sizes provide a particular challenge to the public health system because treatment of severe cases requires significant resources. Advanced warning of increases in incidence could help public health authorities allocate resources more effectively and mitigate the impact of epidemics. In collaboration with the Thai Ministry of Public Health and Bureau of Epidemiology, we have developed a statistical model for infectious disease surveillance that uses data from across Thailand to give early warning of developing dengue epidemics. The model creates forecasts for each of the 77 Thai provinces. For each province, the forecast is based on (1) seasonal dynamics of dengue in the focal province, and (2) observed case counts at recent time-points from the focal province and neighbors demonstrated to be relevant through model selection using historical data. Prior to the beginning of the 2014 dengue season in Thailand, our team defined a process to generate forecasts for dengue in real-time. Beginning in April 2014, we created updated forecasts every two weeks based on the most current data from the Thai Ministry of Public Health database. We will present the results of this real-time forecasting exercise, including evaluating the performance of different forecasting models in predicting different features of the 2014 dengue season in each Thai province. Specifically, we will evaluate the ability of our models to predict the beginning, end, duration, and peak of the dengue epidemic. To our knowledge this is the first time that real-time forecasts of dengue have been attempted in Thailand based on reported case data.

1393

EXPLORING THE IMPACT OF INDIVIDUAL MOSQUITO SALIVARY PROTEINS ON DENV INFECTION IN THE VERTEBRATE

Michael K. McCracken, Rebecca C. Christofferson, Daniel M. Chisenhall, Britton J. Grasperge, Christopher N. Mores

Louisiana State University, Baton Rouge, LA, United States

Dengue virus (DENV) is transmitted during probing by an infectious mosquito concurrent with expectorated saliva. This saliva is composed of numerous proteins with anti-hemostatic and immuno-modulatory capabilities, and was shown previously to aid viral establishment within the vertebrate. IRF3/7 -/--/- (C57BL/6) mice intradermally-inoculated with DENV at sites of contemporaneous mosquito probing exhibited viremias of significantly enhanced magnitude and duration compared to mice unexposed to mosquitoes. This mosquito-driven enhancement was associated with differential regulation of immune transcripts involved in viral recognition and defense at early times post exposure. However, limited work exists on the relationship between individual *Aedes aegypti* salivary proteins and vertebrate infection with DENV. In an effort to characterize the contribution of individual salivary proteins to the enhancement of DENV infection, we have utilized recombinant salivary proteins for examination *in vivo*. One such protein was aegyptin, a known allergen and inhibitor of platelet aggregation. We intradermally-inoculated mice with and without co-inoculation of aegyptin and examined differences in viral titers and circulating leukocytes throughout viremia, along with viral titers and immune parameters at injection sites and draining lymph nodes at 48 hpi. Interestingly, co-inoculation of aegyptin resulted in decreased viral titers at inoculation sites and in circulation at 48 hpi compared to DENV alone, and these decreases were associated with alterations in cytokine concentrations in the lymph nodes of aegyptin-exposed mice. Additionally, co-inoculation of mice that had previously received multiple exposures to aegyptin resulted in further alterations to viremia titers. While co-inoculation of aegyptin did not yield universal enhancement of DENV titers, these results inform on immune pressures faced during DENV infection and support a complex system of interaction between the milieu of salivary proteins expectorated, DENV, and the vertebrate host environment.

1393

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1394

EXTENDING A DETAILED *AEDES AEGYPTI* MODEL TO SIMULATE SINGLE AND COMBINED DENGUE CONTROL IN IQUITOS, PERU

Emily Griffiths, Mathieu Legros, Kenichi Okamoto, Fred Gould, Alun Lloyd

North Carolina State University, Raleigh, NC, United States

Attempts to control dengue include cleaning out water containers, poisoning larvae, and spraying insecticide inside homes. Other control measures like vaccines and engineered mosquitoes are being developed. We do not know which of these new control measures might result in the fewest dengue cases, either alone or in combination. We develop a complex simulation model that can compare single and multiple control interventions. This extends a detailed, stochastic model of *Aedes aegypti* population dynamics (Skeeter Buster) to include the movement patterns and infection histories of individual humans. We use this model to study the effect of various control measures on dengue epidemics in the city of Iquitos, Peru. We show how the speed and size of an epidemic varies with the number of places people visit each day. We also show the effects of spraying insecticide in homes, releasing dengue-resistant mosquitoes, and administering vaccinations. We test for single and pairwise combinations of these interventions, but find little evidence of synergistic effects. Our results suggest that combining control measures while making similar total investments may not prevent as many dengue cases as a single control measure.

1395

ADJUSTING UNDERREPORTED REAL TIME CASE DATA FOR PREDICTION OF DENGUE IN THAILAND

Krzysztof Sakrejda[1], Nicholas G. Reich[1], Derek A. Cummings[2], Paphanij Suangtho[3], Soawapak Hinjoy[3], Sopon Lamsirithaworn[3], Hannah Clapham[2], Henrik Salje[2]

[1]University of Massachusetts, Amherst, MA, United States, [2]Johns Hopkins University, Baltimore, MD, United States, [3]Ministry of Public Health, Nonthaburi, Thailand

Symptomatic cases of dengue virus, including dengue fever and dengue hemorrhagic fever, are an important cause of morbidity in Thailand. Thailand has a comprehensive nationwide case reporting system for Dengue and efforts are currently underway to leverage this data for real-time prediction of epidemic severity. Using case data for real-time prediction across a large geographic area that has multiple distinct administrative units is fraught with challenges. A central challenge is that the administrative units that contribute to the larger reporting system as a whole have heterogeneity in reporting processes which results in a substantial and highly variable interval of time (reporting interval) between when a case record is created and when it becomes available to use for prediction. Currently, dengue prediction models must use weeks-old case counts to assure that most relevant data has been processed through the reporting system. A better understanding of the reporting process in different locations could 1) provide metrics for use in optimizing the reporting system, and 2) make it possible to use the most recent incomplete counts for prediction of dengue epidemic intensity. We have developed and applied time-to-event models to characterize the spatial and temporal variation in reporting intervals and their relationship to case load and other seasonal features. Preliminary results suggest that this problem requires the use of contaminated time-to-event distributions to characterize reporting intervals and a hierarchical approach to combining information from diverse administrative units. We will present our analysis on the reporting process over the course of the 2013 and 2014 dengue seasons in Thailand, as well as our methods for improving the usability of real-time case data.

1396

DENV RNA AND ANTI-DENGUE ANTIBODY INTEGRITY IN CLINICAL SAMPLES ON DRIED BLOOD STABILIZATION PRODUCTS DURING AMBIENT TEMPERATURE SHIPMENT

Theron Gilliland, Jr.[1], Allison Dauner[1], Subhamoy Pal[1], Robert D. Hontz[2], Shuenn-Jue Wu[3]

[1]Naval Medical Research Center/Henry M. Jackson Foundation, Silver Spring, MD, United States, [2]U.S. Naval Medical Research Unit - 6, Lima, Peru, [3]Naval Medical Research Center, Silver Spring, MD, United States

Degradation of RNA and antibodies during specimen transport from collection site to diagnostic facility is a major problem affecting accurate diagnosis of RNA-based pathogens. This is particularly true when shipping may require more than a day of transit, as cold-chain is not always available in low-resource settings. In this study, we used dengue as a model RNA virus to compare the performance of three down-selected commercially available nucleic acid-stabilization products: Biomātrica DNAstāble tubes, ViveBio ViveST tubes, and Whatman FTA Micro Cards. Whole blood specimens collected from acute dengue fever patients (Days 0-4 Post Onset of Symptoms) during routine febrile surveillance in Iquitos, Peru were applied to the nucleic acid-stabilization products and dried overnight. At various time points, the stabilized specimens were shipped under ambient conditions (temperatures ranging from 9.7 to 34.3 °C and relative humidity ranging from 53.4 to 74.6% during shipment) to a diagnostic testing laboratory in Lima, Peru. Anti-dengue antibodies and dengue RNA levels were then tested via IgM ELISA and qRT-PCR, respectively, and compared to matched frozen unloaded controls. Agreements compared to each specimen's matched controls were: 97.3% IgM and 97.4% RNA (DNAstāble); 97.4% IgM and 95.0% RNA (ViveST);

and 81.6% IgM and 82.5% RNA (FTA Micro Cards).Other considerations such as cost, sample volume required, and ease-of-use were also evaluated in this study and should ultimately inform any decision to incorporate commercial sample stabilization products into a downstream diagnostic testing workflow.

1397

CHARACTERIZATION OF A DENGUE VIRUS TYPE 4 OUTBREAK IN SOUTH-CENTRAL MATO GROSSO, BRAZIL

Nayara Zuchi[1], Steven G. Widen[2], Rubing Chen[2], Hilda Guzman[2], Leticia B. Heinen[1], Sandra V. Mayer[2], Thomas G. Wood[2], Marcelo A. dos Santos[3], Jonathan E. Allen[4], Robert B. Tesh[2], Renata Dezengrini-Slhessarenko[1], **Nikos Vasilakis**[2]

[1]Federal University of Mato Grosso, Cuiabá, Brazil, [2]University of Texas Medical Branch Health, Galveston, TX, United States, [3]MT-Laboratory, State Secretary of Health, Cuiabá, Brazil, [4]Lawrence Livermore National Laboratory, Livermore, CA, United States

Dengue viruses (DENV) are by far the most important arboviral pathogens in the tropics globally, putting at risk of infection nearly a third of the global human population. In the current study we characterized the phylogeny and intrahost variation of 26 isolates of dengue virus type 4 (DENV-4) from acute serum samples obtained during an outbreak in South-Central Mato Grosso State (MT), Brazil, in 2012. All 26 isolates located within genotype II in two distinct lineages forming a monophyletic clade. Further confirmation of the co-circulation of two distinct lineages is obtained by analysis of the intrahost virus variation in the acute serum samples. Based on our phylogenetic analyses, there are 6 independent introductions of DENV-4 in Brazil, presumably from Venezuela, Puerto Rico, China, and Southeast Asia. The DENV-4 isolates of the 2012 outbreak in South-Central Mato Grosso State were closely related with two 2010 isolates from the geographically close regions of Amazonas and Roraima and were closely related with strains sampled from Venezuela 2007, indicating the potential origin introduction. The extent and severity of the 2012 DENV-4 outbreak is likely attributed to the lack of immunity in the population.

1398

CALL TO ACTION: A SCREENING TOOL FOR PREVENTION AND TREATMENT OF DENGUE IN TRAVELERS WITH CHRONIC COMORBIDITIES

Koya C. Allen

Kent State University, Kent, OH, United States

There is increased risk of Dengue Hemorrhagic Fever (DHF) in patients with comorbidities such as hypertension, diabetes, allergies and obesity. Lack of preparedness in epidemics can increase mortality rates amongst these patients when preexisting conditions are not identified and guidelines for case management not followed. In travelers, there is limited evidence for predicting clinical course of dengue in patients with chronic comorbidities. The WHO guidelines for treatment, prevention and control of dengue recommends identification of preexisting conditions and offers guidance for treating patients with obesity. Similar guidance is lacking for hypertension, diabetes and allergies. Acetylsalicylic acid (aspirin), Ibuprofen and other non-steroidal anti-inflammatory drugs (NSAID) are contraindicated for dengue; however, the prevalence of individuals on treatment regimens using these drugs is increasing. In an analysis of Behavioral Risk Factor Surveillance System (BRFSS) 2011 data, 25% of adults take aspirin daily and 31% have hypertension, of which 77% are being treated. In addition, 10%, 13% and 60% of adults had diabetes, asthma or obesity respectively. For travelers to dengue endemic regions it is important to develop and provide guidance for chronic disease management and dengue prevention. Post-travel it is important for medical professionals to have strict guidance on case management for potential dengue complications. A travel screening tool can identify high-risk travelers based on VFR status, Cultural Embeddedness, and social determinants of health in conjunction with comorbidities and current treatment. This tool can be implemented in pre-and post-travel consultation. Overall, this research recommends a call to action for researchers to 1) Develop guidelines for treating dengue patients with comorbidities; 2) Research effect of aspirin and NSAID regimens on dengue pathogenesis; and 3) Increase dengue surveillance and control in regions with high chronic disease prevalence and high population susceptibility to dengue, particularly travelers.

1399

INFLUENCE OF MATERNAL TOTAL IGG LEVELS ON TRANSPLACENTAL TRANSFER OF DENGUE VIRUS-SPECIFIC ANTIBODIES

Priscila M. Castanha[1], Eduardo J. Nascimento[1], Marli T. Cordeiro[2], Claudeir D. Silva Jr.[2], Ernesto T. Marques Jr.[1], Cynthia Braga[2]

[1]University of Pittsburgh, Pittsburgh, PA, United States, [2]Aggeu Magalhães Research Center, Recife, Brazil

Placental transfer of maternal dengue IgG antibodies to the fetus is likely to play an essential role in immunity and pathogenesis of dengue infection in infants. In order to investigate the kinetics of dengue-specific maternal antibodies transferred to children in the first two years of life, a birth cohort of 417 children living in an area of intense circulation of dengue virus in the northeast region of Brazil has been established. Here, we carried out a preliminary analysis of 216 dengue seropositive mother-newborn pairs to investigate the transference of total and dengue-specific IgG antibodies via placenta. Maternal and umbilical cord blood samples were obtained during the time of delivery. Serotype-specific antibody profile was determined by PRNT, while in-house ELISA was used to both measure dengue-specific IgG titers and estimate the levels of total IgG in the sera. Antibody titers were log-transformed and used to evaluate the degree of dengue-specific IgG transferred from mothers to infants. The average maternal age was 23.8 years (range, 13-41 years). In maternal sera, 127 out of 216 (58.8%) showed a monotypic profile against DENV3, 25.5% to the combination of DENV3/ DENV4 and 9.8% had detectable neutralizing antibodies against three or more serotypes. Dengue-specific IgG titers were significantly higher in cord blood (4.89±0.52) than in maternal samples (4.69±0.52; p=0.0006). A consistent pattern was also observed when comparing DENV3-specific PRNT titers in infants (2.68±0.83) and mothers (2.49±0.66; p=0.0095). Maternal levels of total IgG were negatively correlated with placental transfer of dengue-specific IgG (r= -0.1818, p= 0.0074) and DENV3 neutralizing antibodies (r= -0.1289, p=0.0586), indicating that very high levels of maternal IgG increases competition among the types of IgG transferred through the placenta. These results further suggest that maternal antibody transfer is influenced by maternal total IgG levels.

1400

CHARACTERIZING GLOBAL AND LOCAL TRENDS IN DENGUE TRANSMISSION: INSIGHT FROM AGE-SPECIFIC SURVEILLANCE DATA

Isabel Rodríguez Barraquer, Derek A. Cummings

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Good characterization of global and local trends in dengue transmission has been challenging. Given that seroprevalence data, the gold standard to measure prior exposure, is very scarce, previous efforts have relied almost exclusively on total case or presence/absence data. However, while this approach has proven useful to define the distribution of dengue at a global scale, relying exclusively on counts may be misleading when looking at trends over time, or at finer spatial scales, due to the poor correlation that exists between infection and symptomatic disease. Here, we propose a framework to estimate yearly forces of infection (yearly probability of a susceptible individual being infected) and basic reproductive number

(R0) of dengue based on the age distribution of cases that are reported to surveillance systems. We use data from 4 countries where age-specific incidence data is publicly available (Thailand, Brazil, Mexico, Colombia) to estimate the force of infection and R0 over a period of 15 years at the province or, where possible, district levels. When available, we compare our estimates to those obtained from age-stratified serological surveys. Preliminary results suggest that age-specific incidence data provides a robust way to characterize dengue transmission at a global and local scale in settings of varying transmission intensity. In addition, they highlight the large heterogeneities in recent dengue epidemiology that exist within countries, provinces and probably even finer spatial scales. This is particularly true for countries such as Brazil, where dengue has recently re-emerged. Proper characterization of global and local trends in dengue epidemiology will be fundamental to target control interventions and design optimal vaccination strategies.

1401

MEDICAL COSTS OF DENGUE FEVER IN MEXICO

Luis Durán-Arenas, Adriana Zubieta-Zavala, Malaquías López-Cervantes

National Autonomous University of Mexico, Delegación Coyoacán, Mexico

In Mexico, dengue fever incidence has varied since its reappearance in 1970s, with peaks in 1980, 1997, and 2009 and >130 000 cases. With high incidences, accurate cost estimates of disease are needed to efficiently use finite treatment and prevention health resources (vaccination and vector control). This study assessed medical cost and cost to the infected individual using a micro-costing approach to overcome a lack of centralized data. The cost per dengue case is derived from health system direct medical costs, patient direct costs, and productivity loss-related indirect costs. Costs were calculated in the SS and the IMSS settings. To derive health system costs, an ideal protocol for dengue fever treatment was based on a review of national and international norms, guidelines, and expert consensus combined with a microcosting tool known as PAATI (program, actions, activities, tasks, inputs). For comparison to real costs, actual tasks and inputs for real dengue fever cases were derived from chart review and health personnel and hospital administrators interviews. Patient direct and indirect costs were derived from patient interviews. Indirect cost was defined as disease-associated productivity loss (to patient and carer). Of chart reviews (N=1440) foreseen in 18 Mexican states, 1293 were obtained (90%) and clinical pathways were obtained for 1168 (81%). For direct medical costs, we observed an increased cost gradient depending setting (ambulatory $92 USD, hospitalized $1644, ICU $9375). We noted a difference between ideal cost and real cost in both SS and IMSS systems. The main difference driver in ideal costs between outpatients and hospitalized patients was cost of professional services (~90% for outpatients and ~100% for hospitalized/ICU patients). Medicine accounts for a fraction of overall cost, yet real expenditure is reduced compared to ideal expenditure for drugs. Direct real medical cost of ambulatory cases of $33 for SS is lower than direct medical costs reported in Brazil ($49),Colombia ($67) whereas medical cost for IMSS system ($92) is higher and comparable to Venezuela ($118). In contrast, hospitalized patient direct medical cost, in relative terms, is higher: $490 and $1644 in SS and IMSS respectively, vs $318 for Brazil, $331 for Columbia ($864 for Venezuela). Real costs and costs associated with ideal treatment are different, particularly for outpatients, pointing to health system failings (both SS and IMSS).

1402

EARLY INDICATORS OF DENGUE AMONG CHILDREN AND ADULTS PRESENTING WITH ACUTE FEBRILE ILLNESS IN PUERTO RICO

Doris A. Andujar-Perez[1], Olga D. Lorenzi-Pena[2], Kay M. Tomashek[2], Frances Gonzalez-Figueroa[3], R. Ivan Iriarte[3], Luisa I. Alvarado-Domenech[3]

[1]Ponce School of Medicine and Health Sciences/Saint Luke's Episcopal Hospital, Ponce, PR, United States, [2]Centers for Disease Control and Prevention, Division of Vector-Borne Disease - Dengue Branch, San Juan, PR, United States, [3]Ponce School of Medicine and Health Sciences/Saint Luke's Episcopal Hospital, Ponce, PR, United States

Early clinical diagnosis of dengue can be challenging because the initial presentation is nonspecific with signs and symptoms similar to those of other acute febrile illnesses (AFI). Rapid diagnostic testing is often not available. Early identification and timely initiation of correct treatment can reduce complications and mortality. To identify early indicators for laboratory-positive dengue, we analyzed data from a sentinel enhanced dengue surveillance system conducted at a large referral hospital in Puerto Rico. Outpatients with fever for <7 days were enrolled and followed through their illness. Serum and nasopharyngeal specimens were collected and tested by RT-PCR and immunodiagnostic methods as appropriate for dengue viruses (DENV-1-4), *Leptospira* spp., *Burkholderia pseudomallei*, 5 enteroviruses, influenza A and B viruses, and 12 other respiratory viruses. Laboratory-indeterminate cases, co-infections and infants were excluded from analysis. Among the 1,580 patients enrolled during May 7, 2012 through May 6, 2013, 570 (36.1%) were hospitalized, 805 (51.0%) were male, and the median age was 21.1 years (range: 1-91 years). There were 617 dengue-positive patients, 611 respiratory infections, 72 infections caused by other viruses or bacteria and 280 cases with no pathogen identified. Five clinical findings were found to be independently associated with a laboratory-positive dengue: retrorbital pain, leukopenia, thrombocytopenia, rash and facial erythema. Sore throat, nasal congestion and cough were less frequent on dengue-positive patients. Clinical and laboratory features that were predictive of dengue were found to vary by patient age. Dengue was associated with: leukopenia, rash and joint pain ($p<0.005$) in children aged <9 years; leukopenia and thrombocytopenia ($p < 0.005$) in individuals aged 10-19 years; and thrombocytopenia, leukopenia, rash, nausea and joint pain ($p < 0.003$) in adults aged ≥20 years. Knowledge of predictors can be used to direct anticipatory guidance.

1403

PRESENCE OF THREE DENGUE SEROTYPES IN OUAGADOUGOU, BURKINA FASO AND ITS PUBLIC HEALTH IMPLICATIONS

Beatriz Parra[1], **Mabel Carabali**[2], Antarou Ly[3], Emmanuel Bonnet[4], Florence Fournet[5], Roch K. Dabiré[6], Clara Beatriz Ocampo[7], Seni Kuanda[8], Isabelle Agier[9], Valéry Ridde[10]

[1]Universidad del Valle, Cali, Colombia, [2]Dengue Vaccines Initiative/ International Vaccines Institute, Seoul, Republic of Korea, [3]Institut de Recherche en Sciences de la Santé, IRSS/CNRST, Ouagadougou, Burkina Faso, [4]Institut de Recherche pour le Développement, Identités et Différenciations de l'Environnement des Espaces et des Sociétés, Paris/ Caen, France, [5]IRSS/Centre Muraz, Bobo-Dioulasso, Burkina Faso/UMR MIVEGEC Institut de Recherche pour le Développement, Montpellier, France, [6]IRSS/Centre Muraz, Bobo-Dioulasso, Burkina Faso, [7]Centro Internacional de Entrenamiento e Investigaciones Medicas, Cali, Colombia, [8]Institut de Recherche en Sciences de la Santé, CNRST, Ouagadougou, Burkina Faso, [9]University of Montreal Hospital Research Centre (CRCHUM), Montreal, QC, Canada, [10]University of Montreal Hospital Research Centre (CRCHUM)/School of Public Health (ESPUM), University of Montreal, Montreal, QC, Canada

The discussion about the presence of febrile non-malaria cases has increased in Burkina Faso. As other febrile diseases, dengue was considered as differential diagnosis due the presence of the vector and previous DENV reports in the country. To explore the virus presence in acute febrile non-malaria cases and *Aedes* mosquitoes in Ouagadougou, an exploratory cross sectional study was performed from December 2013 to January 2014. Five sectors and six correspondent health care centers (CSPS) were selected based on a reported presence of Flavivirus: CSPS 3 and 12 (Dapoya), 8 (Gounghin), 18 (Pissy), 25 (Somgandé) and 28 (Dassasgho). A survey about symptoms was administered to the participants and finger pricks were used to obtain the samples. Each CSPS tested every febrile non-malaria patient for dengue using dengue rapid tests (SD Bioline DengueDuo). Blood spots were obtained in filter paper from all positive results and every tenth negative for further PCR analyses. A parallel entomological survey was conducted in the CSPS's correspondent sectors. From a total of 379 patients tested, 35 (9.2%) were positive for rapid test (60% both IgM/IgG; 21% just IgG and 5% just NS1). 91% were older than 15 years old (range 0-61 years old), 60% were women and 70% came to the CSPS during the first 3 days of fever. From 60 samples tested by RT-PCR, 15 were positive (9 from positive rapid test and 6 from the subsample of negative results). The serotypes observed were DENV2 (Dassasgho and Gounghin), DENV3 (Dapoya, Pissy and Somgande) and DENV4 (Dapoya, Gounghin and Somgande). There was not DENV in the analyzed mosquitoes. The presence of dengue in acute febrile non-malaria patients in Ouagadougou was evidenced. To our knowledge, thought the presence of DENV3 and DENV4 were reported in the region, this is the first time both serotypes are evidenced in Burkina Faso. These findings have important public health implications due the need to prepare the health system and the population for dengue's presence and outbreaks prevention (Additional data will be available at the conference)

1404

EVIDENCE OF RECENT DENGUE EXPOSURE AMONG MALARIA PARASITE-POSITIVE CHILDREN IN THREE URBAN CENTERS IN GHANA

Justin Stoler[1], Rupert K. Delimini[2], J. H. Kofi Bonney[3], Gordon A. Awandare[2]

[1]University of Miami, Coral Gables, FL, United States, [2]University of Ghana, Legon, Ghana, [3]Noguchi Memorial Institute for Medical Research, Legon, Ghana

Dengue fever is increasingly being recognized as an important neglected tropical disease in sub-Saharan Africa, with national burdens generally unknown due to misdiagnosis of cases as malaria. This study screened for evidence of dengue exposure in 222 children aged 2-14 living in Accra, Kintampo, and Navrongo, Ghana, who tested positive on a rapid diagnostic test (RDT) for malaria and were subsequently confirmed to be malaria parasite-positive via blood test. We found presence of dengue-specific IgM antibodies using indirect ELISA methods in 7 children screened across the three sites, and presence of dengue-specific IgG antibodies in 20%, 13%, and 30% for Accra, Kintampo, and Navrongo respectively. The high rates of dengue exposure among children with confirmed malaria may be just the tip of the iceberg in terms of dengue prevalence among the heavy volume of febrile illness patients who do not have confirmed malaria. We discuss demographic correlates of dengue exposure and argue that this study underscores the need for assessing Ghana's baseline dengue burden as well as general clinical knowledge of the disease.

1405

CO-INFECTION WITH DENGUE AND RESPIRATORY VIRUSES AMONG CHILDREN WITH ACUTE FEBRILE ILLNESS, PUERTO RICO

Yajaira Guzman[1], Olga D. Lorenzi[2], Xyomara Jimenez[1], Maria Aranda[1], N. Judith Lopez[1], Ivonne Galarza[1], Jorge L. Muñoz-Jordán[2], Elizabeth Hunsperger[2], Harold S. Margolis[2], Ivan Iriarte[3], Luisa I. Alvarado[1]

[1]Hospital Episcopal San Lucas, Ponce, Puerto Rico, [2]Dengue Branch, Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, San Juan, Puerto Rico, [3]Public Health Program, Ponce School of Medicine and Health Sciences, Ponce, Puerto Rico

Dengue is endemic in Puerto Rico with seasonal increases in incidence that often coincide with increases in other acute febrile illnesses (AFI) caused by respiratory pathogens. Consequently, co-infections are possible, which may complicate both diagnosis upon presentation and the patients' clinical course. Specifically, the presence of respiratory symptoms may reduce early diagnosis of dengue, which is important for early initiation of clinical management that can minimize medical complications and mortality. In May 2012, a sentinel enhanced dengue surveillance system (SEDSS) site was established in a tertiary care hospital in Ponce, Puerto Rico wherein patients with fever for <7 days were enrolled and followed through their illness. Serum, nasopharyngeal and oropharyngeal specimens were collected and tested by RT-PCR for multiple pathogens including: dengue virus subtypes 1-4 (DENV-1-4); influenza A and B viruses, adenovirus, respiratory syncytial virus, metapneumovirus, and parainfluenza viruses. To identify factors associated with co-infection patients with DENV and respiratory virus co-infection (cases) were age-matched to patients infected with DENV only (controls) at a ratio of 1:2. Of 715 case-patients with DENV detected in serum, 30 (4.2%) had evidence of co-infection with a respiratory virus. There were no differences by gender identified among cases and controls. Most (87%) of the co-infections were children and adolescents (<20 years). Cases were more likely than controls to report cough (odds ratio [OR] = 3.17; 95% confidence interval [CI]: 1.2-8.1) or runny nose (OR = 2.58; 95% CI: 1.02, 6.50). Cases were also more likely to have a chest x-rays ordered, although this difference was not statistically significant (OR =1.6; 95% CI: 0.61-4.0). Further analysis will include factors associated with illness severity and clinical outcome. These findings suggest that in areas with endemic dengue and respiratory pathogens, physicians should have a high index of suspicion for co-infections in children and adolescents.

1406

POSITIVE SEROLOGY FOR HANTAVIRUS IN PATIENTS WITH CLINICAL SUSPECTED DENGUE IN CEARÁ, BRAZIL

Danielle M. Lima[1], Raissa M. Fontes[2], Gilberto S. Santos Junior[3], Jeová K. Colares[1], Benedito A. Fonseca[3], Luiz T. Figueiredo[3]

[1]University of Fortaleza, Fortaleza, Brazil, [2]Federal University of Ceará, Fortaleza, Brazil, [3]School of Medicine of Ribeirão Preto-USP, Ribeirão Preto, Brazil

Dengue is considered the most important arbovirus in the world in terms of morbidity and mortality, having broad and unspecific symptoms, ranging from asymptomatic to severe hemorrhagic forms. Thus, it becomes difficult to distinguish it from other febrile syndromes only by clinical and epidemiological criteria. The use of these criteria as the unique basis for diagnosis of dengue can be dangerous and may lead to false diagnoses and inappropriate treatment. Within of the spectrum of similar to dengue acute febrile diseases, some pathogens are not routinely investigated by the lack of resources and ignorance of their existence in the region. In Ceará, the hantavirus was never notified and there is only one report in the regional literature showing the probable existence of this disease in humans in the state. Thus, the aim of this study was to investigate cases of hantavirus in patients suspected of dengue in Ceará. In this study, we evaluated 95 patients, with clinical suspicion of dengue, recruited during the year 2012 in the State of Ceará. The samples were evaluated for hantavirus through ELISA-IgM and ELISA-IgG tests. One (1.05%) patient was positive for hantavirus by ELISA- IgM, detecting current or recent infection by the virus. This patient had moderate symptoms, suggesting that mild or atypical cases of hantavirus should be occurring in the State. Thirty (31.6%) patients were positive by ELISA-IgG. This result suggests that they have recently or previously infected by hantavirus, but this result does not allow to determine whether this virus was the causative agent of febrile syndrome presented by these patients. All patients in this study were questioned about the conduct of recent trips and none reported having left Ceará in recent months, probably acquired the infection locally. For a state that has only one report in the literature for this pathogen in humans, the percentage of people with prior contact with it was very high, showing the need for further investment and research on this disease in Ceará. Financial support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

1407

DESCRIPTION OF FEBRILE ILLNESS AND DENGUE IN INFANTS LESS THAN 90 DAYS OLD

Mariana Tavárez[1], Janice Perez-Padilla[2], N. Judith Lopez[1], Ivan Iriarte[3], Luisa I. Alvarado[1]

[1]Hospital Episcopal San Lucas, Ponce, Puerto Rico, [2]Dengue Branch, Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, San Juan, Puerto Rico, [3]Public Health Program, Ponce School of Medicine and Health Sciences, Ponce, Puerto Rico

Fever in the first 90 days of life presents a diagnostic and therapeutic challenge for pediatricians. Bacterial infections should be identified in order to provide adequate treatment, but sepsis workup is invasive and costly. Differentiation between bacterial and viral etiology is important to prevent unnecessary invasive procedures. Viruses, including dengue (DENV), are believed to be an important cause of fever in endemic countries, but they are not routinely identified and knowledge of their contribution to febrile illness in young infants is limited. This study used data obtained from the Sentinel Enhanced Dengue Surveillance System (SEDSS) established in southern Puerto Rico in May 2012. SEDSS recruits acute febrile illness (AFI) patients, collects clinical data and tests for 22 infectious agents, including DENV, respiratory pathogens and enteroviruses. Reverse transcriptase--polymerase chain reaction and ELISA are used to identify etiologic agents. Of 5,115 patients enrolled during the first year of SEDSS, 48 (0.9%) were infants less than 90 days old. Thirty (62.5%) infants were male, and 9 (18.8%) were less than 30 days old. Twenty four (50%) infants presented on the first day of fever, and most (89.4%) presented within the first 3 days. Most infants (70.8%) were admitted for cultures and treatment, including all patients less than 30 days old. The etiologic agent was identified in 13 (27%) infants: 3 (6.3%) had a bacterial infection, 8 (16.7%) had a viral infection, and 2 (4.2%) had viral/bacterial co-infection. Viruses detected included DENV (n = 2), influenza A virus (n = 4), enterovirus (n = 2), parainfluenza virus-3 (n = 1), and DENV/influenza A virus coinfection (n = 1). Viral infections were more common than bacterial infections in this pediatric cohort, and DENV infection was a rare event. These findings will assist clinicians to understand the causes of fever and the incidence of DENV infection in young infants.

1408

DENGUE VIRUS TYPE 3 CIRCULATION IN A REGION OF THE COLOMBIAN CARIBBEAN DURING AN EPIDEMIC PERIOD

Erwin Camacho-Burgos, Homer Corrales, Sindy Martínez, Kelly Villadiego, Pedro Blanco-Tuirán

Universidad de Sucre, Sincelejo, Colombia

Dengue is an arthropod-borne viral disease which has become a major international public health problem in terms of economic impact, mobidity and mortality. Dengue virus 3 is a recently introduced serotype in Colombia which in addition to the simultaneous circulation of the other serotypes, could be associated with an increased transmission and appearance of severe manifestations risk; however, the local virus surveillance in areas such as the department of Sucre is not constant, leading to the lack of updated information. In the present study we describe the frequency of circulation and phylogenetic characteristics of Dengue virus type 3, present in the department of Sucre, located in the Colombian Caribbean. Clinical data and blood samples from patients with febrile syndrome were collected during the second half of 2013 and early 2014. Molecular detection of DENV was performed by a One-Step RT-PCR and IgM/IgG antibodies against the virus were determined by a capture ELISA. Two C6/36 cell passages were made with the RT-PCR positive samples, for virus isolation. Supernatants were used to amplify the complete E and NS3 genes to be subsequently sequenced in order to perform phylogenetic analysis (Bayesian Inference). 22% of the samples were positive for molecular detection of the virus, whereas 37.7 %, 11.1%, and 24% had IgM, IgG and IgM/IgG antibodies against DENV respectively. Serotypes DENV1, 2 and 3 were detected but DENV3 was the most frequent (60%). Three isolates were obtained corresponding to DENV3 (2) and DENV1 (1). The sequence analysis revealed high similarities between DENV3 isolates that were classified within the genotype III; DENV1 isolate was classified as American/African genotype closely related with Colombian and Venezuelan sequences. The results suggest that most of the Dengue reported cases during this epidemic period were caused mainly by DEN3, but other two serotypes were present. This confirm that the region is a hyperendemic area, which could potentially be a hotspot for Dengue transmission in the Colombian Caribbean.

1409

SENSITIVITY AND SPECIFICITY OF THE WORLD HEALTH ORGANIZATION DENGUE CLASSIFICATION SCHEMES FOR SEVERE DENGUE ASSESSMENT IN CHILDREN IN RIO DE JANEIRO

Gleicy A. Macedo[1], Michelle L. Gonin[2], Sheila M. Pone[1], Oswaldo G. Cruz[1], Flávio F. Nobre[3], **Patrícia Brasil**[1]

[1]Fundação Oswaldo Cruz-Fiocruz, Rio de Janeiro, Brazil, [2]Hospital Municipal Jesus, Rio de Janeiro, Brazil, [3]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

The clinical definition of severe dengue fever remains a challenge for researchers in hyperendemic areas like Brazil. The ability of the traditional (1997) as well as the revised (2009) World Health Organization (WHO) dengue case classification schemes to detect severe dengue cases was

evaluated in 267 children admitted to hospital with laboratory-confirmed dengue. Using the traditional scheme, 28.5% of patients could not be assigned to any category, while the revised scheme categorized all patients. Intensive therapeutic interventions were used as the reference standard to evaluate the ability of both the traditional and revised schemes to detect severe dengue cases. Analyses of the classified cases (n = 183) demonstrated that the revised scheme had better sensitivity (86.8%, P < 0.001), while the traditional scheme had better specificity (93.4%, P < 0.001) for the detection of severe forms of dengue. This improved sensitivity of the revised scheme allows for better case capture and increased ICU admission, which may aid pediatricians in avoiding deaths due to severe dengue among children, but in turn, it may also result in the misclassification of the patients' condition as severe, reflected in the observed lower positive predictive value (61.6%, P < 0.001) when compared with the traditional scheme (82.6%, P < 0.001). The inclusion of unusual dengue manifestations in the revised scheme has not shifted the emphasis from the most important aspects of dengue disease and the major factors contributing to fatality in this study: shock with consequent organ dysfunction.

1410

EVALUATION OF TWO PARAMETERS FOR DENGUE DIAGNOSIS IN HONDURAN PATIENTS

Leda A. Parham, Carlos Sánchez, María José López, Ivette Lorenzana

Microbiology Department, National Autonomous University of Honduras, Hospital Materno Infantil, Tegucigalpa, Honduras

Dengue is an important vector borne disease in tropical and sub-tropical countries. In Honduras during year 2013, around 39,275 cases of dengue fever were registered at national level. Several approaches have been developed for laboratory diagnosis of dengue infections, nevertheless timely diagnosis is a challenge. This study was undertaken to evaluate two different tests to detect dengue virus NS1 antigen (Ag) and dengue IgM antibodies (Ab) manufactured by Standard Diagnostics (SD, South Korea) in samples from patients with dengue infections. The study was carried out in Tegucigalpa, Honduras. The study population consisted of 134 patients clinically classified with dengue hemorrhagic fever according to the WHO criteria. Out of 134 plasma samples, 61 corresponded to patients with ≤5 days of illness and 73 samples to patients with ≥6 days of illness. All samples from patients with ≤5 days of illness, characterized as dengue positive (n=48) or negative (n=13) by RT-PCR, were tested by SD dengue NS1-Ag methods (the rapid test SD Bioline NS1-Ag and SD NS1-Ag EIA); all samples from patients with ≥6 days of illness with positive (n=57) or negative (n=16) result for dengue infection by an in-house IgM-Ab capture EIA, were tested by SD dengue IgM-Ab methods: the rapid test SD Bioline IgM-Ab and SD IgM-Ab EIA. The sensitivity of SD Bioline NS1-Ag was 88% and 85% for SD NS1-Ag EIA. Regarding specificity, although it is 17% for SD Bioline NS1-Ag and 23% for SD NS1-Ag EIA this is not real, the comparison was done with RT-PCR and turn out to be false negative; because 9/10 samples are IgM-Ab positive. For SD IgM-Ab, the sensitivity and specificity was 82% and 88% for SD Bioline IgM-Ab and 88% and 69% for SD IgM-Ab EIA when were compared with the in-house EIA. These results suggest that dengue SD Bioline NS1-Ag method had slightly higher sensitivity than dengue SD NS1-Ag EIA (88% vs 85%). Higher specificity was observed for SD Bioline IgM-Ab than SD IgM-Ab EIA (88% vs 69%). In terms of sensitivity SD IgM-Ab EIA was higher than SD Bioline IgM-Ab (88% vs 82%). Early diagnosis of dengue infection by NS1 antigen could be helpful in the timely management of dengue virus infection, and it might be even superior due to the fact of the known liability of RNA used for molecular testing and confirmed in this study.

1411

SPATIOTEMPORAL CLUSTERING, CLIMATE AND SOCIAL-ECOLOGICAL RISK FACTORS FOR DENGUE IN MACHALA, ECUADOR

Anna M. Stewart Ibarra[1], Angel Munoz[2], Keytia Rivero[3], Efrain Beltran[4], Mercy J. Borbor[5], Julia Finkelstein[6], Raul Mejia[3], Tania Ordonez[4], Cristina Recalde[3], Daniel Ruiz[7], Sadie J. Ryan[8]

[1]SUNY Upstate Medical University, Syracuse, NY, United States, [2]Columbia University, New York, NY, United States, [3]National Institute of Meterology and Hydrology, Guayaquil, Ecuador, [4]Ministry of Health, Machala, Ecuador, [5]Escuela Superior Politecnica del Littoral, Guayaquil, Ecuador, [6]Cornell University, Ithaca, NY, United States, [7]University of Antioquia, Medellin, Colombia, [8]University of Florida, Gainesville, FL, United States

Dengue fever, a mosquito-borne viral disease, is a growing public health problem in Ecuador and throughout the tropics, yet we have a limited understanding of the disease dynamics in these newly emerging regions. The aim of this study was to characterize the spatiotemporal dynamics, climate and social-ecological risk factors associated with the largest dengue outbreak on record (2010) in the coastal port city of Machala, Ecuador. Spatial analysis: Using LISA and Moran's I, we analyzed the spatial distribution of georeferenced dengue cases and found evidence of significant hotspots near the city center. We evaluated whether the presence of dengue transmission was associated with social-ecological variables at the neighborhood level by overlaying data from the 2010 national census and entomological indices. We used a multi-model selection process and found that the best-fit model to predict the presence of dengue included age and gender of the head of the household (older, female), access to piped water in the home, poor housing condition, and distance to the central hospital. Temporal analysis: Using wavelet analysis, we characterized historical patterns of weekly climate and dengue transmission (2003-2010), and we found significant climate effects associated with the outbreak. In conclusion, our findings indicate the potential to develop dengue vulnerability maps that can feed into climate-driven dengue early warning systems to inform vector control interventions. This study provides an operational methodological framework that can be broadly applied to understand local dengue risk.

1412

THE COMPLEX RELATIONSHIP BETWEEN WEATHER AND DENGUE VIRUS TRANSMISSION IN THAILAND AND PERU

Karen M. Campbell

San Diego State University, San Diego, CA, United States

Dengue viruses cause more human morbidity and mortality than any other arthropod-borne virus. Dynamic space-time estimations of risk are needed to guide the development of more effective surveillance-intervention strategies and use of prevention resources. Weather plays an important role in regulating the location and timing of transmission due to direct effects of temperature and humidity on mosquito development cycles, life span, behavior and extrinsic incubation period. We closely examined the relationship between weather dynamics, including temperature, humidity, and rainfall, and dengue virus transmission across all of Thailand by province for 1983-2001 and all of Peru by district for 1994-2012. We quantitatively characterized the role of weather in regulating dengue transmission cycles across both countries. We observed systematic differences in the structure of seasonal transmission cycles of different magnitude, the role of weather in regulating seasonal cycles, necessary versus optimal transmission "weather-space", basis of large epidemics, and predictive indicators that estimate risk. Larger epidemics begin earlier, develop faster and are predicted at seasonal *Onset change-point* when case-counts are low. Temperature defines a viable range for transmission; humidity amplifies the potential within that range. This duality is central to transmission and epidemic magnitude. In Thailand, 80% of 1.2 million severe dengue cases occurred when mean-temperature was 27--29.5°C and mean-humidity was >75%. In Peru, with highly diverse weather

patterns spatially, broadly relevant predictors were developed using a statistical classification approach. Interventions are most effective when potentially large epidemics are identified early. Most cases occur near the local seasonal *Peak*, yet small reductions at epidemic *Onset* can substantially reduce epidemic magnitude. Monitoring the *Quiet-Phase* before *Onset* is fundamental in effectively targeting interventions pre-emptively.

1413

ESTIMATING CROSS-IMMUNITY INTERACTIONS OF DENGUE SEROTYPES USING LONGITUDINAL SEROLOGICAL DATA

Robert C. Reiner[1], Steven Stoddard[1], Brett Forshey[1], Eric Halsey[2], Amy Morrison[1], Tadeusz Kochel[2], Thomas Scott[1]

[1]University of California Davis, Davis, CA, United States, [2]U.S. Naval Medical Research Unit - 6, Lima, Peru

Dengue, a mosquito-borne disease whose incidence and graphic range have increased considerably in the past 50 years, is caused by any of four related but antigenically distinct virus serotypes (DENV-1, DENV-2, DENV-3, DENV-4). Following a DENV infection, in addition to lifelong immunity to the infecting serotype, an individual gains temporary immunity to infections with heterologous viruses. Although temporary cross-immunity (TCI) is a historically demonstrated phenomenon, the strength and duration of this vital component of DENV epidemiology is difficult to estimate. Critically, TCI's primary role in transmission dynamics results in the absence of heterologous infections; something that cannot be explicitly captured using hospital case data. Conversely, large longitudinal serological surveys from the same subset of the population over the course of several years can be used to estimate the risk each individual faces for infection with each serotype and observe the disproportionate decrease (or complete absence) of heterologous infections immediately following a DENV infection. Here we apply a new modeling approach that estimates TCI using a 12-year longitudinal DENV dataset from Iquitos, Peru. The dataset contained information on 14,335 individuals whose blood was assayed by PRNT every 6-9 months (38,416 total samples), and contained interval censored timing for 3,854 serotype-specific infections. We identified 455 individuals that became infected with two different serotypes during their participation in the study. Although the average time between seroconversions was 449 days (2.5 tests on average), 250 of those individuals seroconverted twice in sequential assays. Further analysis, using a spline-based approach previously designed to study the serotypes independently, is currently being leveraged to resolve when, within these testing intervals, infections likely occurred. By modeling a variety of ranges and distributions for the length of cross-immunity, we can estimate the strength of the interactions between DENV serotypes with greater accuracy than was previously possible.

1414

MECHANISMS OF TRAVELING WAVES AND PERIODIC SPATIAL SYNCHRONIZATION OF DENGUE HEMORRHAGIC FEVER INCIDENCE IN THAILAND

Luis Mier-y-Teran[1], Kate Grabowski[1], Justin Lessler[1], Henrik Salje[1], Isabel Rodriguez-Barraquer[1], Donald Burke[2], Surapee Anantapreecha[3], Atchareeya A-Nuegoonpipat[3], Richard Jarman[4], Sopon Iamsirithaworn[5], Simone Bianco[6], Leah B. Shaw[7], Ira B. Schwartz[8], Derek A. Cummings[1]

[1]Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, [2]University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, United States, [3]National Institute of Health, Department of Medical Sciences, Bangkok, Thailand, [4]Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [5]Bureau of Epidemiology, Department of Diseases Control, Ministry of Public Health, Bangkok, Thailand, [6]University of California San Francisco, San Francisco, CA, United States, [7]College of William and Mary, Williamsburg, VA, United States, [8]Plasma Physics Division, Naval Research Laboratory, Washington, DC, United States

Multi-annual periodicity has been observed in multiple time series of dengue including those from Thailand. These time series have been observed to have distinct spatial structuring of the timing of peaks, with spatio-temporal traveling waves and other structures observed. The mechanisms underlying this spatial dependence are not well understood. Here we describe transmission models that explore multiple hypotheses of the mechanism underlying traveling waves and periodic synchronization of dengue incidence observed in Thailand in a 40 year time series of incidence from all provinces in the country. We utilize mechanistic, meta-population models that include migration between patches to understand the incidence synchronization phenomenon. We explore scenarios with varying degrees of patch heterogeneity, migration rates, seasonal forcing and heterogeneity in birth rates to identify the main drivers behind phase structures and synchronization observed in the empirical incidence data. We discuss the potential impact of our observations for the control of dengue.

1415

DENGUE VIRUS INFECTION AMONG MEMBERS OF THE UGANDA PEOPLE'S DEFENSE FORCE

Elizabeth Hunsperger[1], Esther M. Ellis[1], D. Fermin Arguello[1], Joel Montgomery[2], Jeff Borchert[3], John Kayiwa[4], Lutwana Julius Julian[4], Jessica Carrion[1], Kay M. Tomashek[1], Harold Margolis[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]Centers for Disease Control and Prevention, Division of Global Health Protection, GA, United States, [3]Centers for Disease Control and Prevention, Uganda, Uganda, [4]Uganda Virus Research Institute, Uganda, Uganda

Outbreaks of dengue have occurred in East Africa over the last several years. In May 2011, a dengue outbreak was recognized among African Union Mission in Somalia (AMISOM) peacekeepers when several Ugandans were diagnosed with an acute hemorrhagic febrile illness. In response to the outbreak, we conducted a seroincidence study to determine the risk of dengue virus (DENV) infection following Uganda Peoples Defense Force (UPDF) deployment to Somalia. Serum specimens were obtained from 337 participating UPDF soldiers to determine DENV exposure and infection rate pre- and post- deployment. Testing included anti-DENV IgG antibodies by immunoassay and neutralizing IgG antibodies by microneutralization test (MNT). A dengue case was defined as positive for IgG seroconversion and confirmed by MNT. IgG seroconversion was defined as a negative anti-DENV IgG result in the pre-deployment specimen and a positive result in the post-deployment specimen or a 4-fold titer increase. MNT positive titer to only one serotype was classified as a primary DENV infection. Reactivity to multiple DENV serotypes by MNT was classified as a secondary DENV infection. Sixty percent of the UPDF soldiers that deployed to Somalia

had seroconversion by IgG. The MNT results showed that 81% of the IgG positive specimens had neutralizing antibodies specific to DENV. Only 13.3% of the IgG positive specimens had a primary infection to either DENV1, 2 or 3. DENV-3 was the predominant serotype amongst UPDF soldiers. DENV exposure determined by the seroincidence study following UPDF deployment to Somalia matched the identified circulating serotypes in Somalia during the dengue outbreak in 2011. With dengue in the differential diagnosis for acute febrile illness for the UPDF soldiers, a quarantine recommendation should be considered for returning soldiers so as not to introduce DENV into Uganda.

1416

MOLECULAR CHARACTERIZATION OF INFLUENZA A AND B VIRUSES IN CUBA DURING 2006-2010, IMMUNOLOGICAL MARKERS RELATED TO 2009 PANDEMIC DISEASE SEVERITY

Alexander Piñón, Belsy Acosta, Odalys Valdés, Amely Arencibia, Mayra Muné, Yamila Adams, Clara Savón, Suset Oropesa, Angel Goyenechea, Grehete González, Guelsys González

Tropical Medicine Institute Pedro Kourí, Havana, Cuba

During 2006-2010 in Cuba, Influenza and pneumonia were the fourth leading cause of death, with the detection of pandemic influenza A(H1N1) pdm09 in year 2009. Severe disease caused by pandemic virus in persons less than 55 years occurred at highest frequencies than youngest and elder groups worldwide. At that time, scientific community focused on the interaction virus-ecology-host factors. Besides, emergency of genetic variants divergent from vaccine strains and antiviral drug-resistant variants, threaten the effectiveness of prevention and control measures. In Cuba, there are non-previous studies about influenza viruses molecular characterization, we focused in genetic characterization of influenza A and B virus variants circulating during 2006-2010. In addition, the relationship between Influenza pandemic severity with host factors was determined. Study showed seasonal influenza A and B viruses into different genetic variants, some of them genetically divergent from vaccine strain. Different genetic variants of influenza virus A(H1N1) pdm09 were detected, however, they remain the genetic match with vaccine strain. High levels of RANTES and TLR-2, and the presence of CCR5Δ32 suggest their involvement in disease severity produced by the 2009 pandemic virus. M2 channel blocking drugs resistant variants were detected in seasonal influenza A(H3N2) and pandemic A(H1N1)pdm09 strains, and variants resistant to neuraminidase inhibitors emerged during 2008 in seasonal influenza A(H1N1) after permissive mutations gaining. Molecular characterization of influenza virus allowed the detection of emerging genetic variants, with potential to evade antibodies vaccine and become resistant to antiviral drugs. Moreover, results obtained provide useful laboratory criteria for control and prevention policies update by the Ministry of Public Health, and the future perspective new forms of therapy directed to virus-host interactions.

1417

THE MOLECULAR EPIDEMIOLOGY AND PHYLOGEOGRAPHY OF H3N2 INFLUENZA VIRUS IN PERU

Simon Pollett[1], Martha Nelson[2], Matthew Kasper[1], Yeny Tinoco[1], Mark Simons[1], Candice Romero[1], Marita Silva[1], Edward C. Holmes[3], Daniel G. Bausch[1]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]National Institutes of Health, Bethesda, MD, United States, [3]Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, Sydney, Australia

The evolutionary dynamics of H3N2 influenza viruses in tropical regions like Peru remain unclear, including whether lineages persist in the tropics and seed temperate areas. We aimed to test the 'source-sink' model and clarify the migration patterns of H3N2 within and between Peru and the rest of the world. Respiratory specimens from community-based influenza surveillance cohorts were collected from 2010-2012 in four ecologically diverse sites in Peru: Cusco, Tumbes, Puerto Maldonado (PM) and Lima. H3N2 positive specimens (by QIAamp Viral RNA Isolation Kit assay) were randomly selected over time and space and the complete hemagglutinin (HA) gene sequenced and compared with sequences in GenBank and GISAID databases. Alignment and DNA model selection were performed using MEGA and JmodelTest2 software, respectively. A maximum likelihood (ML) tree of all sequences was inferred using RaxML software. A maximum clade credibility (MCC) tree and time to most common recent ancestor (TMCRA) of Peruvian sequences were inferred using BEAST software, with spatial clustering robustness tested by BaTS software. Of 400 specimens selected , 389 were able to be sequenced. ML analysis of Peruvian and 2023 global comparator sequences demonstrated interseasonal extinction of Peruvian clades. Moderate clustering of Peruvian taxa and mixing with global strains were noted at all study sites. A short TMCRA of Peruvian H3N2 taxa was noted (3.8 years), consistent with rapid replenishment of the Peruvian H3N2 gene pool from international regions. The MCC tree of Peruvian taxa revealed a well-supported spatial structure at all sites ($p < 0.01$), although there was also moderate spatial mixing. Spatial clustering was weakest in Lima and PM (mean maximum clade sizes of 8.04 and 8.2, respectively). In conclusion, there is no evidence of a 'sink-source' dynamic or viral persistence in Peru. Rather, our data supporting a model of ever-migrating global metapopulations of H3N2. Peruvian H3N2 strains are replenished by a well-mixed global gene pool each season with gene flow in and out of the country at multiple locations. While spatially structured, there is evidence of H3N2 migration within Peru, particularly at the Lima and PM sites, which is consistent with high fluxes of human movement and/or larger population sizes at these two locations. These findings have implications for pandemic influenza planning in Latin America and beyond.

1418

CYTOKINE RESPONSE OF RHESUS MACAQUES EXPOSED TO LIVE EBOLA ZAIRE VIRUS CHARACTERIZED WITH MAGPIX PARAMAGNETIC BEAD TECHNOLOGY

Matthew Voorhees

United States Army Medical Research Institute for Infectious Diseases, Frederick, MD, United States

Viral Hemorrhagic Fevers (VHFs) are serious, frequently fatal illnesses characterized by fever and unusual susceptibility to bleeding. VHFs are caused by single-stranded RNA viruses from the families Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae. Several aspects of the illnesses contribute to their importance as biological threat agents. Diagnosis is usually made at a reference lab with high risk (BSL3-4) biosafety capabilities. Early diagnosis is critical for proper management of the illness and the prevention of spread. Understanding the pathophysiology of the disease is necessary to be able to develop effective medical countermeasures. To determine if any cytokine responses might convey increased survivability, serum was analyzed from 32 Rhesus macaques that had been exposed to 1, 10, 100, or 1000 pfu of Ebola Zaire virus. Using Luminex's MAGPIX paramagnetic bead platform, the serial bleed live virus samples were analyzed in biocontainment for the development of cytokines important in immune response following infection. Using Life Technologies' Cytokine Monkey Magnetic 29-Plex Panel, time point samples were analyzed from both surviving and non-surviving animals, and has provided data on cytokine responses that correlate with the severity of Ebola virus infection.

1419

RABIES IN IRAQ: 2014 UPDATE

Mashair Z. Ismail[1], Daniel Horton[2], Eman S. Siryan[3], Abdul R. A Wali[2], Husam E. Ab-dullah[2], Emma Wise[2], Katja Voller[2], Graeme Harkess[2], Denise A. Marston[2], Salah F. Abbas[1], Lorraine M. McElhinney[2], Sumaia B. Hassan[4], Anthony Fooks[2]

[1]Central Veterinary Laboratory, Iraq State Company for Veterinary Services, Baghdad, Iraq, [2]Animal Health and Veterinary Laboratories Agency, Weybridge, United Kingdom, [3]Baghdad Veterinary Hospital, Iraq State Company for Veterinary Services, Baghdad, Iraq, [4]Zoonoses Diseases Section, Centers for Disease Control and Prevention, Ministry of Health, Baghdad, Iraq

Rabies control requires the combination of dependable resources, cooperation between veterinary and public health officials and accurate surveillance methods. In prior work we reported trends in human rabies cases between 2001 and 2010 and characterization of animal rabies strains from Baghdad, Iraq. Previously, there had been no systematic surveillance for rabies in animals and no laboratory confirmation of disease or virus strains. Three of 40 animal brains were positive using fluorescent antibody testing and hemi-nested RT-PCR for rabies virus (RABV). Phylogenetic analysis using partial nucleoprotein gene sequences demonstrated that the viruses belonged to a single virus variant and shared a common ancestor with viruses dating back 22 years ago from neighboring countries to the west, north and east of Iraq. These results suggested possible multiple introductions of rabies into the Middle East and regular trans-boundary movement of disease. In the present work, we discuss efforts to improve the surveillance and control of rabies in Iraq over the past several years. In 2012 there were 10 cases of rabies and 12,715 dog bites. In 2013 there were 8 cases of rabies and 15,879 reported cases of dog bites. Although 4000 years have passed since the original disease known as rabies, animals and humans are still dying of this preventable and neglected zoonosis in Iraq.

1420

VALIDATION OF A DUPLEX REAL-TIME RT-PCR ASSAY FOR SIMULTANEOUS DETECTION OF INFLUENZA A AND B VIRUSES

Maria E. Silva, Carlos A. Figueroa, Julia S. Ampuero, Carolina Guevara, Alberto Laguna-Torres, Daniel G. Bausch

Naval Medical research Unit - 6, Bellavista, Peru

Influenza viruses have been the cause of major outbreaks and pandemics with high mortality rates throughout human history. Even though vaccines are available, influenza affects around 15% of planet's population yearly. Disease surveillance is essential for rapid identification of cases, allowing implementation of treatment and control measures. Real-time PCR is a powerful diagnostic technique frequently used in influenza surveillance. We developed a duplex real-time RT-PCR to simultaneously detect influenza A and B viruses to meet the need for rapid and effective diagnostics in an respiratory disease cohort in Peru. A combined set of published primers and probes to detect a highly conserved region of the influenza A virus matrix protein (MP) gene and in-house designed primers and probes to detect the influenza B virus MP gene were optimized for single-step RT-PCR using the ABI7500 Fast real-time PCR system. Two-hundred and sixty eight clinical samples were tested using the newly designed duplex assay. Results were compared with those obtained using single-plex RT-PCR assays for influenza A and B viruses designed by the U.S. Centers for Disease Control and Prevention (CDC). Results from the duplex assay were 97% and 100% consistent with the CDC assay for influenza A and B viruses, respectively. In addition, our duplex assay was able to detect two of three influenza A and B virus co-infections. This assay provides a rapid, accurate, highly sensitive and specific diagnostic test for simultaneous detection of influenza A and B viruses.

1421

BATS IN LYSSA, CORONA AND EBOLA VIRUSES ECOLOGY IN NIGERIA; ONE HEALTH PERSPECTIVE TO INFECTIOUS DISEASE CONTROL

Ignatius C. Nwosuh, Clement Meseko, Benjamin Dogonyaro

National Veterinary Research Institute, Vom, Nigeria

The likelihood of an outbreak of emerging or re-emerging infectious disease is contingent on exposure to known or yet to be described reservoir hosts. In recent years, climate change and habitat alteration increase contact between human and animals in shared environment thereby enhancing interspecies transmission of pathogens. Previous studies have shown evidence of lyssa-viruses in fruit bats in Nigeria. These fruit bats are similar to those observed in other countries where corona and Ebola viruses have been identified. Studies on the risk of human exposure to these bats are critical in protecting public health and animal conservation in the perspective of onehealth. We carried out longitudinal survey of bats including, species identification, their habitats, habits and migration pattern in North Central Nigeria and identified human, climatic, arboreal and ecological factors that are likely to cause exposure to excretions and secretions from these animals by direct field observation. Non-invasive specimens including bat guano and urine were collected for virus detection and isolation by ELISA and culture in mammalian cell lines. Oral interviews and questionnaires survey were also carried out to assess knowledge, attitude and practices with regard to bats. Several species of fruit bats of the order pteropodidae and microchiroptera were found at the forest fringes and within parks and gardens in major cities in North central Nigeria. The choice of habitat is strongly influenced by the forest zones and the presence of forest-like parks and gardens including zoos in the North central. The pattern of migration are also influence by seasonal weather variation such that there is a pattern of movement southward during dry season and northward during rainy season to avoid wetness of the rain and or remain in the south where abundant fruits are available. Bats are reservoir of many emerging and re-emerging pathogens like lyssa-viruses. SARS/MERS corona and Ebola viruses are also considered most likely in these reservoir hosts and because of the interactions of human with bats and games in the forest, game reserve, parks and gardens, the risk of exposure to these pathogens is high. There is therefore an urgent need to design conservation friendly intervention to prevent the pandemics of the future by actions that are taken today.

1422

THE GLOBAL DISTRIBUTION OF CRIMEAN-CONGO HEMORRHAGIC FEVER

Jane P. Messina[1], David Pigott[1], Nick Golding[1], Kirsten Duda[1], John S. Brownstein[2], Peter W. Gething[1], Monica M. Meyers[1], Dylan B. George[3], Simon I. Hay[1]

[1]University of Oxford, Oxford, United Kingdom, [2]Boston Children's Hospital, Boston, MA, United States, [3]Fogarty International Center, Bethesda, MD, United States

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne infection caused by a virus (CCHFV) from the Bunyaviridae family, and while it occurs primarily in animals, it also occurs in humans who work closely with these animals. Healthcare workers in endemic areas are similarly at high risk. There is no safe and effective vaccine against CCHFV which is widely available, and thus treatment for the potentially fatal disease remains primarily supportive. Therefore, an improved understanding of the distribution and level of risk for CCHF is essential for guiding improvements in disease control strategies. Here we undertake an exhaustive assembly of known records of CCHF occurrence worldwide from 1961 to the present, and use a formal modelling framework to map the global distribution of CCHF risk. We do this by first deriving a consensus on country-level presence or absence, and combine this information with the locations of known occurrences and a suite of high spatial-resolution covariates related to climate, urbanisation, agriculture,

and livestock presence to derive the probability of occurrence at a 5km x 5km resolution globally. We find CCHF to be confined to Africa, Eastern Europe, and western Asia, but with spatially heterogeneous levels of risk within these regions. Our new risk map provides novel insights into the global, regional and national threat posed by CCHF, and highlights the need for cohort studies to be carried out in high-risk zones in order to determine the public health burden posed by this neglected disease. We intend for our contemporary risk map to serve as a starting point for a wider discussion about the global impact of CCHF, and for it to help guide improvements in drug and vector-control strategies as well as evaluation of the economic burden caused by this disease.

1423

CHARACTERIZATION OF PUNTA TORO VIRUS RESPONSIBLE OF HUMAN DENGUE-LIKE CASES IN PANAMA

Davis Beltrán[1], Jean-Paul Carrera[1], Yamilka Díaz[1], Patricia V. Aguilar[2], **Sandra L. Lopez-Verges**[1], Robert B. Tesh[2]

[1]Gorgas Memorial Institute of Health Studies, Panama, Panama, [2]University of Texas Medical Branch, Galveston, TX, United States

The genus Phlebovirus (Bunyaviridae family) comprises over 70 antigenically distinct serotypes of viruses with a wide distribution along the tropics. Punta Toro virus (PTV) is part of the Phlebotomus fever viruses (the sand fly group). In the Americas, PTV has been isolated only in Panama from sand flies, slots, human febriles and sentinel hamsters, and viruses from Punta Toro serogroup have been described also in Colombia and Brazil. Many arboviruses, as PTV, cause in humans symptoms similar to Dengue infection; thus the true number of PTV cases could be underestimated in this Dengue endemic country. Up to a 35% seroprevalence for PTV has been reported in Panama before 1988, however there is no recent data about PTV seroprevalence and about the range of clinical illness caused by this virus. The aim of our study is to evaluate the presence of PTV in human acute sera samples referred by the Dengue surveillance program from 1998 to 2013. We inoculated Vero cells with samples that were Dengue negative, and, for now height samples from patients from Western Panama and Panama city induced cytopathic effect in Vero cells. The isolated virus was characterized as PTV by hemaglutinin inhibition assay. Fragments of the segments L, M and S of the genome of PTV were amplified to perform sanger sequencing and the obtained sequences were aligned and analyzed to compare with previous strains isolated in Panama and PTV serogroup from other regions. The phylogenetic trees show that these strains are related to previously described strain GML902878 (isolated from sentinel Sirian hamster in 1976), and are close to Balliet, a strain related to mild disease in hamster models that was isolated in 1966 also in Western Panama. Our preliminary findings suggest that PTV close to Balliet strain circulates continuously in Western and Central Panama and causes undifferentiated febrile symptoms in humans, underlining the fact that many arboviruses like PTV could be responsible of the less than 30% of Dengue-like cases that are negative for dengue in this country.

1424

COVERAGE DURING AN IMMUNIZATION CAMPAIGN PROVIDING INACTIVATED AND ORAL POLIO VACCINES IN REFUGEE CAMPS AND HOST COMMUNITIES, KENYA - DECEMBER 2013

Fredrick M. Makokha[1], Ahmed Unshur[2], Abdullahi Hussein[1], Gedi Mohamed[3], Wences Arvelo[2], Ian Njeru[4], Samuel Amwayi[1], Samuel Okiror[3], Ondrej Mach[5], Concepcion F. Estivariz[6]

[1]Field Epidemiology and Laboratory Training Program, Nairobi, Kenya, [2]Division of Global Health Protection, Centers for Disease Control and Prevention, Nairobi, Kenya, [3]World Health Organization Country Office, Nairobi, Kenya, [4]Ministry of Health, Nairobi, Kenya, [5]Global Polio Eradication Initiative, World Health Organization, Geneva, Switzerland, [6]Global Immunization Division, Centers for Disease Control and Prevention, Atlanta, GA, United States

Poliomyelitis is a highly infectious viral disease, affecting mainly children <5 years of age. Globally, poliomyelitis cases have decreased by 99% since 1988, but outbreaks continue to occur. In May, 2013 an outbreak of wild type poliovirus with 14 cases was reported in Kenya, among them 13 cases were from Dadaab refugee camps and host communities. An immunization campaign providing inactivated poliovirus vaccine (IPV) and oral polio vaccine (OPV) was launched. We conducted a post-campaign coverage survey to assess the impact and guide future use of IPV. We selected 30 blocks in each of the five refugee camp, and 30 villages in the host communities, by probability proportional to size with replacement. We visited nine households in each block and five households in each village, plus a convenience sample of nomad settlements. Within each household we collected data on all children <5 years on IPV and/or OPV; the youngest child age 6 to 59 months was selected for questions about OPV received through routine immunization. Vaccine coverage and 95% confidence intervals were calculated accounting for clustering. We enrolled 1,084 households, including 2,173 children from refugee camps and host communities and 118 from nomad households. Coverage of OPV plus IPV in the December campaign was 92.8 %(90.2%-94.8%) in refugee camps and 95.8%(93.5%-97.3%) in host communities; OPV coverage in the November campaign was 97.2% (95.4%-98.3%) in refugee camps and 97.3%(95.0%-98.5%) in host communities. Among the 118 children <5 years of age from nomadic households, 40(34%) received IPV plus OPV in December, and 37(31%) had received OPV in November. Among caregivers, 1009(99%) reported being aware of the campaign; 766(76%) knew from megaphone announcements, 475(47%) from social mobilizer, 435(43%) from healthcare worker, and 367(36%) heard from the radio. Among 107 children >6 weeks old who missed IPV, 49(46%) caregivers cited not knowing the location of the vaccination. IPV was successfully delivered with high community acceptance both in refugee camps and host communities. Strategies are needed to improve coverage in nomadic populations.

1425

HOW UNPREPAREDNESS FOR AN EBOLA OUTBREAK LEADS TO A WIDESPREAD EPIDEMIC AND COMPLEXITY TO HALT THE EPIDEMIC; AN ANALYSIS AND DESCRIPTION OF ENCOUNTERED OPERATIONAL CHALLENGES DURING A MÉDECINS SANS FRONTIÈRES INTERVENTION IN THE REPUBLIC OF GUINEA AND LIBERIA

Esther Sterk, Gabriel Alcoba, Annick Antierens

Medecins sans Frontieres, Geneva, Switzerland

Suspected cases of hemorrhagic fever were notified in different locations in the Republic of Guinea before the Ebola Zaire virus was identified and the first ever Filovirus epidemic declared in the country on the 22nd of March 2014. The epidemic rapidly spread further to the capital Conakry and cross border to Liberia. In less than 4 months a cumulative total of 163/25 suspected cases and 112/12 deaths were notified in Guinea and Liberia respectively. The attack rate and case fatality observed

are comparable with previous Ebola outbreaks; however the large geographical spread of the disease is unprecedented and leads to complex operational challenges during the interventions put in place by Médecins sans Frontières in collaboration with the Ministries of Health. The first cases were misdiagnosed because of the unfamiliarity of the disease among health staff. Disease confirmation was hampered by the lack of a reference laboratory in Guinea and challenges around sample transport. Rapid geographical spreading was achieved by the high mobility of cases unaware of their status or looking for better perceived health care. Interhuman contact was not minimized because of lack of knowledge and the non-respect of universal precautions. Corpses were moved to different locations and traditional burials greatly contributed to the spread of the epidemic. Misconceptions in the community resulted in difficulties in accepting isolation of patients and lead community members to attack and chase Médecins sans Frontières team members from an intervention site. Filoviridae can achieve an important geographical spread if countries at risk for outbreaks are not prepared. MSF advocates for awareness of viral hemorrhagic fevers, increasing the level of universal precautions at all levels, training of health staff, national laboratory testing possibilities and assuring emergency preparedness at national level. Research and development should be high on the agenda for the availability of rapid diagnostic tests for viral hemorrhagic fever viridae, active and passive immunizations for infected patients and the passive immunization of communities at risk.

1426

KINETICS OF POLIO SHEDDING FOLLOWING ORAL VACCINATION AS MEASURED BY QRT-PCR VERSUS CULTURE

Mami Taniuchi[1], Sharmin Begum[2], Md Jashim Uddin[2], James A. Platts-Mills[1], Jie Liu[1], Beth D. Kirkpatrick[3], Anwarul H. Chowdhury[4], Kh Mahbuba Jamil[4], Rashidul Haque[2], William A. Petri, Jr.[1], Eric R. Houpt[1]

[1]University of Virginia, Charlottesville, VA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]University of Vermont, Burlington, VT, United States, [4]Institute of Public Health, Dhaka, Bangladesh

Measurements of oral polio vaccine (OPV) shedding in stool are useful to evaluate mucosal immunity, to estimate the burden of vaccine strain poliovirus, and for surveillance post-polio eradication. We developed an one-step serotype-specific real-time RT-PCR for detection of Sabin1, Sabin2, and Sabin3 strains along with an extrinsic internal control, MS2, to normalize the targets for extraction and amplification efficiency. Trivalent OPV (tOPV) was administered at week 6, 10, 14, and 52 weeks (at week 39 half of the infants received IPV and the other half tOPV) in a birth cohort study in the Mirpur region of Dhaka, Bangladesh. This assay was used to intensively study OPV shedding kinetics at weeks 14 (n=88 infants; 42 female and 26 male) and 52 (n=182 infants; 84 female and 98 male) post vaccine administration directly from stool specimens collected before the OPV administration (day 0) and on days +4, +11, +18, and +25 after administration. Of the 1350 samples examined (270 infants × 5 time points), sensitivity and specificity of qPCR was 89% and 91%, respectively, when compared to culture. Overall, the PCR detected more shedding than the standard culturing methods. A quantitative relationship was observed between culture+/qPCR+ specimens and culture-/qPCR+ specimens namely the average burden of shedding in viral copy number from the culture+/qPCR+ specimens was higher than in the culture-/qPCR+ specimens (qPCR copies $3.37\times10^{7}\pm1.22\times10^{7}$ versus $3.88\times10^{5}\pm1.45\times10^{5}$, respectively; Mann-Whitney P<0.001 two-tailed). Kinetics of shedding as revealed by qPCR and culture were generally similar at both time points. A qPCR cutoff of approximately 10^{4} viral copies on day 11 or day 18 post OPV could be used to identify the culture-positive shedders after immunization as well as their shedding duration and intensity. qPCR revealed that S3 (6.4%) was most commonly shed followed by S1 (5.1%) and then S2 (1.9%) , and mixed infections occurred in 6.5%. Our findings suggest that this one-step qRT-PCR polio assay can be used to approximate shedding both qualitatively and quantitatively, and will be useful in monitoring OPV efficacy or transmission during eradication efforts.

1427

MECHANISMS OF VIRULENCE OF MONKEYPOX VIRUS: DELETION OF GENOMIC REGIONS AND THEIR EFFECTS IN PATHOGENESIS

Juan G. Lopera[1], Elizabeth A. Falendysz[2], Tonie E. Rocke[2], Jorge E. Osorio[1]

[1]University of Wisconsin, Madison, WI, United States, [2]National Wildlife Health Center, U.S. Geological Survey, Madison, WI, United States

Monkeypox virus (MPXV) causes a human disease similar to smallpox which is endemic in equatorial Africa. However, the emergence of MPXV in 2003 in the Western Hemisphere (USA) demonstrated the potential of these viruses for geographic expansion and worldwide transmission. Understanding the viral genetic factors associated with virulence are of vital importance for surveillance, prevention and treatment of human MPX disease. Using bioinformatics and molecular virology approaches, we identified and evaluated the effects of deletion of two genomic regions in the highly virulent MPXV-Congo strain. *In vitro* and *in vivo* studies indicated that these genomic regions play a significant role in MPXV replication, tissue spread, pathology, and mortality in susceptible CAST/EiJ mice. In this study, we demonstrated that deletion of multiple immunomodulatory (IMM) genes in MPXV is necessary to produce a pronounced attenuating effect, suggesting that targeted genomic regions contain more than one major MPXV virulence factor. More importantly, we observed marked attenuation of virus with simultaneous deletion of two regions (MPXV-ΔR1/R2) which illustrates the additive effect of genomic regions in MPXV pathogenicity. Deletions of MPXV regions in the highly virulent MPXV/Congo genome hindered cell culture growth and significantly reduced morbidity, replication, spread and mortality in infected CAST/EiJ mice. Thus, parental MPX-Congo/Luc+ caused 100% mortality while all mice infected with recombinant MPXVs/Luc+ with deletions of genomic regions survived to infection and did not show clinical signs of disease. Further, serological and histopathological evaluation confirmed that deletion of genomic regions reduced MPXV tissue pathology and elicited strong antibody responses. Our results support the hypothesis that MPXV pathogenesis is not determined by a single gene. Rather, it is the result of the combined effects and interactions of multiple viral and host factors.

1428

REPORT OF A PROSPECTIVE STUDY IN MENINGOENCEPHALITIS IN PERU

Nicanor Mori[1], Joseph R. Zunt[2], Violeta Celis[3], Eduardo Ticona[4], Grimaldo Ramirez[5], Moises Sihuincha[6], Marizabel Rozas[7], Martin Tipismana[8], Matthew Kasper[9], Drake H. Tilley[10], Thanh Ton[2], **Silvia M. Montano**[10]

[1]Hospital Daniel Alcides Carrion, Callao, Peru, [2]University of Washington, Harborview Medical Center, Seattle, WA, United States, [3]Hospital Belen, Trujillo, Peru, [4]Hospital Dos de Mayo, Lima, Peru, [5]Hospital Emergencias Pediatricas, Lima, Peru, [6]Hospital Cesar Garayar Garcia, Iquitos, Peru, [7]Hospital Regional del Cusco, Cusco, Peru, [8]Hospital Cayetano Heredia, Lima, Peru, [9]Division of Global Emerging Infections Surveillance and Response System, Silver Spring, MD, United States, [10]U.S. Naval Medical Research Unit - 6, Callao, Peru

Background: Meningoencephalitis (MEC) is a significant public health problem throughout the world; however, few studies have defined the etiologies outside North America and Europe. The objective of this study was to determine the etiologies of meningoencephalitis in Peru. Methods: We conducted hospital based surveillance at 12 hospitals in five Peruvian cities including Coast, Andean and Jungle geographical areas. Symptoms and medical history was obtained from patients older than 28 days with

suspected viral MEC; serum, CSF, rectal and nasopharyngeal swabs were also collected. Follow-up visits were conducted 14 days after presentation. Samples were tested for HSV-1 and 2, HIV, and 18 other viruses. HSV MEC was defined as confirmed (HSV detected by PCR in CSF) or probable (HSV detected in serum or IgG sero-conversion). Results: We enrolled 911 subjects since February 2009. 522 subjects (57.3%) were male; average age was 25.9 years (range 29 days - 86yrs). 31 patients (3.8%) died; 77 (8.5%) were co-infected with HIV. 112 subjects (12.3%)were infected with herpes simplex virus (91 confirmed, 21 probable). HSV sequence was available for 94 participants, of whom 84 (89.3%) had HSV-1 and 10 (11.7%) had HSV-2. Tuberculous meningitis was confirmed in 2 cases and suspected in 13 cases. Seven participants developed meningitis secondary to coxsackievirus. Ten cases were secondary to bacterial meningitis, seven cases were caused by Epstein-Barr virus, six cases by enterovirus, 19 cases by Cryptococcus neoformans, 2 cases by Treponema pallidum, one case by cytomegalovirus, and one by adenovirus. 26 participants exhibited co-infection. Overall, an infectious cause of MEC was identified in 296 (22.6%) participants. Conclusions: This is an updated report on the etiology of community-acquired meningoencephalitis in Peru. HSV infection remains the most common pathogen identified. Unfortunately, 77.3% of cases remain undiagnosed. Additional molecular studies are being implemented to discover the etiological cause of cases that had no pathogen detected.

1429

EVIDENCE OF INTRA-SUBTYPE, INTER-VACCINE CLADE REASSORTANTS OF H3N2 IN GLOBAL SURVEILLANCE SAMPLES

Irina Maljkovic-Berry[1], Melanie C. Melendrez[1], Anthony Hawksworth[2], Gary Brice[2], Patrick Blair[2], Eric Halsey[3], Maya Williams[3], Stefan Fernandez[4], In-Kyu Yoon[4], Leslie Edwards[5], Robert Kuschner[1], Xiaoxu Lin[1], Stephen J. Thomas[1], Richard G. Jarman[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]U.S. Naval Health Research Center, San Diego, CA, United States, [3]U.S. Naval Medical Research Unit - 6, Lima, Peru, [4]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [5]Department of State, Washington, DC, United States

Inter-species and inter-subtype genetic reassortment (antigenic shift) has played a major role in the evolution of pandemic influenza, generating viruses to which there is little to no immunity in the general population, resulting in severe disease symptoms and global spread (i.e. 1918 pandemic). Antigenic drift, on the other hand, has been suggested as being mainly responsible for the evolution of the more mild seasonal influenza, generating substitutions conferring gradual escape from previously acquired immunity and giving rise to the need of a constantly updated influenza vaccine. Recent evidence suggests, however, that the evolution of seasonal influenza is in addition substantially affected by intra-subtypic reassortment. Here we report identification of H3N2 intra-subtype reassortment variants derived from global influenza surveillance samples collected since 2009. Full genome and segment phylogenetic analyses show that the reassorted variants derive from two slightly divergent (0.1-1.2%) but well defined vaccine clades, A/Victoria/361/2011 and A/Perth/10/2010. In addition, we identified variants with segments originating from different geographical areas. Our results illustrate the ability of H3N2 to reassort segments (i.e. HA, NP, PA, NA and NS) between both geographically and antigenically defined clades. Although intra-subtypic reassortment of H3N2 occurs frequently, appearance of persistent reassorted variants originating from antigenically distinct clusters is a rare event that has previously been shown capable of producing unusually severe seasonal influenza. It thus becomes important to follow whether the A/Victoria/361/2011-A/Perth/10/2010 reassorted variants found in this study have the capacity to become fixed in the population. Intra-subtype reassortment adds yet another layer of complexity as vaccines circulate with wild type diversity potentially altering the trajectory of influenza viral evolution.

1430

THE EFFECT OF IMMUNIZATION ON MEASLES INCIDENCE IN THE DEMOCRATIC REPUBLIC OF CONGO

Reena H. Doshi[1], Calixite Shidi[2], Nicole A. Hoff[1], Jean-Jacques Muyembe[3], Emile Okitolonda[4], Anne W. Rimoin[1]

[1]University of California Los Angeles Fielding School of Public Health, Los Angeles, CA, United States, [2]Ministry of Health, Expanded Programme on Immunization, Kinshasa, Democratic Republic of the Congo, [3]National Institute of Biomedical Research, Kinshasa, Democratic Republic of the Congo, [4]Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo

Measles continues to be one of the largest causes of vaccine-preventable disease mortality among children under five, despite the fact that a safe and efficacious vaccine is readily available. While global vaccination coverage has improved tremendously, measles outbreaks persist through sub-Saharan Africa. Since 2010, the Democratic Republic of Congo (DRC) has seen a resurgence of measles outbreaks, mainly attributed to severe deficiencies in Routine Immunization (RI) at the Health Zone level, where only 22% of reported vaccine coverage rates reach higher than 90%. We used available data from the 2011-2012 IDSR system for measles suspected cases counts reported weekly by health zone to investigate the decline in measles incidence post-immunization (by health zone) with one dose of measles containing vaccine (MCV1) with and without the addition of Supplementary Immunization Activities (SIAs) in the provinces of Kasai-Oriental and Equateur. The impact of measles immunization by health zone was modeled using negative binomial regression. At the provincial level, in Kasai-Oriental, the mean incidence was 452.7 per 100,000 in 2011, while the mean incidence declined to 167 per 100,000 in 2012. In Equateur, the mean incidence was 15 per 100,000 in 2011, while the mean incidence increased to 148.7 per 100,000 in 2012, despite a September 2011 SIA. However, multivariate modeling at the health zone level showed that each 1% increase in MCV1 coverage was associated with a .4% decrease in incidence. Furthermore, the lack of an SIA in each health zone was associated with a 3.4% increase in incidence. While the mean yearly incidence of measles did increase in Equateur following an SIA these are provincial level estimates. Differences may be explained partially by the fact that vaccine effects are not immediate and the selective age categories of mass campaigns. Repeated occurrences of large-scale outbreaks in DRC suggest that vaccination coverage rates are grossly overestimated and signify the importance of the re-evaluation of measles virus dynamics and prevention and control strategies.

1431

DISCOVERY, CHARACTERIZATION AND ECOLOGY OF A NOVEL HEPATITIS A-LIKE VIRUS IN WILD OLIVE BABOONS (*PAPIO ANUBIS*), UGANDA

Andrew J. Bennett[1], Samuel D. Sibley[1], Michael Lauck[1], Geoffrey Weny[2], David Hyeroba[3], David H. O'Connor[1], Tony L. Goldberg[1]

[1]University of Wisconsin Madison, Madison, WI, United States, [2]Kibale EcoHealth Project, Kanyawara, Uganda, [3]Jane Goodall Institute, Entebbe, Uganda

Hepatitis A (HAV; family Picornaviridae; genus Hepatovirus) is an RNA virus that causes acute inflammatory disease of the liver in humans and nonhuman primates, and is commonly transmitted through the fecal-oral route. Most often associated with food-borne outbreaks resulting from fecal-contamination, more rarely humans have acquired HAV from the handling of infected non-human primates in captivity. Conversely, recent studies discovering high HAV antibody seroprevalence in wild non-human primates have implicated reverse zoonotic transmission in areas of sub-Saharan Africa where human-nonhuman primate contact and conflict occur frequently. We discovered and characterized by Next-Generation Sequencing (NGS) a novel Simian Hepatitis A-like virus in the blood of a wild olive baboon (*Papio anubis*) in Kibale National Park, Uganda. Furthermore, RT-PCR diagnostics detected viral RNA in the feces of 40% of

baboons sampled at the time of blood collection, suggesting the shedding of potentially infectious viral particles into the environment by wild baboons in western Uganda. Additional screening by field-deployable PCR shows non-random distribution of the virus among individuals and groups. Our results implicate this nonhuman primate as a potential zoonotic source of Hepatitis A-like viruses. This study demonstrates the value of NGS for discovering potential reservoirs of zoonotic pathogens, and supports the supposition that HAV-like viruses circulate naturally in wild nonhuman primates.

1432

VALIDATION OF A MULTIPLEX RT-QPCR ASSAY FOR DETECTION OF INFLUENZA A AND B IN CLINICAL SAMPLES

Pool Marcos[1], Victoria Gutierrez[1], Nancy Rojas[1], Jorge Achata[1], Sila Ruiton[1], Emelda Gallardo[1], Luis Segura[2], Marco Galarza[1], Maribel Huaringa[1]

[1]Instituto Nacional de Salud, Lima, Peru, [2]Universidad Peruana Unión, Lima, Peru

Influenza viruses and their subtypes are common worldwide and produce outbreaks, often along with other respiratory viruses. In Peru, a nationwide surveillance program performs diagnosis and reports to CDC on the outbreak status and emerging influenza variants. Our aim was to develop a multiplex real-time assay for simultaneous detection of Influenza Virus A and B as well as identification of the Influenza Virus A(H3N2) and (H1N1) pdm09 variants as a way to improve the diagnosis speed. The assay consists of two one-step multiplex real-time PCR reactions (RT-qPCR). We discriminated between Influenza A and Influenza B using the matrix gene of Influenza A virus and the nucleoprotein gene of Influenza B virus. We determined subtypes H3N2 and H1N1pdm09 of Influenza A Virus using the hemagglutinin gene. The RT-qPCR reactions were standardized by amplification of serial template dilutions from isolates provided by the CDC. To validate the assay, we analyzed 109 selected clinical samples from patients collected during 2013. Samples were previously analyzed as part of a diagnosis screening and tested positive for influenza viruses. No cross-reaction was recorded with other respiratory viruses potentially present in clinical samples like Adenovirus, Parainfluenza 1, 2 and 3, Human Respiratory Syncytial Virus and Metapneumovirus. No cross-reaction was found either between samples carrying H1N1pdm09 and H3N2. The cutoff was determined at Cq ≤35 for diagnostic test on both reactions. All Influenza A(H3N2), Influenza A(H1N1)pdm09, and Influenza B clinical samples were diagnosed with 100% concordance. The assay was 100% specific for the detection of influenza subtypes in the sample analyzed. This assay is faster and more cost effective than the one reaction per tube setup previously used in the Peruvian surveillance program.

1433

EVALUATION OF RISK FACTORS FOR HIGH RESPONSE TO ROTAVIRUS IGA AT BASELINE

Deok Ryun Kim[1], Ayan Dey[1], Anuradha Sinha[2], Bisakha Haldar[2], Byomkesh Manna[3], Suman Kanungo[2], Ranjan K. Nandy[2], Mohammad Ali[4], William A. Petri[5], Thomas F. Weirzba[1]

[1]International Vaccine Institute, Seoul, Republic of Korea, [2]National Institute of Cholera and Enteric Diseases, Kolkata, India, [3]National Institute of Cholera and Enteric Diseases, Seoul, India, [4]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [5]University of Virginia, Charlottesville, VA, United States

Poor immune responses to rotavirus vaccination were observed in infants in developing countries with sero-protection of roughly 40% after vaccination. The reasons for these lower immune responses are not well understood. Therefore, it is important to measure the preexisting factors which may effects response to vaccine. We evaluated the risk factors for influencing rotavirus IgA in infants living in the urban slums of Kolkata, India. We recruited 372 infants who were 6 weeks of age, and collected their blood samples and mother's breast milk at the time of enrolment. Considering skewed distribution of rotavirus IgA titers, quantile regression that estimates conditional median or other quantiles of the response variable was used to evaluate the risk factors for rotavirus IgA response at 6 weeks. Covariates, such as mother's breastmilk at 6 weeks, mother's height, mother's BMI, and monthly household expenditure (in 1000 INR), a proxy for socio-economic status, were selected for the multivariable model. The 25th quantile regression model yielded that infant rotavirus serum IgA at baseline was significantly influenced by mother's nutritional status, which was also supported by the 50th quantile regression and the ordinary regression methods indicating the relationship is stable. Mother's BMI influenced infant rotavirus IgA titers by an average of 2.5 titers/ 10 unit of BMI in ordinary regression, and 5 titers/10 unit of BMI in 50th quintile regression analysis. However, the 25% quantile regression explained only 1.2 titers per 10 unit of mother BMI. Household socioeconomic status was found significant in lower quartile but not in higher quantile suggesting the relationship with the IgA serum level is not stable. The study identifies mother's nutritional status influence baseline serum level for rotavirus IgA in infants in India, thus this need to be considered while evaluating immunogenicity of the rotavirus vaccines. The results also suggest that the quantile regression is a useful statistical tool as it provides flexiblity to detect trends in skewed data.

1434

RENAL PATHOLOGY IN THE RHESUS MACAQUE/ *PLASMODIUM COATNEYI* MODEL FOR SEVERE MALARIA

Eric Lombardini[1], Imerbsin Rawiwan[1], Peter Weina[2], Arthur Brown[1], Gareth Turner[3]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Walter Reed Army Institute of Research, Forest Glen, MD, United States, [3]MORU, Bangkok, Thailand

Severe falciparum malaria in adults often results in a spectrum of renal pathology ranging from minimal tubular degenerative changes to severe tubular epithelial degeneration and necrosis with hemoglobinuria, cellular casts and proteinosis, consistent with acute renal failure. The pathogenesis of disease is theoretically linked to damage to the endothelial damage within the microcirculation, inflammatory mediators, hemodynamic disturbances and hemolysis. *Plasmodium coatneyi* is one of the non-human primate malarias which serves as an animal model for *Plasmodium falciparum* induced disease in humans. We examined and described the renal pathology of 40 retrospective cases of *P. coatneyi* infection in rhesus macaques. Macroscopic evaluation of the samples was conducted by board certified veterinary and medical pathologists and were correlated with available antemortem clinical data to include terminal parasitemias. In these animals there was significant capillary sequestration within the renal interstitium as well as within the glomerular tufts as well as irregular thickening of the glomerular mesangium.. Furthermore, and closely correlating with the degree of parasitemia, there was increasing severity of vacuolar tubular epithelial degeneration and necrosis, intratubular proteinosis, hemoglobin and cellular casts, as well as parasitized erythrocytes, erythrocytic and histiocytic hemozin pigment and interstitial hemorrhage, fibrin and edema. Interestingly, there is relative absence of inflammation within the affected tissues. These findings are for the most part consistent with those described in adult humans diagnosed with malaria associated renal failure (MARF). As a result of the correlation of the antemortem symptomology, clinical and histopathologic findings in these retrospective samples, we demonstrate the utility of this animal model for specific use in the examination of acute renal failure in severe malaria.

1435

INCREASED LEVELS OF S-NITROSYLATION IMPROVES OUTCOMES IN A MODEL OF EXPERIMENTAL CEREBRAL MALARIA

Robyn E. Elphinstone[1], Jonathan S. Stamler[2], Kevin C. Kain[1]

[1]Sandra Rotman Centre for Global Health, University Health Network-Toronto General Hospital, Tropical Disease Unit, Department of Medicine, University of Toronto; Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada, [2]Institute of Transformative Molecular Medicine, Case Western Reserve University, Cleveland, OH, United States

Decreased nitric oxide (NO) bioavailability is associated with disease severity and worse clinical outcomes in malaria infection. Traditionally NO was thought to act primarily through guanylate cyclase and the production of cGMP; however, there is now extensive evidence for NO to function via a post-translational modification, S-nitrosylation. S-nitrosylation of proteins has been shown to regulate a wide variety of cellular signaling processes and aberrant S-nitrosylation may thus contribute to many disease processes. We hypothesize that increasing bioavailable NO via S-nitrosylating strategies will improve clinical outcome in malaria. In order to test this hypothesis we examined experimental cerebral malaria (ECM) infection in S-nitrosoglutathione reductase (GSNOR) knockout C57BL/6 mice. GSNOR is an enzyme that reduces S-nitrosoglutathione and, therefore, reduces the amount of S-nitrosothiol (SNO), including S-nitrosylated proteins. The deletion of this enzyme results in increased levels of SNO, thereby increasing NO bioactivity in hematopoietic, endothelial and other host compartments. In the ECM model we infected GSNOR knockout mice or their wild type counterparts with 10^6 red blood cells infected with *Plasmodium berghei* ANKA (PbA). In ECM, mice with deletion of the GSNOR enzyme had significantly improved survival compared to wild type control mice ($p<0.0001$), despite significantly increased parasitemia ($p<0.0001$). The prolonged survival in GSNOR null animals was accompanied by improved Rapid Murine Coma and Behavioural Scores (RMCBS) compared to controls. We are currently investigating the effects of the GSNOR deletion on markers of endothelial dysfunction and blood brain barrier integrity during infection. Moreover utilizing bone marrow transplantation strategies, we are determining whether protection is dependent upon S-nitrosylation of hemoglobin, regulators of endothelial WPB exocytosis or other non-hematopoietic compartments. These experiments will help define whether interventions to increase NO bioavailability through SNOs improves outcome in severe malaria.

1436

CHARACTERIZATION OF *PLASMODIUM VIVAX* BLOOD TRANSMISSION STAGES

Nicanor III Obaldia[1], William Otero[2], Jose E. Calzada[3], Pierre-Yves Mantel[1], Manoj Duraisingh[1], Dyann Wirth[1], Matthias Marti[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Antimalarial Drugs and Vaccines Evaluation Center, Tropical Medicine Research, Panama City, Panama, [3]Instituto Conmemorativo Gorgas de Estudios de la Salud, Panama City, Panama

Development of new tools for the detection of *Plasmodium vivax* sub-patent gametocytemia or asymptomatic carriage in semi-immune individuals is fundamental for the eradication agenda. However, *P. vivax* gametocytes are poorly characterized and only a few markers known. In this study we develop new tools for the characterization and field detection of *P. vivax* gametocytes. Using a down-selection based on orthology to *P. falciparum* gametocyte markers we selected a series of putative *P. vivax* gametocyte markers for antibody production and generation of qRT-PCR primers. Epitope-specific rabbit polyclonal antibodies and exon-exon spanning primer sets were tested in samples from *P. vivax* infected Aotus monkeys. Exon-Exon primers against two putative late stage gametocyte markers, PVX_117730 and PVX_117900, were successfully optimized using synthetic cDNA probes and validated in the Aotus monkey model. In these samples typical *P. vivax* gametocyte morphology, including macrogametocytes and exflagellating microgametocytes, were identified on Giemsa stained smears. Indeed, comparison with Pvs25 demonstrated stage specificity and similar sensitivity as this gold standard for the PVX_117900 primer set. Marker gene expression was detected in infected blood samples directly collected from the animals, or after prolonged *ex vivo* culture. Importantly, indirect immunofluorescence (IFA) assays with PVX_117900 antibodies, labeled *P. vivax* parasites showing gametocyte morphology in spots obtained from Percoll gradient bands, though at low frequency. qRT-PCR analysis of longitudinal sampling upon experimental *P. vivax* infection in Aotus supported previous field observations that asexual parasitemia is positively correlated with gametocytemia. Experiments are underway to apply the qRT-PCR and antibody assays to investigate P.vivax transmission during human infection and to perform histological studies in the monkey model. This work represents an excellent starting point for further characterization of *P. vivax* gametocytes *in vitro* and during infection.

1437

HOST CONTROL OF PARASITE GROWTH IN *PLASMODIUM BERGHEI* INFECTION

Miles P. Davenport[1], Shannon E. Best[2], Ismail Sebina[2], Chelsea L. Edwards[2], Kylie James[2], Deborah Cromer[1], Ashraful Haque[2], David Khoury[1]

[1]The University of New South Wales, Sydney, Australia, [2]QIMR Berghofer Medical Research Institute, Brisbane, Australia

Early infection with *Plasmodium berghei* leads to rapid parasite growth, which slows around day 5-6 of infection. Although splenic clearance is thought to be an important factor in host control of parasite growth, few studies have directly measured parasite clearance. We developed a novel protocol to study the clearance of *P. berghei* infected red blood cells (RBC) *in vivo*. Fluorescently labelled RBCs infected with GFP+ *P. berghei* ANKA (PbA-GFP+) were transfused from donor mice into recipient C57BL/6 mice, and their clearance monitored by regular sampling over the subsequent 24 hours. We compared clearance in two groups of recipient mice; one group of naïve mice, and a second group of mice that were infected with PbA-GFP- 5-days prior. Flow cytometric analysis allowed us to distinguish donor vs. host RBC, and donor vs. host parasites. We used modelling to estimate parasite growth and clearance rates in the naïve vs. 5-day-infected animals, and observed faster clearance and reduced growth of donor parasites in the 5-day-infected animals. However, the changes during infection appeared more complex than simply an increase in clearance in the infected animals. Instead, our modelling suggested that there were differences in both the life-stages of parasites recognised and cleared in infected vs. naïve animals, and in the susceptibility of RBC in these animals. We further analysed the clearance data, focusing on clearance of parasites of different life-stages. We found evidence that trophozoites were more highly targeted in 5-day-infected animals, consistent with a shift towards clearance of earlier life stages over the first 5 days of infection. We also analysed the susceptibility of recipient RBC, by comparing the donor RBC (which were the same in the two groups) parasitemia vs. recipient RBC parasitemia. This showed much higher rates of infection of RBC in naïve mice, consistent with a greatly reduced susceptibility of host RBC in 5-day-infected recipients. This approach provides novel insights into the mechanisms of innate control of parasite growth *in vivo*.

1438

NON-INVASIVE MEASURES OF INCREASED INTRACRANIAL PRESSURE IN MALAWIAN CHILDREN WITH CEREBRAL MALARIA

Erin M. Fedak[1], Samuel D. Kampondeni[2], Michael J. Potchen[3], Montfort B. Gushu[2], Ian J. MacCormick[4], Carol A. Rowley[5], Karl B. Seydel[6], Terrie E. Taylor[6]

[1]Nationwide Children's Hospital, Columbus, OH, United States, [2]University of Malawi College of Medicine, Blantyre, Malawi, [3]University of Rochester, Rochester, NY, United States, [4]University of Liverpool, Liverpool, United Kingdom, [5]National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [6]Michigan State University, East Lansing, MI, United States

Brain swelling seen on magnetic resonance imaging (MRI) is the best clinical predictor of mortality in Malawian children with retinopathy-positive cerebral malaria (CM). Identifying more affordable and feasible non-invasive measures of raised intracranial pressure (ICP) would facilitate recognition of this high risk group, and simplify the conduct of interventional clinical trials. During the malaria season of 2014 (January - June), we carried out serial MRI every 12-24 hours on children with retinopathy positive CM while they were in coma. Two radiologists independently assessed overall brain volume (BV) on an 8-point scale; any discrepancies were resolved by consensus. The BV scores on admission were compared to papilledema (present/absent) and opening pressure at the time of lumbar puncture (mm cerebrospinal fluid). Two non-invasive measures were assessed on admission and at intervals thereafter: optic nerve sheath diameter (ONSD) measured using ultrasound, and pupillometry (NeurOptics). When increased BV was defined as an MRI score of >6, patients with increased BV were more likely to have papilledema than those without increased BV (Fisher's exact, p<0.04). Using the same cut-off, pupillometry, ONSD and opening pressure had AUROCs of 0.35 (95%CI: 0.18-0.53), 0.66 (95%CI: 0.37-0.96), and 0.84 (95%CI: 0.61-1), respectively. Longitudinal analyses of ONSD, pupillometry and BV and case studies in which BV changed significantly over the course of the hospital stay are in progress to determine the natural history of each of the surrogate markers in relation to MRI findings. The findings to date suggest that ultrasound measures of optic nerve sheath diameter, presence of papilledema, and opening pressure are the most useful surrogates for increased BV as determined by MRI.

1439

VITAMIN D INSUFFICIENCY IS COMMON IN UGANDAN CHILDREN AND IS ASSOCIATED WITH SEVERE MALARIA

Sarah Cusick[1], Robert Opoka[2], Troy Lund[1], Chandy John[1], Lynda Polgreen[1]

[1]University of Minnesota, Minneapolis, MN, United States, [2]Makerere University, Kampala, Uganda

Vitamin D plays a role in the immune response to infectious diseases. Activation of the vitamin D receptor in macrophages results in increased production of the anti-microbial peptides cathelidicin and beta defensin, while vitamin D supplementation reduces the inflammatory response and thus severity of influenza infection in animal models. Therefore, we hypothesized that children with severe malaria would have lower concentrations of plasma 25-hydroxy vitamin D (25[OH]D) than healthy children in a malaria-endemic region. To test this, we measured 25(OH)D in plasma by chemiluminescent immunoassay on samples collected from 40 children between the ages of 18 months and 12 years with severe malaria (20 with cerebral malaria, 20 with severe malarial anemia) and 20 healthy community children (CC) in Kampala, Uganda. We found that low plasma 25(OH)D was widespread: 95% of children with severe malaria (38 out of 40) and 80% of CC (16 out of 20) had insufficient vitamin D levels [25(OH)D < 30 ng/mL]. Of note, 20% of children with severe malaria, but no CC, had 25(OH)D levels < 15 ng/ml. Mean plasma 25(OH)D concentrations were significantly lower among children with severe malaria than among CC [mean (se): 21.2 (1.0) vs. 25.3 (1.6) ng/mL, p=0.03]. In addition, after adjusting for weight-for-age z-score (a measure of overall nutritional status), we found that the odds of having severe malaria declined by 9% [OR: 95% CI = 0.91: 0.83, 1.0] for every 1 ng/mL increase in plasma 25(OH)D. In conclusion, we describe for the first time an association between low vitamin D and severe malaria. These preliminary results suggest a possible role for vitamin D in the etiology of severe malaria. Confirmation of the findings of the present study will set the stage for a clinical trial of vitamin D treatment as a preventative or adjunctive therapeutic intervention to decrease the severity of malarial infection.

1440

THE REMODELLING OF NASCENT RETICULOCYTES BY *PLASMODIUM VIVAX* AND ITS PATHOLOGICAL CONSEQUENCES

Bruce Russell[1], Benoit Malleret[1], Wenn Chyau Lee[2], Rou Zhang[1], Rossarin Suwanarusk[3], Yee Ling Lau[2], Francois Nosten[4], Georges Snounou[5], Laurent Renia[3]

[1]National University of Singapore, Singapore, Singapore, [2]University of Malaya, KL, Malaysia, [3]Singapore Immunology Network, Singapore, Singapore, [4]Shoklo Malaria Research Unit, Mae Sod, Thailand, [5]Département de Parasitologie, Hôpital Pitié-Salpêtrière, Paris, France

The pathobiology of *Plasmodium vivax* infections is poorly understood. As malaria parasite red cell tropism defines the course of invasion and the pathology of the resultant disease, we have focused our efforts to determine the fine specificity of *P. vivax* invasion. To do this, we used a novel field flow cytometry approach to sample the different subsets of infected reticulocytes from vivax malaria patients and a range of *ex vivo* assays. Thus, we were able to determine the fine scale tropism of *Plasmodium vivax* for nascent reticulocytes (Heilmeyer Classes I to III) .Importantly nascent reticulocytes are rare in the peripheral blood, suggesting a cryptic role for bone marrow where such target cells are abundant.Subsequent *ex vivo* culture studies of *P. vivax* (with multiple rounds of maturation and invasion) allowed us demonstrate rapid modification of membrane structure and cytoplasm of the nascent reticulocyte. The shear modulus, immunophenotype and nanostructure of the infected reticulocyte membrane were significantly altered within 3 hours of invasion. We also employed microfluidic and micropipette aspiration methods to investigate the biomechanical implications of *P. vivax* development in the reticulocytes.One key finding of these studies was that *P.vivax* rosetting (a process we recently determined is mediated by reticulocyte glycophorin C) may play a significant role in the in disappearance of vivax schizont from circulation. Interestingly, the rate of *P. vivax* rosetting is clearly affected by certain antimalarial treatments.

1441

RESPONSE OF *PLASMODIUM FALCIPARUM* TO OXIDATIVE STRESS

Anthonia M. Hananiya[1], Vasiliki Pappa[1], Kris Subramanaim[1], Rebecca Greenstein[1], Kirk Dietsch[2], Johan Ankarlev[3], Laura Kirkman[3], Johanna Daily[1]

[1]Albert Einstein College of Medicine, Bronx, NY, United States, [2]Weill Cornell Medical College, New York, NY, United States, [3]Weil College of Medicine, New York, NY, United States

Malaria remains a major cause of morbidity and mortality around the world. Severe malaria and malaria-related mortality are due to the human malaria parasite *Plasmodium falciparum*. Host response to the parasite involves the production of reactive oxygen species (ROS). Malaria encodes antioxidant enzymes, however a full understanding of the parasite response to ROS is lacking. ROS could also induce genetic changes in the parasite that could be beneficial for parasite survival. We first examined transcriptional change in the parasites after 4 hour ROS and identified upregulation of stress response pathways including Response to Heat (GO.

0009408), DNA Repair (GO.0006281) and (GO.0006281 DNA repair). We set out to characterize the parasite response to ROS and determine if *P. falciparum* can adapt to increased levels of ROS. To examine the effect of ROS on growth we cultivated the 3D7 strain of *P. falciparum in vitro* in human erythrocytes at 4% hematocrit in supplemented RPMI media with and without ROS. All experiments began at ~2% parasitemia. Parasite growth curves were determined by microscopy of daily smears. Oxidative stress in the form of continuous extracellular generation of hydrogen peroxide was provided by supplementing the culture with 1 mM Xanthine and increasing concentrations XO. 100 U/ml of superoxide dismutase (SOD) was added throughout to enhance formation of hydrogen peroxide from superoxide radical anions generated by the XO-catalyzed oxidation of X. We identified the lethal dose of XO and determined if the parasite could adapt to sub-lethal concentrations of XO. Parasites treated with sub-lethal concentrations of XO demonstrated a decrease in parasite growth from day 3 to day 4. On day 6 of treatment we observed that the treated parasites demonstrated similar growth as compared to untreated control. Our data suggests that parasites exposed to varying concentrations of exogenous ROS showed decrease in growth, however they were able to adapt and grow normally after a few days. We will examine these adapted parasites genetically through transcriptional analysis to characterize their ROS adaptation and examine if genetic rearrangement occurs. Taken together this work explores the impact of host physiology on the biology of the parasite to inform severe disease models of pathogenesis.

1442

POPULATION GENETIC STRUCTURE OF THE ZOONOTIC MALARIA PARASITE *PLASMODIUM KNOWLESI* IN MALAYSIA

Paul Divis[1], Balbir Singh[2], Fread Anderios[3], Shamilah Hasim[4], Asmad Matusop[5], Samuel Assefa[1], Craig Duffy[1], David Conway[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]UNIMAS, Kuching, Malaysia, [3]Sabah Public Health Department, Kota Kinabalu, Malaysia, [4]Institute for Medical Research, Kuala Lumpur, Malaysia, [5]Sarawak Health Department, Kuching, Malaysia

Human cases of *Plasmodium knowlesi* malaria have been seen in many parts of Southeast Asia, with the largest number in Malaysia, following the first major focus of infections reported from Malaysian Borneo 10 years ago. This parasite is recognized as being zoonotic with wild long-tailed and pig-tailed macaques incriminated as the main reservoir hosts. In order to explore the previous and current transmission of infections, and address whether it may have been adapting to humans recently, it is important to study the population genetic structure of the parasite. In this study, a set of 10 microsatellite loci with tri-nucleotide repeats in the reference *P. knowlesi* genome were developed and validated for genotyping of natural inections by hemi-nested PCR assays. Using these markers, we analysed more than 300 *P. knowlesi* isolates from patients at 9 different sampling sites in Sarawak and Sabah states of Malaysian Borneo and mainland Peninsular Malaysia. All loci were polymorphic in all sampling sites, with more than 100 alleles in total scored across all 10 microsatellite loci, but most individual human isolates had single genotype infections. The mean genetic diversity across the loci was moderate to high, with no significant difference between different sampling sites (He values between 0.65 and 0.75). Levels of multi-locus linkage disequilibrium were very low in all sampling sites, indicating that recombination commonly occurs between different parasite genotypes in mosquito vectors, many of which presumably feed on macaques with multiple genotype infections. Pairwise genetic differentiation was more marked between sites in Borneo and mainland Peninsular Malaysia (FST > 0.10) as compared to differences among sites within Borneo. The human *P. knowlesi* genotype data are compared to isolates of wild macaques in Sarawak to test if there is any restriction in gene flow between the different hosts. These results are being used to strategically plan sampling from selected sites for whole genome sequence analysis in order to scan for possible evidence of host adaptation, and to study recombination in more detail.

1443

DIVERSITY OF ERYTHROCYTE INVASION PATHWAYS USED BY *PLASMODIUM FALCIPARUM* IN AREAS OF CONTRASTING INFECTION ENDEMICITY IN WEST AFRICA

Paul Bowyer[1], Lindsay Stewart[1], Harvey Aspeling-Jones[1], Henrietta Mensah-Brown[2], Ambroise Ahouidi[3], Alfred Amambua-Ngwa[4], Gordon Awandare[2], **David Conway**[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]University of Ghana, Legon, Ghana, [3]Universite Cheikh Anta Diop, Dakar, Senegal, [4]MRC Unit, Fajara, Gambia

Plasmodium falciparum uses a variety of alternative ligand-receptor interactions in order to invade red blood cells. The diversity of these pathways has traditionally been investigated by assessing the ability of parasite isolates to invade red blood cells that have been enzyme treated to selectively remove receptors. To date a variety of assay formats have been reported in different studies, but a standardised assay has not been applied to compare across population samples from diverse locations. Here we investigate *P. falciparum* invasion phenotypes from clinical isolates sampled in three sites on a gradient of transmission intensity in West Africa, using a single assay format. This is the first large-scale comparative analysis of erythrocyte invasion by clinical isolates from different endemic countries assayed in a single laboratory. Assays were performed on over 100 *P. falciparum* isolates from Ghana, Guinea and Senegal, that were cryopreserved at source and thawed so that the laboratory operator of the invasion assay was blinded to the sample source. These isolates were phenotyped for their ability to invade erythrocytes treated with neuraminidase, trypsin, chymotrypsin or a combination of these enzymes, in the first round of invasion following thawing but prior to adaption to culture. RNA was isolated for qRT-PCR from the schizont stage of a subset of these *ex vivo* cultured isolates in order to determine the relative expression levels of parasite invasion ligand genes. The data are analysed to explore the hypothesis that particular invasion pathways are selected in areas of high infection endemicity where there is strong acquired immunity against the parasite ligands, compared with areas of lower endemicity where immune selection is weaker.

1444

IRON DEFICIENCY ANEMIA AND *PLASMODIUM FALCIPARUM* GAMETOCYTOGENESIS

Martha A. Clark[1], Morgan M. Goheen[2], Raj S. Kasthuri[2], Carla Cerami[2]

[1]Harvard School of Public Health, Boston, MA, United States, [2]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Iron deficiency anemia and malaria are overlapping public health concerns in large parts of the developing world. Clinical and epidemiological studies have revealed that iron deficiency is protective against malaria infection in children and pregnant women. Our comparison of *Plasmodium falciparum* growth in iron-deficient and iron-replete RBCs *in vitro* has revealed that *P. falciparum* erythrocytic stage infection is attenuated in iron-deficient RBCs. We hypothesized that the inhospitable environment of iron-deficient RBCs, which inhibits asexual erythrocytic stage *P. falciparum* propagation, may additionally impact the rate and magnitude of *P. falciparum* gametocytogenesis. Here we report the results of our study of (i) the time to and (ii) the degree of *P. falciparum* gametocytogenesis in iron-deficient as compared to iron-replete RBCs *in vitro*. Our study of iron-deficiency and *P. falciparum* provides an invaluable model for studying *P. falciparum* pathogenesis and transmission.

1445

EXAMINING SELECTION ON *PLASMODIUM FALCIPARUM* AT DIFFERENT ENDEMIC SITES WITHIN GHANA

Craig Duffy[1], **Samuel Assefa**[1], James Abugri[2], Nicholas Amoako[3], Thomas Ayorigiya[4], Bronwyn MacInnis[5], Dominic Kwiatkowski[5], David Conway[1], Gordon Awandare[2]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]University of Ghana, Legon, Ghana, [3]Kintampo Health Research Centre, Kintampo, Ghana, [4]Navrongo Health Research Centre, Navrongo, Ghana, [5]Wellcome Trust Sanger Institute, Cambridge, United Kingdom

Populations of the human malaria parasite *Plasmodium falciparum* in West Africa are highly polymorphic while being closely related due to relatively unrestricted gene flow within the region. However, selection on individual local populations may vary significantly due to differences in transmission seasonality, drug pressure and levels of acquired immunity. Adaptation of populations will be a balance between local selective pressures and gene flow from neighbouring regions, which may quickly erode signatures of local selection. This will occur most extensively when populations are separated by only short distances, such as within a single country. Population specific selection has been demonstrated to differ between countries within West Africa, but the subtle differences that may exist between populations within a single country have not been investigated. In this study, the genomes of 101 *P. falciparum* clinical isolates from two different Ghanaian sites (Kintampo and Navrongo) separated by ~350km were sequenced and analysed. Transmission in Kintampo, in the forested centre of the country, is high throughout the year, while Navrongo, near the northern border with Burkina Faso, experiences high but seasonal transmission. Scans for evidence of directional selection identified several signatures apparently unique to Ghana, in that they were not seen previously in other West African countries. Patterns of balancing selection were similar in the two Ghanaian populations with high Tajima's D scores observed at loci expected to be exposed to host immune responses. Comparative analysis of the two populations indicated a very close relationship, with a mean FST of ~0.01 and only a small minority of SNPs with FST > 0.1, indicating few loci that may be under divergent selection and which will be discussed.

1446

DEVELOPMENT AND VALIDATION OF AN *IN VITRO*, CELL-FREE METHOD OF CULTURING MOSQUITO-STAGE *PLASMODIUM FALCIPARUM*

Tatyana Savransky, Lindsey S. Garver, Jack L. Williams, Silas A. Davidson

Walter Reed Army Institute of Research, Silver Spring, MD, United States

Between ingestion of gametocytes by an *Anopheles* mosquito and deposition of sporozoites by that mosquito into the skin of a human host, *Plasmodium falciparum* parasites transition through multiple life-cycle stages. These developmental stages are uniquely present in the mosquito vector and present a plethora of molecular and mechanistic targets for interruption of malaria transmission. However, it can be technically difficult and/or laborious to study these developmental stages and the targets they present because they rely on the mosquito for stage progression and growth. We have developed a cell-free method for culturing mosquito-stage *P. falciparum* (NF54 strain) *in vitro*. By seeding with gametocytes from blood-stage cultures, this proprietary method is capable of producing viable ookinetes, oocysts and sporozoites that maintain GFP expression and exclude trypan blue. Immunofluorescent staining with mAb against circumsporozoite protein (CSP) indicates the sporozoites obtained through this method uniformly express CSP while a liver stage development assay indicates they are able to infect cultured human hepatocytes and progress to liver stage, still expressing GFP. Comparative gene expression and mosquito infectivity assays between culture-derived and mosquito-derived parasites are underway.

1447

COMPARABLE DEVELOPMENTAL AND MORPHOLOGIC STAGES OF *IN VITRO* CULTURED *PLASMODIUM FALCIPARUM* SUPPLEMENTED WITH TWO COMMERCIALLY AVAILABLE SERUM SUBSTITUTES

Jourdan Posner, Sandra P. Chang, William L. Gosnell

University of Hawaii at Manoa, Honolulu, HI, United States

Current culturing techniques for the *in vitro* culture of *Plasmodium falciparum* are well established. Historically, media used for the *in vitro* culture of this organism consisted of RPMI 1640, a classic well-defined basal cell culture medium, and the addition of human serum. The use of human serum as a supplement is an absolute requirement for parasite growth, but is problematic due to biosafety concerns and lot variability. For that reason, ALBUMAX© II, a lipid-rich bovine albumin serum supplement, is now used in parasite culture medium in place of human serum. It's effectiveness as a human serum substitute has been well documented, but its composition is uncharacterized. This is an issue with research investigating parasite metabolomics and proteomics, which require well-defined *in vitro* growth parameters. Recently, MP Biomedicals released a highly purified microbiological grade bovine serum albumin for use in cell culture. The objective of this study was to compare ALBUMAX© II and MP Biomedical's Microbiological Grade BSA as human serum substitutes in *P. falciparum in vitro* culture. Identical culture conditions supplemented with either ALBUMAX© II or MP Biomedical's Microbiological Grade BSA were prepared and ran simultaneously. Short-term cultures analyzed by Giemsa staining and flow cytometry revealed similar growth trends with similar proportions of parasite developmental stages. Ring-stage and trophozoite survival assays were performed to examine merozoite invasion of erythrocytes and their subsequent development. Long-term cultures with either human serum substitute were also maintained to verify similar growth trends.

1448

CO-LOCALIZATION OF PFCSA-L AND VAR2CSA ON SURFACE KNOBS OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES THAT BIND THE PLACENTAL RECEPTOR CSA

Harold T. Obiakor[1], Patricia A. Gonzales Hurtado[1], Sanjay Desai[2], Michal Fried[1], Patrick E. Duffy[1]

[1]Laboratory of Malaria Immunology and Vaccinology/National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States, [2]Laboratory of Malaria and Vector Research/National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States

Placental malaria (PM) is a major cause of disease in pregnant women and their infants; it results from sequestration of *Plasmodium falciparum*-infected erythrocytes (*Pf*-IE) in the placenta via specific binding to chondroitin sulfate A (CSA). Over successive pregnancies, women become resistant to PM as they acquire antibodies against the novel protein PfCSA-L and the variant surface antigen VAR2CSA, *Pf*-IE surface proteins that bind to CSA. Here, we describe the association of PfCSA-L with VAR2CSA, and provide evidence that both protein exist in complexes at the *Pf*-IE surface. In earlier studies, we reported that PfCSA-L binds with high affinity (K_D = 6.6 x 10^{-9} M) to human placental CSPG by Surface Plasmon Resonance (SPR). We also reported that VAR2CSA and PfCSA-L interact on *Pf*-IE surface knobs (by DuoLink analysis), and that the DBL2X domain of VAR2CSA binds to PfCSA-L with subnanomolar affinity (K_D = 8.6 x 10^{-10} M). Here, we report that immune blots using PfCSA-L monoclonal antibodies detect only the PEXEL cleaved form of PfCSA-L (PfCSA-L_{PC}) in *Pf*-IE membrane preparations. Urea extraction of *Pf*-IE membranes suggests that both VAR2CSA and PfCSA-L are anchored by protein-protein (rather than protein-lipid) interactions on the IE surface, suggesting that they exist in complexes. However, VAR2CSA is resistant to alkaline sodium carbonate extraction while PfCSA-L is mostly extractible, indicative of integral and peripheral membrane proteins, respectively.

Preliminary analysis of co-immunoprecipitation and proteomics analysis confirmed direct association of PfCSA-L and VAR2CSA on the surface knobs of *Pf*-IE. These findings suggest that PfCSA-L interacts with VAR2CSA on surface knobs of *Pf*-IE, where they contribute to the CSA-binding phenotype. We are attempting to immunize rats with recombinant PfCSA-L and VAR2CSA DBL2X complexes to generate antibodies against neo-epitopes that may be capable of blocking *Pf*-IE binding to CSA. As a highly conserved protein of small size (~25 kDa), PfCSA-L appears to be a valuable component of a placental malaria vaccine.

1449

PLASMODIUM FALCIPARUM TOPOISOMERASE II

Devaraja Mudeppa, Shiva Kumar, Sreekanth Kokkonda, John White, Pradipsinh Rathod

University of Washington, Seattle, WA, United States

Type II topoisomerases, which are well-studied drug targets for many infectious agents and cancer, remain poorly understood in the human malaria parasite *Plasmodium falciparum*. Conventional efforts to express this enzyme have been challenging, as with many important malaria proteins. Here we report expression of full-length *Plasmodium falciparum* topoisomerase II (PfTopoII) in a cell-free wheat-germ protein expression system. Electrophoresis of *in vitro* expressed, radiolabeled PfTopoII pointed to a single 169 kDa entity on an autoradiogram. Soluble PfTopoII from translated lysates displayed a magnesium-dependent, ATP-dependent, and salt-sensitive supercoiled plasmid relaxation activity, and also DNA decatenation activity. A partially truncated PfTopoII construct retained full TopoII function and was more stable. PfTopoII was purified on a DNA affinity column, and a simple and sensitive fluorescence-based screen was established to conveniently track PfTopoII decatenation reactions. Preliminary work with existing TopoII inhibitors pointed to selective inhibition of PfTopoII compared to human TopoII. The availability of pure, functional PfTopoII opens up exciting paths to discovery of new classes of antimalarial agents.

1450

THE EBL-1/GLYCOPHORIN B LIGAND-RECEPTOR INTERACTION DEFINES A DOMINANT *PLASMODIUM FALCIPARUM* INVASION PATHWAY

Selasi Dankwa[1], Usheer Kanjee[1], Rays H. Jiang[2], Amy K. Bei[3], Markus Ganter[1], Mischka Moechtar[1], Christof Grüring[1], Ambroise D. Ahouidi[3], Daouda Ndiaye[3], Tandakha N. Dieye[4], Souleymane Mboup[3], Manoj T. Duraisingh[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]University of South Florida, Tampa, FL, United States, [3]Cheikh Anta Diop University, Dakar, Senegal, [4]Aristide Le Dantec Teaching Hospital, Dakar, Senegal

Parasite invasion of red blood cells (RBCs) is an obligatory step in malaria pathogenesis. *Plasmodium falciparum*, the parasite that causes the most virulent form of malaria, has evolved multiple proteins known as invasion ligands that bind to specific RBC receptors to facilitate invasion of human RBCs. The EBA-175/Glycophorin A (GPA) and RH5/Basigin ligand-receptor interactions, referred to as invasion pathways, are under consideration as vaccine targets and have been the subject of intense study to the neglect of others. For this study, we chose to focus on the little-studied EBL-1/Glycophorin B (GPB) invasion pathway because polymorphisms in GPB are prevalent in malaria-endemic regions, suggesting selection from malaria pressure. Through bioinformatic analysis, we have also recently identified considerable variation in GPB transcript levels in individuals from Benin. To elucidate the relative importance of the EBL-1/GPB invasion pathway vis-à-vis the well-described EBA-175/GPA and EBA-140/Glycophorin C (GPC) invasion pathways, we used an *in vitro* RBC culture system to deplete GPA, GPB or GPC via lentiviral transduction of erythroid progenitor cells. We assessed invasion efficiency using a panel of wild type *P. falciparum* lab strains and invasion ligand knockout lines, as well as *P. falciparum* Senegalese clinical isolates and short-term culture-adapted isolates. Our results indicate that the EBL-1/GPB and EBA-175/GPA invasion pathways are of similar and greater importance than the EBA-140/GPC invasion pathway, suggesting a hierarchy of RBC receptor usage in *P. falciparum*.

1451

EFFECTS OF HETEROZYGOUS SICKLE HEMOGLOBIN ON *PLASMODIUM FALCIPARUM* ERYTHROCYTIC GROWTH INDICES *IN VITRO*

Morgan M. Goheen[1], Steve M. Taylor[2], Nirmish Shah[2], Martha A. Clark[1], Carla Cerami[1]

[1]University of North Carolina Chapel Hill, Chapel Hill, NC, United States, [2]Duke University, Durham, NC, United States

Heterozygous hemoglobin S, resulting in sickle cell trait (HbAS), reduces the risk of severe *Plasmodium falciparum* infection in African children by 90%. The precise mechanisms by which HbAS confers this protection from malaria remain poorly understood. Elucidating these mechanisms may enable new strategies to neutralize the parasite therapeutically. Among other impacts of HbAS, it has been reported that parasite growth and invasion in HbAS RBCs is attenuated only at low oxygen tensions (≤5% O2), yet much of this work was done several decades ago with less detailed techniques for evaluating parasite growth and invasion. In effort to further define the phenotype of *P. falciparum* infection in patients with HbAS, we quantified parasite cellular phenotypes while cultivating *in vitro* in erythrocytes containing HbAS or normal adult hemoglobin (HbAA). Specifically, using flow cytometry-based assays, we separately examined the effect of HbAS erythrocytes on overall parasite growth, merozoite invasion of RBCs, and merozoite production (parasite erythrocyte multiplication rate). In addition, we used microscopic analyses to compare the timing of parasite maturation and development in HbAS and HbAA RBCs. With our HbAS versus HbAA RBC invasion analyses, we also report development of a simple two-color invasion assay allowing for direct comparison of parasite invasion into two cell populations labeled with the same fluorophore at differing concentrations. Finally, we investigated the impact of alpha-thalassemia upon the distinct cellular phenotypes in HbAS erythrocytes, because clinical data indicate that the co-inheritance of alpha-thalassemia attenuates the protection against severe malaria conferred by HbAS. These investigations leverage novel tools to refine our understanding of an ancient relationship between parasite and host. Further investigations of this relationship can improve our fundamental understanding of *P. falciparum* pathogenesis and enable the development of strategies to treat and prevent severe malaria.

1452

MICROVASCULAR TISSUE REOXYGENATION IN MALAWIAN CHILDREN WITH CEREBRAL MALARIA

Carol A. Rowley[1], Allison K. Ikeda[1], Karl Seydel[2], Terrie Taylor[2], Hans Ackerman[1]

[1]National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [2]Michigan State University, East Lansing, MI, United States

Impaired vasodilation and parasitized red blood cell adherence to blood vessel walls is thought to contribute to the pathophysiology of severe malaria, resulting in poor tissue perfusion. Decreased rates of skeletal muscle reoxygenation have been observed in Indonesian adults with severe malaria compared to healthy controls, but studies of tissue perfusion have not previously been performed on children with severe malaria. We measured rates of gastrocnemius-soleus tissue reoxygenation following a 3-minute femoral artery occlusion in Malawian children with cerebral malaria (CM) on each of 3 days following admission and at a 28-day follow-up visit. Children with uncomplicated malaria (UM) were assessed as controls. Children with cerebral malaria had lower maximum reoxygenation rates than uncomplicated malaria patients (median[IQR]; CM admission: 0.75[0.59-1.01] vs. UM: 1.22[1.12-1.34] % O2 saturation/second, p = 0.016). Maximum reoxygenation rate increased on day 3 compared to admission (CM day 2: 0.84[0.63-1.10] % O2 saturation/

second, p = 0.176 vs. CM admission; CM day 3: 1.07[0.98-1.33] % O2 saturation/second, p = 0.005 vs. CM admission). In addition, peak reoxygenation rate increased further at the 28-day follow-up visit (CM Follow-Up: 1.99[0.59-2.74] % O2 saturation/second, p = 0.039 vs. CM admission). Children with cerebral malaria appear to have an acutely diminished capacity to reoxygenate hypoxic tissue.

1453

RETINAL MICROCIRCULATION DYNAMICS DURING AN ACTIVE MALARIAL INFECTION

Emile Gordon[1], Ilker Ersoy[2], Stefan Jaeger[3], Michael Waisberg[4], Mirna Pena[1], George Thoma[3], Sameer Antani[3], Susan K. Pierce[1], **Kannappan Palaniappan**[2]

[1]Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]Department of Computer Science, University of Missouri-Columbia, Columbia, MO, United States, [3]Lister Hill National Center for Biomedical Communications, U.S. National Library of Medicine, National Institutes of Health, Bethesda, MD, United States, [4]University of Virginia, Department of Pathology, Charlottesville, VA, United States

The development of effective adjunctive therapies to treat cerebral malaria (CM) would have significant clinical impact in Africa where nearly a million children die each year due to CM infections. A better understanding of the cellular and molecular mechanisms underlying CM can lead to improved therapies and vaccines. Microcirculation in the retinal vasculature provides a window to image dynamic changes taking place in the central nervous system during CM disease progression. We have introduced a new video microscopy imaging modality using high resolution fundoscopy (HRF) and optical coherence tomography (OCT) to visualize the course of a *Plasmodium berghei* infection in a murine model of CM. Using OCT measurements the *in vivo* retinal cross-sections of infected mice do not seem to be enlarged or edematous in comparison to uninfected mice. Bright field fundoscopy reveals flowing hyper-reflective clumps that are confined to the retinal vasculature. We are actively investigating the nature of these clumps using fluorescently tagged parasites and flow cytometry to determine whether the size and behavior of the hyper-reflective bodies is correlated with disease severity and parasitemia. Infected mice are easily distinguished from uninfected controls based on the presence of hyper-reflective clumps, which suggests that HRF has diagnostic potential for establishing an individual's infection status. Using fundoscopy we detected a CM-specific increase in the number of GFP-positive immune cells (monocytes, macrophages, granulocytes) in the retina of LysM-GFP mice as the infection progressed. These preliminary data suggest that the retinal microcirculation can serve as a diagnostic window for malarial infection, and using video analysis of the microcirculation dynamics can aid in quantitatively characterizing the development of cerebral malaria under different treatments.

1454

AMPLICON DEEP SEQUENCING OF *PLASMODIUM VIVAX* MEROZOITE SURFACE PROTEIN-1: FROM GENES TO STRAINS TO INDIVIDUALS TO POPULATIONS

Christian M. Parobek[1], Jessica T. Lin[2], Nicholas J. Hathaway[3], Nick Matinyan[4], Deen Garba[4], Mark Janko[5], Oksana Kharabora[2], Suwanna Chaoratanakawee[6], Panita Gosi[6], Charlotte A. Lanteri[6], Chanthap Lon[6], Jeffrey A. Bailey[3], David L. Saunders[6], Jonathan J. Juliano[2]

[1]University of North Carolina at Chapel Hill, Curriculum in Genetics and Molecular Biology, Chapel Hill, NC, United States, [2]University of North Carolina at Chapel Hill, Division of Infectious Diseases, Chapel Hill, NC, United States, [3]University of Massachusetts Medical School, Program in Bioinformatics and Integrative Biology, Worcester, MA, United States, [4]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [5]University of North Carolina at Chapel Hill, School Department of Geography, Chapel Hill, NC, United States, [6]U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences, Department of Immunology and Medicine, Bangkok, Thailand

Malaria parasites have numerous hypervariable surface antigens. Deep sequencing of genes and the SNPs that encode this variability is a compelling new tool to answer questions about within-host diversity, between-strain competition, population genetics, and the origin of recurrences. Amplicon deep sequencing has been used to investigate these issues in malaria and other organisms. However, several questions regarding this approach remain. First, it is unclear how these results relate to other genotyping methods, such as microsatellites. Second, it is unclear how deep sequencing of individuals correlates to deep sequencing of pooled samples (PoolSeq). Here, we explore these issues while investigating the *Plasmodium vivax* population of Northern and Western Cambodia. Using ion semiconductor sequencing, we sequenced a short hypervariable fragment of the *P. vivax* merozoite surface protein-1 42-kDa domain to investigate the within-host diversity, population diversity and population structure of *P. vivax* using a cohort study (n=108 isolates) and a cross sectional survey (n=159 isolates). In these populations, we identified 67 and 35 unique haplotypes, respectively, and a total of 47 SNPs. Comparing amplicon deep sequencing to a three-locus neutral microsatellite genotyping approach on a subset of 50 isolates, we found that amplicon deep sequencing is more sensitive for resolving and following mixed infections (average MOI of 3.6 vs 2.1 variants per isolate, p<0.001). Direct comparison of PoolSeq to standard individual deep sequencing found that pooled deep sequencing of clinical isolates provides an accurate picture of parasite diversity and captured the great majority (88%) of the diversity within the population. Lastly, different population structures are seen between provinces and within provinces in Northern and Western Cambodia, suggesting that highly structured *P. vivax* populations exist within this region. This study addresses several knowledge gaps concerning the use of amplicon deep sequencing and further demonstrates its utility for studying the genetic epidemiology of malaria.

1455

VARYING INTERACTION BETWEEN *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN AND DUFFY-POSITIVE RED CELLS

Sushma Krishnan[1], Niraj H. Tolia[2], Lenore Carias[1], Brian T. Grimberg[1], Christopher L. King[1], Peter A. Zimmerman[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Washington University, St. Louis, MO, United States

Plasmodium vivax invasion of erythrocytes is known to be dependent on the interaction between *Plasmodium vivax* Duffy Binding protein region II (PvDBPII) and the Duffy antigen (Fy) present on erythrocytes. However increasing evidences indicates an alternative Fy-independent invasion pathway maybe available. To better understand the mechanisms by which the PvDBP interacts with host erythrocytes we examined binding

of conformationally correct recombinant PvDBPII to Duffy-positive host erythrocytes. We found levels of PvDBPII binding to a single individual's erythrocytes highly reproducible, but we also observed considerable variability in binding among erythrocytes from different individuals of the same Duffy genotype. Some of this inter-individual variation was attributable to the Duffy polymorphism on the N-terminal region of the Fy (Fy^a/Fy^a = 2197.38 ± 608.15 vs Fy^b/Fy^b = 4713 ± 483.16). However, PvDBPII binding between two *FY*B/*B* individuals showed considerable (e.g. individual 1 vs individual 2 = 4713 ± 483.16 v/s 12924 ± 749.79). The results seem to suggest that polymorphisms in other erythrocyte membrane proteins and/or their post-translational modifications could influence interaction between PvDBP and host erythrocytes, thus modifying susceptibility to vivax malaria.

1456

PHARMACOVIGILANCE DURING CAMPAIGN OF SEASONAL MALARIA CHEMOPREVENTION IN SENEGAL, 2013

Ibrahima Diallo, Mamadou L. Diouf, Moustapha Cisse, Alioune B. Gueye, Medoune Ndiop, Mady Ba

Senegal National Malaria Control Program, Dakar, Senegal

In 2013, the Senegal National Malaria Control Program (NMCP) implemented seasonal malaria chemoprevention (SMC), an intervention recommended by the World Health Organization (WHO) in 2012 in areas of seasonal malaria in which at least 60 % of cases occur over a period of four months. In Senegal, SMC was implemented in a door to door campaign by community health volunteers administering a dose of sulfadoxine-pyrimethamine (SP) and amodiaquine (AQ) under directly observed therapy to children three months to ten years and leaving an additional two doses of AQ for the guardian to administer. The campaign took place in four districts with almost 60,000 children in the target age group, and was accompanied by communications, advocacy, and social mobilization activities. While the drugs used in SMC are generally considered safe and effective, they can cause adverse events that may be minor, moderate, or in very rare cases, severe. The implementation of pharmacovigilance of antimalarials was an important step for the development of the pharmacovigilance program in Senegal and helped reorganize the national system, with the appointment of a national focal point at the Directorate of Pharmacies and Laboratories. Senegal has been able to establish a single system that takes into account all medicine programs and other products available in the country. Senegal was the 95th member of the WHO International Drug Monitoring Program and transmits notifications to the Uppsala Monitoring Center via VigiFlow software. During the 2013 SMC campaign, notices of adverse events were collected by the NMCP and processed by the Anti-Poison Center. Of the 115,547 treatments of SP+AQ administered to 59,420 children under 10 years, 20 adverse events notifications were sent to the NMCP. Adverse effects reported were mostly minor: abdominal pain, nausea, vomiting, urticaria, etc. All notifications were made by health post nurses. The Anti-Poison Center, which is responsible for determining imputability, judged that imputability was possible in 18 cases, improbable for one case, and uncategorized for one case, in which a single dose of amodiaquine was given. No severe adverse events were notified. In 2014, SMC will be implemented in 16 districts in the four regions of Kédougou, Tambacounda , Kolda and Sédhiou, targeting nearly 600,000 children. The pharmacovigilance system will be strengthened to ensure that adverse events will be notified and tracked.

1457

SELECTION OF ANTIRETROVIRAL TREATMENT (ART) IMPACTS ANTIMALARIAL PHARMACOKINETICS AND TREATMENT OUTCOMES IN HIV-MALARIA CO-INFECTED CHILDREN IN UGANDA

Sunil Parikh[1], Norah Mwebaza[2], Richard Kajubi[2], Joshua Ssebuliba[2], Sylvia Kiconco[2], Liusheng Huang[3], Qin Gao[3], Abel Kakuru[2], Jane Achan[2], Francesca Aweeka[3]

[1]Yale School of Public Health, New Haven, CT, United States, [2]Makerere University, Kampala, Uganda, [3]University of California San Francisco, San Francisco, CA, United States

HIV-infected children on protease-inhibitor (PI)-based ART have a lower risk for malaria compared to those on NNRTI-based ART. We evaluated the pharmacokinetics (PK) and pharmacodynamics (PD) of artemether-lumefantrine (AR-LR) in Ugandan children aged 0.5 to 8 years, providing the first intensive PK/PD data in HIV-infected children receiving lopinavir/ritonavir (LPV/r) or NNRTI [nevirapine (NVP) or efavirenz (EFV)]. HIV-uninfected children served as controls. Intensive PK (area under the concentration-time curve, AUC) and PD for 28 and 42 days, respectively was done. AR, active dihydroartemisinin (DHA), and LR in capillary plasma were measured by LCMSMS for 121 children (n=30 LPV/r; 28 NVP; 15 EFV and 48 controls). Lower AR AUC was seen with all ART groups compared with controls [geometric mean (GM) ratio; LPV/r 0.79 (ns); NVP 0.36 ($p<0.001$); EFV: 0.42, ($p=0.003$)] while DHA was reduced only in children on EFV [GM ratio 0.27, ($p<0.001$)]. For LR, AUC was 2-fold higher for children on LPV/r and 3-fold lower for children on EFV-based ART ($p<0.001$ for both). Median Day 7 LR level was 3.4-fold higher and 3.9-fold lower with LPV/r and EFV, respectively. Cumulative 28 day risk of parasitologic failure was 12%, 27%, and 33% for children on LPV/r, NVP, and EFV, respectively. Multivariate regression indicates altered malaria risk was largely due to distinctions in LR AUC ($p=0.016$). Moreover, day 7 levels were associated with 28 day risk of recurrent parasitemia (hazard ratio 0.60, $p=0.001$). Notably, 14/15 children on EFV had day 7 levels in the lowest quartile, and 13/15 had LR AUC in the lowest quartile. Use of AL in the setting of LPV/r-based ART resulted in a significant increase in LR exposure, largely explaining a reduced risk of malaria. In contrast, EFV-based ART results in significant reduction in exposure to all drugs; AR, DHA and LR; with LR reduction strongly associated with increased risk of parasitologic failure. These intensive PK/PD data demonstrate altered exposure and response supporting reevaluation of guidelines for antimalarial treatment of HIV-infected children, especially in the setting of EFV-based ART.

1458

ARTEMETHER-LUMEFANTRINE EXPOSURE FOLLOWING TREATMENT IN MALARIA-INFECTED CHILDREN AS COMPARED WITH ADULTS IN UGANDA

Norah Mwebaza[1], Richard Kajubi[1], Liusheng Huang[2], Joshua Ssebuliba[1], Xixi Cheng[2], Sylvia Kiconco[1], Jane Achan[1], Myaing Nyunt[3], Francesca Aweeka[2], **Sunil Parikh**[4]

[1]Makerere University, Kampala, Uganda, [2]University of California San Francisco, San Francisco, CA, United States, [3]University of Maryland, Baltimore, MD, United States, [4]Yale School of Public Health, New Haven, CT, United States

Artemether-lumefantrine (AR-LR) is currently the most widely adopted artemisinin combination therapy world-wide. We evaluated the comparative pharmacokinetics (PK) and pharmacodynamics (PD) of AR-LR in the context of developmental changes occurring in children 6 months to 8 years and compared PK results to data from adults. All individuals were treated for malaria in a high endemic region of Uganda and enrolled for intensive PK evaluations for AR-LR with 42 day follow-up. Exposure was estimated to 21 days [area under the concentration-time curve (AUC)]. Artemether (AR), its active metabolite dihydroartemisinin (DHA), and the long-acting partner drug, LR were quantitated from capillary plasma by LC/

MS/MS. Thus far, intensive PK/PD evaluations have been completed and analyzed for 26 children 8 months to 4 years, 22 children 4 to 8 years, and 11 adults (16 to 56 years) (n=30 enrolled). As expected, parasite densities (parasites/μL) at the time of presentation were significantly different between age ranges [Geometric mean (GM) 20,615, 6232 and 604 parasites/μL in < 4 years, ≥4 to 8 years, and adults, respectively). For the artemisinins (AR and DHA), no significant changes in exposure (maximum concentration or AUC) were observed in children as compared with adults. For LR, a trend toward reduced exposure (AUC) and day 7 (D7) levels were observed for children compared to adults [AUC GM 272 vs 316 hr•ug/mL (p=0.15); D7 median 339 vs 450 ng/mL). Notably, LR levels on day 21 were significantly lower in younger children compared to adults (p=.04). These results suggest overall comparable exposure in children as compared with adults, although sample sizes are limited in the very young (n=8 less than 2 years) and adults (n=11). Enrollment is ongoing in both population and intensive PK studies, and final results will be presented.

1459

THE AFFORDABLE MEDICINES FACILITY-MALARIA (AMFM) IN GHANA: FACTORS ASSOCIATED WITH PRIVATE RETAILER'S ADHERENCE TO THE RECOMMENDED RETAIL PRICE

John H. Amuasi[1], Beth Virnig[1], Graciela Diap[2], The AMFm Independent Evaluation Team[3]

[1]University of Minnesota School of Public Health, Minneapolis, MN, United States, [2]Drugs for Neglected Diseases initiative, Geneva, Switzerland, [3]Komfo Anokye Teaching Hospital, London School of Hygiene & Tropical Medicine, ICF International, Kumasi, Ghana; London, United Kingdom; Rockville, MD, United States

The Affordable Medicines Facility-malaria (AMFm) was initiated as a pilot in 7 countries aimed at increasing availability, reducing prices, increasing market share and increasing use of co-paid quality-assured artemisinin-based combination therapies (QAACTs). As part of the AMFm supporting interventions to facilitate the high level subsidy on QAACTs reaching consumers, the AMFm green leaf-logo was widely publicized, along with a recommended retail price (RRP) in Ghana. Using data from the 2011 endline survey of the Global Fund-commissioned AMFm independent evaluation, we explored factors associated with outlets stocking some co-paid QAACTs at RRP, and those stocking all at RRP in the private-for-profit health sector in Ghana. Analyses accounted for the complex survey design. We used multivariate logistic regressions to determine the association between being aware of the RRP and correctly specifying it, and the probability of stocking some or all QAACTs at RRP. Among the 545 outlets making up our sample, and which stocked at least 1 co-paid QAACT, 1,440 co-paid QAACTs were audited, with a mean number of 2.3 per outlet (95% CI: 2.1, 2.4). Twenty-four percent of outlets stocked no co-paid QAACTs at RRP, while 68% had some, but not all their co-paid QAACTs at RRP. Almost half of all the co-paid QAACTs audited were available at RRP. Many more outlets stocked some co-paid AL over ASAQ (93 % vs. 46%). Knowledge of the RRP was associated with a much higher predicted probability of stocking some co-paid QAACTs at RRP than stocking all at RRP (83% vs. 41%), although it was a strong predictor of both outcomes (p<0.001 for both). The type of co-paid QAACT being stocked (ASAQ/AL/both) was an important predictor of an outlet stocking both some (p=0.014) and all (p=0. 005) co-paid QAACTs at RRP. Malaria prevalence was also associated with stocking some co-paid QAACTs at RRP (p=0.013). Our study shows that retailer's adherence to the RRP for co-paid QAACTs can be high when knowledge about the RRP is present. Information on the AMFm subsidy needs to be disseminated to retailers with greater focus on those areas of high malaria prevalence, such as the northern savanna zone. All recommended policy interventions should be coupled with regular monitoring of prices and other indicators in the market in order to accurately measure the trend of the effects of the interventions.

1460

EFFECTIVENESS AND TREATMENT ADHERENCE TO ARTEMETHER-LUMEFANTRINE UNIT DOSED BLISTER-PACKS VERSUS STANDARD BLISTER-PACKS IN THE TREATMENT OF UNCOMPLICATED MALARIA: A RANDOMIZED CONTROLLED EQUIVALENCE TRIAL

Joaniter Nankabirwa[1], James Tibenderana[2], Samuel Gonahasa[1], Bonnie Wandera[1], Grace Nakanwagi[2], Mark Montague[2], Myers Lugemwa[3], Kirstie Graham[4], Sylvia Meek[4]

[1]Makerere University College of Health Sciences, Kampala, Uganda, [2]Malaria Consortium, Kampala, Uganda, [3]Ministry of Health, Kampala, Uganda, [4]Malaria Consortium, London, United Kingdom

Pre-packing drugs for treatment of uncomplicated malaria, into colour coded unit doses for particular age or weight groups has been shown to improve adherence; however, it creates challenges regarding procurement and administration thus reducing the benefits gained. This study sought to determine whether effectiveness and treatment adherence to standard blister-packs would be equivalent to unit dosed blister-packs. Between February and October 2010, an open label randomised controlled trial was conducted in 846 children aged 6-59 months living in a high malaria transmission setting in Uganda. Enrolled children were randomised to two study arms, receiving either unit dosed or standard blister-packs, and followed for 28 days. Outcome measures were risk of clinical and parasitological failure over 28 days' follow-up and adherence to prescribed treatment. Analyses were conducted on an intention-to-treat basis. The cure rate unadjusted by genotyping was 44.6% in the unit dosed blister-packs treatment arm compared to 41.5% for standard blister-packs (risk difference (RD) 3.1, 95% confidence interval (CI) -3.1, 9.9 p=0.375). Unadjusted risk of clinical failure was 28.7% in both treatment arms and unadjusted risk of parasitological failure was 26.7% and 29.8% in the unit dosed and standard blister-packs arms respectively (RD -3.1, CI -9.2, 3.2, p=0.330). There was no difference in adherence between the two treatment arms. Effectiveness and treatment adherence were equivalent in the two study arms. This study questions the value of unit dosed packaging given the challenges associated with ensuring uninterrupted supply, and highlights the importance of good provider-patient communication for treatment adherence. The findings suggest that standard blister-packs would improve quality of care through improved reliability of supply without compromising effectiveness and adherence to antimalarials. The implications of this study are broader than antimalarials and further research is needed to confirm and explore the potential impact of these findings.

1461

BIOLOGICAL STABILITY OF DIHYDROARTEMISININ IN PHYSIOLOGICAL CONDITIONS

Nicoletta Basilico[1], Silvia Parapini[1], Piero L. Olliaro[2], **Donatella Taramelli**[1]

[1]University of Milan, Milan, Italy, [2]UNICEF/UNDP/World Bank/WHO Special Programme on Research & Training in Tropical Diseases, World Health Organization, Geneva, Switzerland

Artemisinin derivatives are the most effective antimalarials available to-date. Dihydroartemisinin (DHA) is a drug on its own and also the main metabolite of other artemisinins. These molecules, characterized by the presence of an endoperoxide pharmacophore, are highly unstable; they degrade very quickly in the presence of ferrous iron or organic solvents. Less documented is the stability of DHA, measured as antimalarial activity, when incubated *in vitro* with blood components or in different cultures conditions. We investigated this problem by incubating DHA in PBS, plasma, serum or erythrocytes lysate for different lengths of times, at different temperatures and pHs. Chloroquine, a 4-aminoquinoline antimalarial and artesunate were also used to verify if drug instability was related to the presence of the endoperoxide. Residual activity of the drugs was evaluated by determining *Plasmodium falciparum* viability with the

pLDH method. A significant reduction of the antimalarial activity of DHA was seen after incubation in plasma or serum and to a lesser extent with erythrocytes lysate or PBS: 3-hour incubation in plasma was sufficient to double the IC50 of DHA, whereas activity was almost completely lost after 24h. The serum-enriched mediums (10% human serum or 10% albumax) customarily used for *in vitro* cultures also affected DHA efficacy. DHA activity was partially preserved at 4°C or at room temperature, but was lost at 40°C. Similarly, increasing pH from 7.2 to 7.6 reduced DHA efficacy. Artesunate behaved in a similar way to DHA, whereas chloroquine was unaffected in any of the tested *in vitro* conditions. These results suggest that particular care has to be taken in conducting and interpreting *in vitro* studies, and in storing these compounds. Moreover, conditions such as fever, hemolysis or acidosis associated with malaria severity may contribute to artemisinins instability and reduce its effectiveness.

1462

SYNTHESIS AND BIOLOGICAL TESTING OF 2,5-SUBSTITUTED PYRIMIDINES

Victor E. Zottig[1], Stanton Q. Smith[2], Thomas A. Bradshaw[2], Brent S. Clark[2], Zachary A. Ratka[2], Patricia J. Lee[1], Norma E. Roncal[1], David A. Donkin[1], Eric K. Kim[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Virginia Military Institute, Lexington, VA, United States

Heterocyclic containing compounds have historically represented important structures in medicinal chemistry. Malaria is a debilitating and life-threatening mosquito borne disease that affects millions of people a year. From the 1600's to WWII the traditional treatment for malaria used the aromatic heterocylic compound, quinine. Vinamidinium salts are known for their ability to make aromatic heterocycles. We report on the synthesis of pyrimidines from vinamidinium salts and their biological activity against Malaria. We have prepared two vinamidinium salts and then synthesized a series of pyrimidines from each salt. These two series of pyrimidines were evaluated for anti-malarial activity.

1463

MALARIA PLACENTAL INFECTION AND INTERMITTENT PREVENTIVE TREATMENT IN SUBURBAN KINSHASA, DR CONGO

Serge M. Kayembe[1], Fabrice Matuta[1], Aimée K. Kahindo[1], Solange E. Umesumbu[2], Thierry L. Bobanga[1]

[1]University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [2]Malaria National Control Program, Kinshasa, Democratic Republic of the Congo

Malaria is a major health threat and an obstacle in the path of economic development of individuals, communities and nations. It is the leading cause of mortality and morbidity in the DRC. Pregnant women and children under 5 years are the most vulnerable groups. For pregnant women, it may be responsible for premature abortion, fetal growth retardation and infant and maternal death. In the DRC, the NMCP recommends that all pregnant women receive two doses of IPT with sulfadoxin pyrimethamin during ANC. This study was undertaken to 1) determine the proportion of pregnant women who took the IPT, 2) Estimate the frequency of placental malaria infection at N'djili Reference Hospital, and3) Determine the frequency of chorionitis A cross sectional study was conducted among 223 women delivered at the maternity HGR N'DJILI in Kinshasa, women who accepted to participate in the study after informed consent in a period from September 2013 to March 2014. Blood sampling was performed for making a thick and a thin smear in women at childbirth, placenta prints was made and a sample was preserved in formalin for histological analysis. In addition an interview was conducted to obtain information about IPT. 223 women were included in the study. The age group most represented was 18-25 years with 45.4 %. Primiparous were 46.4 %. TPI 1 was observed in 76.9 %, while the taking of IPT 2 was observed at 23.1%. 28.7% of women took the first dose at fifth month and 44.3 % on the sixth month or after . All multiparous took the first dose of IPT, in secondiparous and primiparous group the taking was 66. 6 %. The difference between the two group was highly significant p <0.0001 For the second dose, 68.5% take up to 8th month and 16.6% have taken at nineth month 77.8% of GE examined was positive positive with *Plasmodium falciparum* 73.1% of placental prints were positive with trophozoites and 7.4% with trophozoites and schizonts of *Plasmodium falciparum*. Diiference between women with IPT and women without IPT, was significant p< 0.001 Histopathological findings will be available in late May In conclusion, the plasmodium infection in pregnant women and placental infection are very high among pregnant women in peri -urban environment Kinshasa Intermittent preventive treatment in pregnant women is not unfortunately respected Increased awareness should be held to a greater commitment to the strategy of prevention against malaria

1464

DIRECTLY OBSERVED THERAPY: REVIEW OF BEST PRACTICES AND THE APPLICATION TO MALARIA TREATMENT

Jennifer A. Wegbreit[1], Thang Duc Ngo[2], Anh Quy Nguyen[2], Xa Xuan Nguyen[2], Nicholas Martin[3], Duong Thanh Tran[2], Hien Nguyen[4], Colin Ohrt[1]

[1]University of California San Francisco, San Francisco, CA, United States, [2]National Institute of Malariology, Parasitology and Entomology, Hanoi, Vietnam, [3]Naval Medical Research Center-Asia, Singapore, Singapore, [4]U.S. Embassy, Hanoi, Vietnam

Directly observed therapy (DOT) is the standard of care for tuberculosis treatment and it is used for HIV/AIDS treatment in many settings. These are complex treatment regimens for which high adherence rates have been achieved. Yet, for malaria, where treatment regimens range from three to 14 days, DOT is perceived to be too difficult to implement. We are conducting a literature review of DOT best practices for tuberculosis, malaria and any other applicable disease treatment regimens. We are examining DOT for malaria across a wide variety of treatment protocols. In addition to the literature review, we will interview key informants, including community health workers and village health workers, who have implemented DOT for malaria and other diseases. The aim of the interviews is to better understand the most effective implementation strategies and any challenges encountered. We will examine factors that have promoted and hindered high treatment adherence. The standard interview questionnaire captures structured data from key informants and includes information on DOT implementation strategies, contextual information, treatment regimens, and factors leading to success and failure. It also includes questions about treatment seeking behaviors and access to health services at the community level. The National Malaria Control Program (NMCP) in Vietnam has experience implementing DOT for *Plasmodium falciparum* infection in tier 1 provinces of the containment zone. In 2014-2015, Vietnam's National Institute of Malariology, Parasitology and Entomology (NIMPE), has funding from the Global Fund to implement DOT in areas where multi-drug resistant malaria is emerging. In coordination with NIMPE, we plan to pilot the best practices identified in the literature review and key informant interviews. By June of 2014, we will have completed and analysed 20 structured interviews. The literature review and key informant data will be presented as well as the plan to pilot implementation of DOT.

1465

CHEMOGENETIC PROFILE ANALYSIS OF *PLASMODIUM FALCIPARUM* TO COMPOUNDS FROM THE MMV MALARIA BOX

Kenneth O. Udenze[1], Anupan Pradhan[1], Geoffrey Siwo[2], Zhang Min[1], Asako Tan[2], Naresh Singh[1], Dennis E. Kyle[1], Michael T. Ferdig[2], John H. Adams[1]

[1]Department of Global Health, University of South Florida, Tampa, FL, United States, [2]Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, United States

Drug resistance in *Plasmodium falciparum* requires improved use of existing drugs and enhanced methods for discovery of new drugs with unique targets and mechanisms of action. Chemogenetic profiling of *P. falciparum* mutants is a new approach to identify and prioritize drugs with novel targets and/or modes of action and is potentially a way to predict drug combination therapies with optimal synergistic anti-parasite activity. Isogenic mutants of *P. falciparum* were created by *piggyBac* transposon insertion whereby each mutant parasite carries a unique signature of affected metabolic pathways that can alter responses to drugs. An important advantage of this approach is the precise nature of the chemical-genetic profile, since single mutations are created in an identical genetic background (a clone of NF54). *piggyBac* mutant clones with insertions in identifiable links to specific GO pathways were profiled for responses to a subset of compounds from the malaria box. The wild type and *piggyBac* mutant parasites were allowed to grow for 72 hours in a range of growth inhibitors and then quantified using a DNA dye (SYBRGreen I). The different *piggyBac* mutants varied in their susceptibility to inhibitors, demonstrating unique signatures related to the specific mutation allowing us to map associations among inhibitors and mutants. Cluster and network analyses of chemicogenetic profiles to malaria box drug susceptibility profiles linked drugs with common mechanisms of action and provided potential insights into metabolic pathways targeted by the antimalarial drugs.

1466

OVERCOMING PERSISTENT BARRIERS TO THE SCALE UP OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY (IPTP): PERSPECTIVES OF POLICYMAKERS, HEALTHCARE PROVIDERS AND PREGNANT WOMEN

R. Matthew Chico[1], Joyce Wamoyi[2], Daniel Chandramohan[1], John Changalucha[2], Jayne Webster[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]National Institute of Medical Research, Mwanza, United Republic of Tanzania

The World Health Organization recommends intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) for pregnant women resident in areas of moderate (stable) or high malaria transmission to prevent the adverse consequences of malaria infection during pregnancy. Despite efforts over the past decade to scale up coverage, less than one-quarter of women receive two doses. To identify persistent barriers to the scale-up of IPTp, as well as the potential to scale-up alternative regimens and/or alternative strategies, semi-structured in-depth interviews (IDIs) and focus group discussions (FGD) were conducted among healthcare providers and pregnant women in Tanzania. A total of 64 pregnant women participated in FGDs, while 28 pregnant women were included in IDIs; 14 healthcare providers participated in IDIs and, separately, 11 policymakers were interviewed. Participant responses were coded and analysed using NVivo 10.0. Content analysis was used to derive a range of themes. A major barrier to the acceptability of IPTp-SP across those interviewed was side-effects. The risk of side-effects discourages some healthcare providers from providing treatment given the ethos of 'do no harm' and the fact that most pregnant women at antenatal presentation are either not infected or have an asymptomatic infection and do not feel ill. Perceptions and experiences of side effects of SP are likely to shape whether or not replacements drugs may be brought to scale given that all current candidates involve multi-day regimens. The risk of side-effects might be more acceptable to many (but not all) policymakers, health care providers, and pregnant women if: a more efficacious therapy than SP is used in IPTp, a replacement for SP is simultaneously protective against malaria and curable sexually transmitted infections, as may be the case with azithromycin-based combination therapies, or women are screened for malaria and only women who are found to be parasitemic are then given treatment.

1467

IN VIVO EFFICACY AND SAFETY OF ARTEMETHER/LUMEFANTRINE VS. DIHYDROARTEMISININ-PIPERAQUINE FOR TREATMENT OF UNCOMPLICATED MALARIA AND ASSESSMENT OF PARASITE GENETIC FACTORS ASSOCIATED WITH PARASITE CLEARANCE OR TREATMENT FAILURE

Deus Ishengoma[1], Celine Mandara[1], Samuel Gesase[1], Esther Ngadaya[2], Sigsbert Mkude[3], Renata Mandike[3], Ally Mohamed[3], Janeth Mgamba[4], Peter Mbuji[4], Ritha Njau[5], Martha Lemnge[1]

[1]National Institute for Medical Research, Tanga, United Republic of Tanzania, [2]National Institute for Medical Research, Dar es Salaam, United Republic of Tanzania, [3]National Malaria Control Program, Dar es Salaam, United Republic of Tanzania, [4]Ministry of Health and Social Wellfare, Dar es Salaam, United Republic of Tanzania, [5]World Heath Organization, Dar es Salaam, United Republic of Tanzania

Antimalarial efficacy studies are recommended by the World Health Organisation to monitor the efficacy of artemisinin based combination therapy (ACT) and possibly detect evolution/emergency of tolerance/resistance to these drugs. Currently, Artemether/Lumefantrine (AL) is the only ACT which is being used in Tanzania and thus, testing of new ACTs such as dihydroartemisinin-piperaquine (DP) is important because alternative drugs are urgently required. This study will be an open-label randomized trial and aims to assess the efficacy of AL versus DP; and the role of parasite genetic/genomic factors that might be associated with treatment outcome among patients with uncomplicated malaria treated with these ACTs. The study will be conducted from May 2014 and will recruit 600 children aged 6 months to 10 years with uncomplicated falciparum malaria at Muheza Designated District Hospital and Ujiji Health Centre in Tanga and Kigoma regions respectively (150 patients per treatment arms at each site). Follow up will be done for 63 days and the primary end point will be parasitological cure on day 28 for AL and 42 for DP (non-adjusted and adjusted by PCR to correct for new infections). The secondary end points will include: parasite clearance after 72 hours, parasitological cure on day 14, extended parasitological cure on day 42 for AL and 63 for DP, improvement in haemoglobin level at day 28 compared to day 0, reduction in gametocyte carriage at day 14 and day 28 Vs 0, occurrence and severity of adverse events, and genomic profile of *P. falciparum* malaria parasite. Preliminary results will be presented and discussed, and study will provide important data to the National Malaria Control Program (NMCP) to be used in the ongoing review of treatment guidelines. The information will also support NMCP to recommend DP as the second line antimalarial drug for the treatment of uncomplicated malaria in Tanzania.

1468

BURIED LEGACY? AN ANALYSIS OF PSYCHIATRIC TOXICITY OF PRE-CHLOROQUINE ANTIMALARIALS

David Adams[1], Daniel Mauck[2]

[1]AASU/Georgia Regents Medical University, Savannah, GA, United States, [2]AASU, Savannah, GA, United States

The toxicity of synthetic anti-malarials such as primaquine, chloroquine, and mefloquine have been well documented. Their side effects range from hemolytic anemia in the case of primaquine to psychiatric disturbances in the case of chloroquine and mefloquine. The toxicity profiles of their

pre-WWII predecessors, however, have received relatively little attention. Pamaquine and mepacrine, synthetic anti-malarials developed by German industrial chemists in the 1920s and 1930s, proved invaluable for malaria control among Allied and Axis troops alike. Pamaquine and mepacrine, however, were not without their own toxicity issues. As their use expanded, first in far-flung colonial outposts and subsequently in Asian and European theatres, reports began to emerge about unexpected psychiatric toxicity. Despite these warnings, their central role as anti-malarials continued throughout the Second World War. This presentation will examine the factors that spurred their widespread use despite a growing number of contemporary reports that recommended cautious use among high-risk individuals.

1469

SEVERE MALARIA MORTALITY AND MANAGEMENT IN THREE GENERAL REFERENCE HOSPITALS IN KINSHASA, DEMOCRATIC REPUBLIC OF CONGO

Solange E. Umesumbu[1], Billy S. Kunyu[2], Adonis T. Ntoka[3], Malene B. Masukidi[3], Marilyne L. Mvuata[3], Thierry L. Bobanga[3]

[1]National Malaria Control Program, Kinshasa, Democratic Republic of the Congo, [2]National Institute of Medical Research, Kinshasha, Democratic Republic of the Congo, [3]University of Kinshasa, Kinshasa, Democratic Republic of the Congo

Malaria remains a global problem and remains a major public health concern for the countries of Sub-Saharan Africa, particularly the Democratic Republic of Congo (DRC). It is one of the leading causes of morbidity including severe form occurs in individuals lacking premunition or those who have lost over several years without exposure , particularly children under 5 years and pregnant women . This form of malaria is based on high hospital mortality in a pediatric setting, requiring proper care, effective and consistent with national policy. This study aimed to describe the forms of severe malaria to determine the molecules used for the care and describe the evolution of children hospitalized for severe malaria. This descriptive study was conducted in the pediatric wards of General Reference Hospitals of Makala (GRHM), Kintambo (GRHK) and Universitary Clinics of Kinshasa (UCK) for the periods from 01 January 2011 to 13 July 2013 (2.5 years) by collecting information on archived records. Severe malaria cases in anemic and neurological forms were the most encountered respectively 58.59 % and 35.35 % in UCK; 62.2% and 30.8 % in GRHK; 65.5 % and 23 % in GRHM. Pulmonary and haemoglobinuric forms were also observed. Injectable quinine infusion was the most commonly used antimalarial molecule in 91.92 %; 89.7 % and 97.5% of cases respectively at UCK, GHRK and GHRM. The evolution after treatment showed a mortality of 33.3% (UCK), 23.4 % (GRHK) and 39 % (GRHM). But healing was observed in most cases at 67% (UCK), 67.1 % (GRHK) and 61 % (GRHM) without sequelae but 1.01% has sequelae In conclusion, the predominant shape was anemic form followed by neurological form, quinine antimalarial infusion was administered to support these and therapeutic evolution post cure was recovery in most cases, all times with considerable mortality. The ideal is an early treatment of malaria cases to avoid severe cases.

1470

ARE WE ACHIEVING SUFFICIENT POPULATION COVERAGE OF ARTEMISININ-COMBINATION THERAPY AMONG CHILDREN WITH MALARIA IN AFRICA? A SYSTEMATIC ANALYSIS OF DATA 2003-2012

Adam Bennett[1], Josh Yukich[2], Thomas P. Eisele[2]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States

Artemisinin-based combination therapies (ACTs) are highly effective for curing uncomplicated malaria and preventing progression to severe disease. Funding for ACTs has dramatically increased and most countries in Africa have promoted ACT as first-line treatment since around 2005. Since this time, there has been a major rise in global ACT procurement. However, because of the challenges of reliably measuring the population coverage of ACTs among children with confirmed uncomplicated malaria, to date continent-wide changes in treatment coverage have not been quantified in a rigorous manner. Data from household surveys with parasite testing using antigen-detection rapid diagnostic tests (RDTs) are increasingly available, which provide a period prevalence estimate of infection that overlaps with two-week fever history. We combined data from 71 national household surveys (DHS, MIS, and MICS) to estimate the annual proportion of children with uncomplicated malaria (fever + parasite infection measured by RDT) receiving ACTs for all countries in sub-Saharan Africa 2003-2012. We used an individual-level logistic regression model including local PfPR, child age, household wealth, urban/rural, and insecticide-treated net (ITN) possession to predict RDT status for children in surveys without parasite testing. We used ACT distribution data combined with country-level covariates and temporally-correlated random effects in generalized linear regression models within a Bayesian framework to predict coverage to countries and years without data. Scale-up of treatment with ACTs among all children with uncomplicated malaria has been modest, reaching only 18% (95% Credible Interval 13%-22%) by 2011-2012, with highly variable coverage by country. The primary barriers to treatment with an ACT appear to be low treatment-seeking rates and inadequate access to health services, as coverage is much higher amongst children for whom care was sought. Additionally, children were more likely to receive an ACT in the public sector than in the private sector, but RDT+ children were only slightly more likely to have received an ACT than RDT- children, indicating a high degree of presumptive treatment. Improved access to health services and increased availability of ACTs in the private sector, coupled with increased demand for fever treatment, is critical for preventing severe disease and deaths among children with uncomplicated malaria.

1471

CHANGES IN THE AVAILABILITY AND AFFORDABILITY OF ACTS IN THE RURAL WEST AFRICAN PRIVATE RETAIL SECTOR: TWO AND A HALF YEARS POST AFFORDABLE MEDICINES FACILITY - MALARIA (AMFM)

Evelyn K. Ansah

Ghana Health Service, Accra, Ghana

The four main objectives of Affordable Medicines Facility - malaria (AMFm) were to: (i) to increase Artemisinin Combination Therapy (ACT) affordability; (ii) to increase ACT availability; (iii) to increase ACT use, including among vulnerable groups; and (iv) to "crowd out" oral artemisinin monotherapies. Ghana was among the nine countries which piloted the first phase of the strategy. The majority of adults and children with febrile illness, including the poorest are treated in the private retail sector. The study assessed changes in ACT availability in private retail shops 2 months before, 2 months after and 2.5 years after the arrival of the first co-paid ACTs in Ghana in August 2010. We also assessed prices of antimalarials (AM) in the shops 2.5 years after AMFm in a rural district in Ghana with an original fixed co-paid ACT price of GHC1.50. Supply, stock-out and cost issues were explored during the last survey in February 2013. Fifty-three chemical shops and 3 pharmacies out of 62 shops participated in the study. Overall, there were 398, 388 and 442 different brands of AMs in the shops during the 3 censuses. ACTs increased over the period, comprising 16.6%, 42.5% and 47.7% of AM in stock respectively. There appeared to be a slight reversal with regards to the market share of non artemesinin therapies from 34.2%, and 6.9% to 9.5% in the most recent census. Artemisinin monotherapies comprised of 9.5%, 4.6% and 3.4% AM available in the 3 time periods. Stocks of Herbal based AM preparations were relatively high forming 40-45% of all stock of AM. This did not change much over the period, constituting 39.7%, 45.9% and 39.4% of AMs respectively. For both children and adults, ACTs were the most sold AM type. Overall, 55.4% (31/56) of shops had experienced stock-outs of quality assured ACTs (QAACTs) in the preceding 2 months,

most of them (12/31; 38.7%) for a 1-2 week period. Sixteen of the 56 shops (28.6%) had no stock of QUAACTs. Buying and selling prices of QAACTs had increased by 40-100% and shopkeepers attributed this mainly to the scarcity of the commodity. In order to prevent reversal of the gains in malaria control over the last decade, consistent supply of QAACTs to the private retail sector must be assured.

1472

EFFECTIVENESS OF PROVIDER AND SCHOOL INTERVENTIONS ON THE TREATMENT PROVIDED TO FEBRILE PATIENTS ATTENDING PUBLIC PRIMARY HEALTH CENTRES AND MEDICINE RETAILERS IN SOUTHEASTERN NIGERIA

Obinna Onwujekwe[1], Lindsay Mangham-Jeffries[2], Bonnie Cundill[2], Neal Alexander[2], Julia Langham[2], Ogochukwu Ibe[1], Benjamin Uzochukwu[1], Virginia Wiseman[2]

[1]University of Nigeria, Enugu, Nigeria, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

A formative survey for this study found that less than 1% of patients were tested for malaria, ACTs were received by only 22.4% of all patients and 37.9% of patients received SP. There was hence the need to improve appropriate treatment malaria by designing and implementing useful interventions. The interventions were evaluated using a three-arm cluster randomized trial in a real-life setting. The three arms were: the Control; Intervention arm 1; and Intervention arm 2. In the control arm there was only normal practice with supply of rapid diagnostic tests (RDTs) with basic instruction. In arm 1 there was provider intervention with supply of RDTs. In arm 2 there was provider intervention and school-based community intervention. The interventions were evaluated using a patient exit survey, log of malaria tests conducted, provider survey and a household survey. Within each stratum and arm, a point estimate of the proportion of patients treated according to guidelines was calculated. The implementation of the interventions differed in some stratum within the same arms. There was a general increase in testing compared to formative study, but the number of patients that were tested was still low across the three different arms despite the availability of RDTs in the facilities and there were no significant differences by arm. A large proportion of patients asked for a specific medicine and 96% of those who asked for a specific medicine got what they asked for. There was also no evidence of a difference between the intervention arms and control in the proportion of test positive patients receiving an ACT. It was found that 63% of those not tested asked for a medicine compared with 19% of those tested. The interventions did not make significant improvements in the intervention arms compared to the control arm. The reasons for this may include the real life setting of the project, the non-uniform implementation of the interventions in some arms and the relative differences in the gap between implementation and evaluation of the intervention in some clusters.

1473

HIGH FREQUENCY OF SUBMICROSCOPIC GAMETOCYTE CARRIAGE AFTER THE TREATMENT OF UNCOMPLICATED MALARIA WITH ACTS

Marielle K. Bouyou-Akotet[1], Denise P. Mawili-Mboumba[1], Rosalie Nikiema Ndong Ella[1], Eric Kendjo[1], Brigitte Charron[2], Valérie Lameyre[2], Maryvonne Kombila[1]

[1]Université des Sciences de la Santé, Libreville, Gabon, [2]Access to Medicine, Gentilly, France

Studying the parasite reservoir is a tool for monitoring the effectiveness of control strategies. The gametocyte carriage is more common in patients with asexual forms, and the occurrence of recrudescence or reinfection in patients could also be favored and contribute to the maintenance of a large reservoir of parasites. The aim of this study was to determine the prevalence of submicroscopic gametocytaemia in patients treated for uncomplicated malaria. Gametocytes carriage and density were estimated by Pfs25mRNA amplification using QT-NASBA in samples obtained at enrolment and during the follow-up (day 21 to day 42 post-treatment) in samples of children treated with either artesunate-amodiaquine (ASAQ) or artemether-lumefantrine (AL). Data were analyzed according to the study visit, the presence of asexual parasites, the type of treatment and the treatment response. Samples from 48 children were analyzed; 23 were treated with ASAQ and 25 with AL. They had 147 visits, all corresponding to treatment failure with either ASAQ or AL. None of the patients had a microscopic gametocytaemia. Overall, the frequency of SMG carriage was 51%, comparable at day 0 between the ASAQ (53%) and the AL (56%) patients (p=0.6). During the post-treatment visits, it was of 58% and 44% respectively in the ASAQ and in the AL groups respectively (p= 0.4). When pair samples of 23 children were analyzed, the gametocytaemia was positively correlated with the asexual form density the day of treatment failure (rho=0.4 in the ASAQ group and 0.5 in the AL). Logistic regression analysis showed that recrudescent infection (aOR: 12.9[1.1-14.9]) were independent risk factors for SMG carriage whereas no association was found with the type of treatment, age and number of episode. The frequency of SMG carriage is high after ACT treatment whatever the combination used. A strong association between the presence of gametocytes and a recurrent infection is also observed.

1474

LACK OF SIGNIFICANT PHARMACOKINETIC INTERACTIONS BETWEEN PIPERAQUINE AND NEVIRAPINE- OR EFAVIRENZ-CONTAINING ANTIRETROVIRAL REGIMENS IN *PLASMODIUM FALCIPARUM* NEGATIVE HIV-INFECTED MALAWIAN ADULTS STABILIZED ON HAART

Victor Mwapasa[1], Fraction Dzinjalamala[1], Jane Mallewa[1], Mavuto Mukaka[1], Saye Khoo[2], Robert Heyderman[3], David Lalloo[4]

[1]Malawi College of Medicine, Blantyre, Malawi, [2]University of Liverpool, Liverpool, United Kingdom, [3]Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi, [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

In sub-Saharan Africa (SSA), most HIV-infected (HIV+) individuals on antiretroviral therapy (ART) are exposed to malaria. Currently, Dihydroartemisinin-piperaquine (DPQ) is being rolled out in SSA but no studies have examined the pharmacokinetics (PK) and safety of DPQ in HIV+ individuals taking ART containing Nevirapine (NVP) or Efavirenz (EFV). We conducted an open label clinical trial to compare the maximum concentration (Cmax) and area under concentration-time curve (AUC) of piperaquine (PQ) in antiretroviral naive HIV+ individuals and those taking NVP and EFV-based ART. In step 1 of the trial, malaria uninfected adults (n=6/ART group) received half the standard dose of DPQ (2 tablets of 40/320mg each for participants) at times 0, 24 and 48hrs. In Step 2, another group of malaria uninfected adults (n=15/ART group) received a standard dose of DPQ (4 tablets of 40/320mg each). Data-rich PK blood sampling were performed at the following times after dosing; 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48, 60, 72, 96, 120, 144, 168, 336, 504 and 672 hrs. PQ levels were measured using HPLC-UV assays. We also assessed treatment emergent hematological and biochemistry abnormalities. The baseline demographic characteristics and CD4 cell counts were similar across the three groups. In step 1, compared with the ART-naïve group, there was a non-significant trend towards higher PQ AUC in the NVP-ART group and lower PQ AUC in the EFV-ART group. Similarly in Step 2, median PQ AUC was non-significantly lower in the EFV-ART group (15 µg/mL.hr, range: 2.6-25.6) than the ART-naïve group (22.6 µg/mL.hr: range: 11-37, p=0.052). The median PQ AUC in the NVP-ART group (29.9 µg/mL.hr, range: 14.2-80.9) was similar to the ART-naïve group (p>0.16). Cmax for PQ was similar across the three groups. In step 2, there were transient cases of grade 1 or 2 transaminitis in the ART-naïve arm and treatment-emergent grade 3 or 4 neutropenic episodes across the study arms. However, these abnormalities were not clinically significant nor persistent. Thus, there are limited PK interactions between DPQ and EFV or NVP-based ART.

1475

OVER-TREATMENT WITH ACTS AND FALSE ANTIMALARIAL DRUG HISTORY DETECTED THROUGH SERUM DRUG CONCENTRATION STUDIES

Akinwumi A. Akinyede[1], Peter Ojobor[2], Aderonke Ayanniyi[2], Olufunsho Awodele[2], Austin Adaje[2], Patrick Orumbie[3]

[1]University of Lagos & Babcock University, Lagos/Illishan-Remo, Nigeria, [2]University of Lagos, Lagos, Nigeria, [3]Lagos University Teaching Hospital, Lagos, Nigeria

Artemisinin Combination Therapies (ACTs) were recommended as first line therapy for patients with uncomplicated malaria fever in regions where chloroquine resistant strains of *Plasmodium falciparum* were found in the last 10 years. However, there is an emerging trend such that doctors' prescriptions contain more of chloroquine and other antimalarial agents either as monotherapy or in combination with ACTs forming triple therapies because physicians make presumptive diagnosis of resistance to ACTs. Antimalarial drug use histories of 18 adults with clinical diagnosis of uncomplicated malaria were taken in the staff clinic of the Lagos University Teaching Hospital, Nigeria. Blood samples were also tested for malaria parasitaemia and artemether/lumifantrine concentration on Day 0(pre-treatment) and Day 4 (day after completion of treatment).All patients declined the use of any antimalarial (including ACTs) during the 2 week period preceding the study and were malaria parasite negative on Days 0 and 4. Artemether was not detectable in the blood samples taken on Days 0 and 4. However, lumefantrine was detected in all blood samples taken on Days 0 and 4. The mean concentration of lumefantrine on Days 0 and 4 were 330.0µg/l±33.77 (SEM) and 349.7µg/l±18.39(SEM) respectively, these values were not significantly varied p> 0.05.This study exposed the wide spread use of artemether/lumefantrine among this group of patients before presentation at the clinic and underscores the need for confirmation of malaria parasitaemia before drug treatment. The patients' drug use history is also unreliable. Our findings may be a pointer to the fact that presumptive diagnosis of malaria resistant to ACTs should be halted even in malaria endemic regions such as Nigeria, these patients should have their blood tested for malaria parasites and blood drug concentration.

1476

HSP90, A POTENTIAL DRUG TARGET AGAINST *PLASMODIUM FALCIPARUM*

Claribel Murillo Solano, Shantanu Guha, Juan C. Pizarro

Tulane University, New Orleans, LA, United States

The prevalence of drug resistance represents a major threat against current efforts to control malaria. Therefore, the development of new drugs or the identification of new drug targets against *Plasmodium* is a priority to reduce the impact of malaria. Towards that end, we evaluated the *in vitro* effect against *P. falciparum* of available inhibitors against its heat shock protein 90 (HSP90); this chaperone is a key component of the parasite stress response and protein folding machinery. We determined the EC50 for several chaperone inhibitors *in vitro* with a fluorescent-based assay against two *P. falciparum* reference strains 3D7 and W2. The same method was used to determine their anti-Plasmodial effect in combination with current anti-malarial drugs. Moreover, the cytocidal activities of these compounds were evaluated in long term cultures following a bolus dosage exposure. The tested compounds were highly active against the malaria parasites with EC50 values between 10-7 to 10-5 M, and some of the compound-drug combinations displayed synergistic interactions inhibiting parasite growth. In parallel, we have cloned all the four genes coding for Hsp90 family members from *P. falciparum* and generated constructs to express the protein chaperones in bacteria. The recombinant proteins have been used to assay the inhibitors specificity in biochemical assays, aimed at determine their mechanism of action. The recombinant chaperones were screened against additional compound libraries to identify new compounds with potential anti-plasmodial activity. Our preliminary results, lead us to conclude that the *P. falciparum* Hsp90 chaperones is an appealing new drug target to combat malaria.

1477

EVALUATION OF ARTEMETHER PLUS LUMEFANTRINE TREATMENT FAILURES IN WEST AFRICA

Daouda Ndiaye[1], Davis Nwakanma[2], Baba Dieye[1], Yaya Ndiaye[1], Lansana Sangare[3], Fatou Joof[2], Clarissa Valim[4], Sarah K. Volkman[4], Dyann F. Wirth[4], Donald J. Krogstad[5]

[1]University Cheikh Anta Diop, Dakar, Senegal, [2]MRC Unit, Fajara, Gambia, [3]University of Bamako, Bamako, Mali, [4]Harvard School of Public Health, Boston, MA, United States, [5]Tulane University Health Sciences Center, New Orleans, LA, United States

Malaria caused by *Plasmodium falciparum* is a serious concern for public health and development in sub-Saharan Africa. To address these problems, a number of African countries have adopted artemisinin-based combination therapies (ACTs) as their first-line treatment for uncomplicated malaria. We have examined the effectiveness of Coartem for uncomplicated *Plasmodium falciparum* malaria in Gambissara in The Gambia, Dioro in Mali and Thiès in Senegal. These studies have enrolled participants 2-20 years of age with 2,000 to 199,999 asexual parasites per µl of blood who had no evidence of severe or complicated malaria. Primary endpoints include asexual parasite counts <25% of baseline by day 3, clearance of asexual parasites by day 7 and the absence of recurrent infection between days 8 and 42. Secondary endpoints included asexual parasite clearance times, *ex vivo* determinations of susceptibility and resistance to individual antimalarials; testing for drug resistance markers and for presumptively neutral markers (SNPs). From September 2011 to February 2013, we performed an open enrollment, multicenter study of the standard 3 day course of artemether + lumefantrine (AL) for uncomplicated *Plasmodium falciparum* malaria according to World Health Organization (WHO) guidelines. Follow-up visits were performed on days 1, 2, 3, 7, 14, 21, 28, 35 and 42 to evaluate clinical and parasitological results. These studies have now enrolled 328 subjects with uncomplicated *P. falciparum* malaria, who have been treated with arthemeter plus lumefantrine and followed for recurrent infection or other evidence of treatment failure. Of the 328 subjects enrolled, 19 have been lost to follow-up and 13 have developed recurrent infections between days 8 and 42. However, there have been no early treatment failures (on or before day 7). Twelve of the 13 subjects with recurrent infections had parasites at the time of recurrence with different genetic markers (using the SNP-based barcode). However, 1 subject had parasites with similar markers at the times of diagnosis and recurrence together with delayed parasite clearance on day 3. The isolates from this patient also had IC50s above the mean values for both artemether and lumefantrine. Apart from that subject, the results obtained thus far provide no evidence for artemisinin or Coartem resistance at the community level in The Gambia, Mali or Senegal.

1478

INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY WITH MEFLOQUINE IN HIV-INFECTED WOMEN RECEIVING COTRIMOXAZOLE PROPHYLAXIS: A MULTICENTER RANDOMIZED PLACEBO-CONTROLLED TRIAL

Raquel Gonzalez[1], Meghna Desai[2], Eusebio Macete[3], Peter Ouma[2], Mwaka Kakolwa[4], Salim Abdulla[4], John Aponte[1], Helder Bulo[3], Abdunoor M. Kabanywanyi[4], Abraham Katana[2], Sonia Maculuve[3], Alfredo Mayor[1], Arsenio Nhacolo[3], Kephas Otieno[2], Golbahar Pahlavan[1], María Rupérez[1], Esperança Sevene[3], Laurence Slutsker[5], Anifa Vala[3], John Williamsom[2], Clara Menéndez[1]

[1]Barcelona Centre for International Health Research (CRESIB), Barcelona, Spain, [2]Kenya Medical Research Institute/Centers for Disease Control and Prevention Research and Public Health Collaboration, Kisumu, Kenya, [3]Manhiça Health Research Center (CISM), Manhiça, Maputo, Mozambique, [4]Ifakara Health Institute, Dodoma, Dar Es Salaam, United Republic of Tanzania, [5]Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States

Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) is recommended for malaria prevention in HIV-negative pregnant women, but it is contraindicated in HIV-infected women due to potential interactions with cotrimoxazole prophylaxis (CTXp). We studied the safety and efficacy of mefloquine (MQ) in women receiving CTXp and long-lasting insecticide treated nets (LLITNs). A total of 1071 HIV-infected women from Kenya, Mozambique and Tanzania were randomized to receive either three doses of IPTp-MQ (15 mg/kg) or placebo given at least one month apart; all received CTXp and a LLITN. IPTp-MQ was associated with nearly halved maternal parasitemia (RR, 0.51 [95%CI, 0.29; 0.90]; p=0.021), and placental infection (RR, 0.53 [95%CI, 0.30; 0.93]; p=0.028), and reduced incidence of all-cause and non-obstetric hospital admissions (RR, 0.65 [0.41.; 1.03]; p=0.065; and RR, 0.59 [0.37; 0.95]; p=0.031; respectively). There were no differences in the prevalence of adverse pregnancy outcomes between groups. Drug tolerability was poorer in the MQ group compared to the control group (29.6% referred dizziness and 23.9% vomiting after the first IPTp-MQ administration). HIV viral load at delivery was higher in the MQ group compared to the control group (p=0.048). The rate of perinatal mother to child transmission (MTCT) of HIV was increased in women who received MQ (RR, 1.95 [95%CI 1.12;3.39]; p=0.018). An effective antimalarial added to CTXp and LLITNs in HIV-infected pregnant women can improve malaria prevention and maternal health through reduction in hospital admissions. The translation of this information into policy actions that reduce malaria in this particularly vulnerable group should be prioritized. However, MQ was not well tolerated, limiting its potential for IPTp and indicating the need to find alternatives. MQ was associated with an increased risk of MTCT of HIV, which warrants a better understanding of the pharmacological interactions between antimalarials and antiretroviral drugs.

1479

SIMULATIONS TO INVESTIGATE NEW INTERMITTENT PREVENTIVE THERAPY DOSING REGIMENS FOR DIHYDROARTEMISININ-PIPERAQUINE

Jesmin P. Lohy Das

Uppsala University, Uppsala, Sweden

A fixed-dose combination of dihydroartemisinin (DHA) and piperaquine (PQ) with monthly dosing has been suggested as a new promising alternative for Intermittent Preventive Therapy (IPT). Alternative dosing regimens for DHA-PQ was explored based on simulations with a previously developed in silico model describing the concentration-effect relationship for the malaria preventive effect. The model was developed in application to placebo controlled monthly versus bimonthly dosing regimen study of 1000 healthy male subjects in Northern Thailand. The simulations compared the clinically investigated monthly dosing regimen (120 mg DHA, 960 mg PQ dosing on three consecutive days repeated every month) to novel dosing regimens (120 mg DHA, 960 mg PQ once weekly). The usefulness of initial loading doses and robustness towards different levels of compliance was investigated for both weekly and monthly regimens. Among placebo recipient, the predicted yearly malaria incidence was 52%. In perfect compliance, the annual malaria incidence was less than 1% for weekly dosing compared to approximately 3% for the once monthly dosing regimen. Under the assumption of poor treatment compliance (60%), the weekly dosing of initial 3 day loading dose was predicted to contain the incidence below 3% compared to >15% for any monthly loading dose strategy in a year. Clinical trial simulations were applied to investigate the necessary sample size to confirm the predicted advantage with weekly compared to monthly dosing if a study was to be carried out under similar conditions as the original study. A samples size of 966 subjects (483+483) was needed to have 80% power to demonstrate a statistically significant benefit of weekly dosing over monthly dosing in a 9 months clinical trial. To have the same power to demonstrate non-inferiority (25% margin) a sample size of 684 subjects (342+342) was needed.

1480

PREGNANCY LOWERS THE EXPOSURE OF DIHYDROARTEMISININ: WHAT IS NEXT, INCREASE THE DOSE OR EXTEND THE TREATMENT?

Frank L. Kloprogge, Joel Tarning

Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand

Our work, comprising paired comparisons as well as literature comparisons between pregnant and non-pregnant women, has shown that the exposure of dihydroartemisinin is decreased during pregnancy after oral administration of dihydroartemisinin, artesunate and artemether. This is worrisome as these drugs are the first line treatment during the second and third trimester of pregnancy in many countries and decreased exposures can result in therapeutic failures and an accelerated development of resistance. The aim of this study was to evaluate the pharmacokinetics and pharmacodynamics of the artemisinin drugs in the treatment of uncomplicated malaria in pregnant women and investigate different optimised dose regimens. In-silico Monte-Carlo simulations, based on the pharmacokinetic exposures and pharmacodynamic parasite reduction, were used to evaluate the effect of a dose increase and treatment extension for oral administration of dihydroartemisinin, artesunate and artemether in pregnant women with uncomplicated *Plasmodium falciparum* malaria. Simulations indicated that it was possible to achieve similar dihydroartemisinin exposures in pregnant women compared to non-pregnant patients after an increased dose. However, this would also result in higher peak concentrations which may result in toxic side effects. An extended treatment could compensate for the lower dihydroartemisinin exposures during pregnancy without this increase in peak levels, but it may also result in lower adherence. This study suggests an optimised dose regimen for pregnant women with uncomplicated *P. falciparum* malaria. New pharmacokinetic studies evaluating the suggested dose optimisations are needed to enable an evidence-based dose optimisation.

1481

PKPD RELATIONSHIPS BETWEEN PLASMA PIPERAQUINE LEVELS AND CARDIAC QTC PROLONGATION IN MALARIA PATIENTS ADMINISTERED DIHYDROARTEMISININ-PIPERAQUINE IN CAMBODIA SUGGEST A CONVENTIONAL 3-DAY REGIMEN IS SAFER THAN A COMPRESSED 2-DAY REGIMEN

Pattaraporn Vanachayangkul[1], Jessica Manning[1], Chanthap Lon[1], Michele Spring[1], Mary So[2], Sok Sommethy[2], Sut-Thang Pann[3], Soklyda Chann[1], Mali Ittiverakul[1], Sabaithip Sriwichai[1], Nillawan Buathong[1], Worachet Kuntawunginn[1], Winita Ta-aksorn[1], Paktiya Teja-Isavadharm[1], Eugene Soh[4], Charlotte Lanteri[1], Jaranit Kaewkungwal[5], Douglas Tang[6], Mark Haigney[4], Louis Cantelena[4], David Saunders[1]

[1]Department of Immunology and Medicine, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Royal Cambodian Armed Forces, Phnom Penh, Cambodia, [3]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [4]F. Edward Hebert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [5]Center for Excellence in Bioinformatics (BIOPHIC), Mahidol University, Bangkok, Thailand, [6]Fast Track Biologics, Bethesda, MD, United States

Dihydroartemisinin-piperaquine (DP), presently the firstline therapy for uncomplicated *Plasmodium falciparum* and *P. vivax* malaria in Cambodia, is widely used as a standard 3-day dosing regimen (360 mg dihydroartemisinin & 2880 mg piperaquine). However, piperaquine can prolong the QTc interval, resulting in cardiotoxicity that is undetected in countries like Cambodia lacking electrocardiograms. We conducted 3 clinical studies to explore the cardiotoxicity risk of administering DP for malaria treatment and prevention in Cambodia. Comparison of the 3-day versus a compressed 2-day DP regimen (total dose equivalent to 3-day DP), the latter used by the Cambodian military for malaria treatment, revealed both regimens had similar efficacy with mild correlation of plasma piperaquine-QTc prolongation. In a follow-on randomized, double-blind, placebo-controlled study evaluating 2-day DP as a monthly malaria prevention therapy, the trial was halted after 4 out of 69 volunteers met a pre-specified safety endpoint of >500 ms QTcF prolongation. Two-day DP had moderate correlation of plasma piperaquine with QTc prolongation (spearman rho = 0.6706, p-value < 0.0001), with strong correlation in the 4 halted volunteers (spearman ρ = 0.8990, p-value < 0.0001). In an ongoing 3-day DP treatment trial, we observe greater treatment failures and piperaquine IC_{50}s relative to our treatment study conducted 3 years prior, and note mild correlation of piperaquine-QTc prolongation (spearman Rho = 0.3954, p-value < 0.0001). A significant correlation between piperaquine-QTc prolongation was observed in a larger proportion of volunteers given 2-day DP (36 out of 47, 76.6%) relative to 3-day DP (13 out of 50, 26%). Mean plasma piperaquine C_{max} at 4 hours post-1st dose of 2-day DP (633.6 ng/ml) was significantly higher than for 3-day DP (127.3 ng/ml). Mean QTcF after 4 hr post-1st dose of 2-day DP (440.9 ms) was also significantly higher than 3-day DP (405.1 ms). Our findings suggest risk for cardiotoxicity can be mitigated by using 3-day DP, rather than a compressed regimen, with additional precautions of fasting and avoiding co-administration of other QT-prolonging medications.

1482

DEVELOPMENT OF PEDIATRIC FORMULATION FOR TREATMENT OF *PLASMODIUM FALCIPARUM* MALARIA: COARTEM® (ARTEMETHER-LUMEFANTRINE) DISPERSIBLE

Quique Bassat[1], Salim Abdulla[2], Bernhards Ogutu[3], Abdoulaye DJimde[4], Kirstin Stricker[5], Kamal Hamed[6], Heiner Grueninger[5]

[1]Barcelona Centre for International Health Research, Barcelona, Spain, [2]Ifakara Health Institute, Dar-es-Salaam, United Republic of Tanzania, [3]Walter Reed Project-Center for Clinical Research, Kenya Medical Research Institute, Nairobi, Kenya, [4]Malaria Research and Training Center, University of Bamako, Bamako, Mali, [5]Novartis Pharma AG, Basel, Switzerland, [6]Novartis Pharmaceuticals Corporation, East Hanover, NJ, United States

Pediatric artemisinin-based combination therapy formulations have the potential to improve effectiveness and accuracy of dosing in young children. Coartem® (artemeter-lumefantrine; AL) Dispersible was developed in partnership with the Medicines for Malaria Venture for the treatment of uncomplicated *Plasmodium falciparum* malaria and is the first pediatric antimalarial to receive Swissmedic approval and meet WHO specifications for use in infants and children ≥5 kg. Search using PubMed, Ovid and clinical trial registry databases for AL dispersible in children revealed 6 original and 5 review articles involving 674 infants/children. In a palatability study, sweet tasting cherry was the preferred flavor by children for AL dispersible. Pharmacokinetic profile of AL dispersible was comparable to AL crushed tablets. Efficacy and safety of dispersible formulation versus crushed tablet was evaluated in a large, randomized, multicenter study in 5 sub-Saharan African countries. Efficacy and acceptability of AL dispersible were also compared to dihydroartemisinin-piperaquine (DP) pediatric in an open-label, randomized study in Kenya. A total of 674 children were randomized in both studies to receive AL dispersible with mean age 38.5 months, body temperature 38.2°C and parasite density 38,202-53,921/µl. 28- and 42-day PCR-corrected cure rates were 97.8% and 96.4%; similar to AL crushed tablets and DP in respective studies. Acceptability of AL dispersible was significantly better than DP pediatric (ease of use: p=0.007; taste of medicine: p=0.001). 28-day PCR-corrected cure rate was not related to food intake; however, consumption of milk/low fat meal increased lumefantrine bioavailability compared to no food. Efficacy of AL dispersible was comparable in children with different body weights. Median parasite and fever clearance times were 34.3 and 7.9 hours (n=447). Safety profile of AL dispersible was comparable to crushed tablets. AL dispersible was specifically tailored for the pediatric population and offers a convenient formulation with efficacy and safety similar to that of standard crushed AL tablets.

1483

EFFICACY, SAFETY AND POPULATION PHARMACOKINETICS OF THE ARTESUNATE MEFLOQUINE (ASMQ) FIXED DOSE COMBINATION VERSUS ARTEMETHER LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN AFRICAN CHILDREN

Sodiomon Sirima[1], Bernhards Ogutu[2], John Lusingu[3], Ali Mtoro[4], Zakayo Mrango[5], Alphonse Ouedraogo[1], Jean Baptiste Yaro[1], Kevin Omondi Onyango[6], Samwel Gesase[3], James Samwel Ngocho[5], Joelle Vanraes[7], Nathalie Strub[7], Gwenaelle Carn[8]

[1]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [2]The Kenya Medical Research Institute, Kisumu, Kenya, [3]National Institute for Medical Research, Korogwe, United Republic of Tanzania, [4]Ifakara Health Institute, Bagamayo, United Republic of Tanzania, [5]National Institute for Medical Research, Kilosa, United Republic of Tanzania, [6]The Centre for Clinical Research - Kenya Medical Research Institute, Nairobi, Kenya, [7]Drugs for Neglected Diseases initiative, Geneva, Switzerland, [8]Centre National de Recherche et de Formation sur le Paludisme, Geneva, Switzerland

Artemisinin-based combination therapies (ACTs) are recommended by WHO to treat uncomplicated *Plasmodium falciparum* malaria. Artesunate

(AS) and Mefloquine (MQ), in loose or fixed-dose combination (ASMQ), was the first ACT used extensively in Asia and Latin America but there is very limited data in Africa. Our objective was to evaluate the efficacy and safety of ASMQ in comparison with the standard of care Artemether-Lumefantrine (AL), and to study the population-pharmacokinetics (PK) in African children.The clinical trial was conducted in children aged from 6 months to 5 years in Burkina Faso, Kenya and Tanzania. Febrile children with *P. falciparum* density between 2,000 and 200,000 asexual parasites/µl were randomized to receive (a) ASMQ for 3 days [6 to 11 months old: one 25mg/55 mg tablet once daily (OD); 12 to 59 months old: two tablets OD], or (b) AL for 3 days [children 5-15 Kg: one 20mg/120mg tablet BID; 15-25 Kg: two tablets BID]. All children were followed for 60 days after treatment period. The primary efficacy outcome is the cure rate based on the PCR-adjusted results by Day 63. Cure rates at 28 and 42 days are also evaluated. Patients with parasitaemia during the follow-up period were switched to the other treatment arm and followed for a further 60 days or until second recurrence. Safety was assessed during the first follow-up period (up to Day 63) and during the second one if recurrence occurred. 945 patients were randomised by June 2013. Under blinded conditions the overall safety profile did not reveal any unexpected signals. The data base lock is expected mid 2014. The population PK results of ASMQ were presented in 2013, and showed a large inter-patient variability in children; clearance and volume of distribution of MQ in children is lower than in adult patients but the terminal elimination half-life and mean absorption time are of similar magnitude.

1484

A PHASE III, RANDOMIZED, OPEN LABELLED, ACTIVE CONTROLLED, MULTI CENTER, SUPERIORITY TRIAL OF ARTIMIST™ VERSUS INTRAVENOUS QUININE IN CHILDREN WITH SEVERE OR COMPLICATED FALCIPARUM MALARIA, OR UNCOMPLICATED FALCIPARUM MALARIA WITH GASTROINTESTINAL COMPLICATIONS

Stephen Rulisa[1], Patrick Ansah[2], Sirima, Ouagadougou, Burkina Faso B. Sodiomon[3]

[1]Kigali University Teaching Hospital, Kigali, Rwanda, [2]Navrongo, Navrongo, Ghana, [3]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso

Malaria is a serious, infectious disease. About half the world's population (3.3bn) live in areas that have some risk of malaria transmission, 36.4% (1.2bn) of which are living in regions considered at high risk The Phase III trial was carried out in malaria endemic areas of Rwanda, Burkina Faso and Ghana over a 22-month period from November 2010 to September 2012 151 subjects were randomised and enrolled. A total of 151 subjects were analysed in the Safety Analysis Population, 141 subjects in the Modified Intention to Treat (MITT) Population, and 137 subjects in the Per Protocol (PP) Population The study's primary objective was to demonstrate that sub lingual (under the tongue) ArTiMist™ was superior to IV quinine in reduction of the parasite counts by >90% within 24 hours in children with severe or complicated falciparum malaria, or uncomplicated falciparum malaria with gastrointestinal complications. The primary objective for this study showed that ArTiMist™ demonstrated superiority over iv quinine in both efficacy populations. For the MITT population 66 of the 70 subjects (94.3%) treated with ArTiMist™ and 28 of the 71 subjects (39.4%) treated with quinine had parasitological success. The absolute difference (95% CI) between treatments, without correcting for the factor site, was 54.85 (42.25 - 67.45) % which was statistically significant ($p < 0.0001$). For the PP population 65 of the 68 subjects (95.6%) treated with ArTiMist™ and 28 of the 69 subjects (40.6%) treated with quinine had parasitological success. The absolute difference (95% CI) between treatments, without correcting for the factor site, was 55.01 (42.44 - 67.58) % which was statistically significant ($p < 0.0001$). Following sublingual administration of ArTiMist™, absorption is rapid with mean Cmax following the first dose reaching 333.2 ng/mL and 83.3 ng/mL in 1.0 h and 1.5 h for artemether and dihydroartemisinin (DHA), respectively. In conclusion, sub-lingual ArTiMist™ was superior to IV quinine, demonstrating significantly faster parasite killing and fewer early treatment failures. PK analysis demonstrated that ArTiMist™ was rapidly absorbed in children with severe or complicated falciparum malaria, or children with uncomplicated malaria with gastrointestinal complications

1485

INHALED NITRIC OXIDE FOR THE ADJUNCTIVE TREATMENT OF SEVERE MALARIA: A RANDOMIZED CONTROLLED TRIAL

Michael Hawkes[1], Andrea Conroy[2], Laura Hermann[2], Chloe McDonald[2], Hani Kim[2], Sarah Higgins[2], Sophie Namasopo[3], Chandy John[4], Christopher Miller[5], W. Conrad Liles[6], Robert Opoka[7], Kevin Kain[2]

[1]University of Alberta, Edmonton, AB, Canada, [2]University of Toronto, Toronto, ON, Canada, [3]Jinja Regional Referral Hospital, Jinja, Uganda, [4]University of Minnesota, Minneapolis, MN, United States, [5]University of British Columbia, Vancouver, BC, Canada, [6]University of Washington, Seattle, WA, United States, [7]Makerere University, Kampala, Uganda

Severe malaria remains a major cause of childhood mortality globally. Exogenous inhaled nitric oxide (iNO) reduces endothelial activation, protects the blood-brain barrier, and improves survival in pre-clinical studies of experimental cerebral malaria. We conducted a randomized, blinded, parallel-arm, controlled trial of iNO at 80 ppm by non-rebreather mask vs room air placebo as adjunctive treatment in children (age 1 to 10 years) with severe malaria. Blinding of trial clinicians, nurses, parents, children, laboratory technicians and statistician was achieved by using room air placebo, also administered by mask by a dedicated and unblinded team that monitored dose-dependent adverse effects (methemoglobinemia) but did not participate in clinical care. The primary outcome was the rate of improvement in angiopoietin-2 levels (a biomarker of malaria severity and convalescence). 180 children were enrolled; 88 were assigned to nitric oxide and 92 to placebo (all received IV artesunate). The median [IQR] rate of change of Ang-2 over the first 72 hours of hospitalization was similar between groups: -2.2 [-3.1 to -1.2] ng/mL/day in the iNO group vs -1.9 [-3.7 to -0.56] ng/mL/day in the placebo group; p=0.68). The mortality at 48 hours was similar between groups (6/87 [6.9%] in the iNO group vs 8/92 [8.7%] in the placebo group; OR 0.78, 95% CI 0.26-2.3; p=0.65). Methemoglobinemia (>10%) was higher in the iNO group (5/88 [5.7%] vs 0/92 [0%]; p=0.026). Incidence of neurologic sequelae (<14 days), acute kidney injury, hypoglycemia, anemia and hemoglobinuria were similar between groups (p>0.05 for all comparisons). Clinical recovery times (time to eat, sit, localize pain, fever resolution, recovery of consciousness, and hospital discharge) were similar between group (p>0.05 for all comparisons). Parasites cleared quickly in both groups, with no difference in parasite clearance kinetics (p>0.05). No patient in either group had recrudescence of patent parasitemia at day 14 of follow-up. Inhaled nitric oxide at 80 ppm administered by non-rebreather mask was safe but did not accelerate endothelial stabilization, as reflected by circulating levels of Ang-2, in children with severe malaria. Alternative methods of delivering NO to the endothelium (e.g., higher dose, donor molecules, routes of administration) may be necessary to achieve a more potent biological effect and an impact on clinical outcomes.

1486

ALLOMETRIC SCALING OF PYRONARIDINE PHARMACOKINETIC PARAMETERS IN PEDIATRIC MALARIA PATIENTS

Amal Ayyoub[1], Janthima Methaneethorn[1], Stephan Duparc[2], Isabelle Borghini-Fuhrer[2], Jang-Sik Shin[3], Lawrence Fleckenstein[1]

[1]University of Iowa, College of Pharmacy, Iowa City, IA, United States, [2]Medicines for Malaria Venture, Geneva, Switzerland, [3]Shin Poong Pharmaceuticals, Seoul, Republic of Korea

Pyramax® is a pyronaridine/artesunate combination for the treatment of uncomplicated malaria in adult and pediatric patients. A granule formulation of this combination is being developed for treatment of

uncomplicated *Plasmodium falciparum* and *P. vivax* malaria in pediatric patients. The population pharmacokinetics of pyronaridine (PYR) were evaluated in pediatric malaria-infected patients participating in six Pyramax® clinical trials. A total of 1085 blood PYR concentrations were available from 349 malaria patients younger than 16 years of age with mild to moderate uncomplicated malaria. Blood PYR concentrations were measured using a validated LC-MS method. Non-linear mixed effects modeling was used to obtain the pharmacokinetic and variability parameter estimates. PYR concentrations were well described by a two-compartment model with first order absorption and elimination. Allometric scaling was implemented to address the effect of body weight on clearance and volume parameters. The final parameter estimates of PYR apparent clearance (CL/F), central volume of distribution (V2/F), peripheral volume of distribution (V3/F), inter-compartmental clearance (Q/F) and absorption rate constant (Ka) were 377 L/day, 2230 L, 3230 L, 804 L/day and 17.9 day-1, respectively. The corresponding percent coefficient of variation of inter-individual variability for CL/F, V2/F, V3/F and Ka were 40.7%, 99.6%, 50.6% and 65.8%, respectively. Covariate model building conducted using forward addition ($p<0.05$) followed by backward elimination ($P<0.001$) yielded two significant covariate-parameter relationships: age on V2/F and formulation on Ka. Evaluation of bootstrapping, visual predictive check, and condition number indicated that the final model displayed satisfactory robustness, predictive power, and stability. Simulations of PYR concentration-time profiles generated from the final model show similar exposures across pediatric weight ranges, supporting the proposed labeling for weight-based dosing of Pyramax® granules.

1487

EFFECTS OF BREATHING NITRIC OXIDE AS AN ADJUNCTIVE TREATMENT FOR CHILDREN WITH CEREBRAL MALARIA

Juliet Mwanga-Amumpaire*[1], Ryan W. Carroll[2], Elisabeth Baudin[3], Elisabeth Kemigisha[1], Dan Nyehangane[1], Pierre De Beaudrap[4], Jean-François Etard[3], David Bangsberg[2], Kenneth Bloch[2], Yap Boum II[1], Warren Zapol[2]

[1]Epicentre Research Base of Mbarara/Mbarara University of Science and Technology, Mbarara, Uganda, [2]Massachusetts General Hospital/Harvard Medical School, Boston, MA, United States, [3]Epicentre, Paris, France, [4]Epicentre Research Base of Mbarara, Mbarara, Uganda

Children with cerebral malaria (CM) have reduced plasma and urine levels of NO biometabolites. Two studies have reported breathing NO reduces mortality, inflammation, and CNS pathology in *Plasmodium berghei*-infected mice. Therefore, from Sept 2011 to February 2014, we completed a phase-II open-label clinical trial assessing the efficacy and safety of inhaled NO (INO) as an adjunctive treatment for cerebral malaria in pediatric patients. A total of 92 children, aged 3 months – 9 years, with CM were enrolled in the study at the Mbarara Regional Referral Hospital in Uganda. Patients were randomly assigned to receive either inhaled NO (INO), or nitrogen (N_2) via nasal cannula (INOPulse, TM, Ikaria, USA) for at least 24 hours. All patients received IV artesunate and were monitored continuously for changes in metHb% levels. The primary endpoint was the change in plasma Angiopoietin-1 (Ang-1) over 48 hours. Plasma Ang-1 levels increased over 48 hours in both treatment groups, but there was no difference between the groups. There was a decrease in plasma angiopoietin-2 and plasma cytokine levels (TNF-α, IFN-γ, IL-1β, IL-6, IL-10, and MCP-1) over 48 hours in both study arms, but no significant difference between the treatment groups. Total mortality was 12.0%. Seven (15.2%) patients died in the N_2 group, and 4 (8.7%) patients died in the INO group. Five patients in the N_2 group and 6 in the INO group had developed neurological sequelae by the time of discharge. For patients who received INO, the average hourly dose of INO delivered over the initial 48 hours was 1.13 ± 0.30 mg/kg/hr (N=39, mean $\pm$ SD). There was no difference in the baseline metHb% levels among patients in both study arms. There was an increase in metHb% in patients treated with INO, up to 4.1 ± 2.3% (N=33, mean $\pm$ SD) at 12 hours, which remained at safe elevated levels up to 72 hours. MetHb levels were unchanged throughout the treatment period in the N_2 group. This pilot trial of INO as an adjuvant therapy for CM demonstrates the safety and feasibility of delivering INO in a low-resource setting but there was no statistically significant evidence for efficacy.

1488

EXPERIMENTAL VIVAX TRANSMISSION TO *ANOPHELES* (EVITA), A CLINICAL TRIAL TO ASSESS MOSQUITO TRANSMISSIBILITY IN PARTICIPANTS INOCULATED WITH BLOOD STAGE *PLASMODIUM VIVAX*

Paul Griffin[1], Suzanne Elliott[2], Silvana Sekuloski[1], Cielo Pasay[1], Maggy Sikulu[1], Leon Hugo[1], Tom Churcher[3], Jetsumon Prachumsri[4], Ivinson Karen[5], Chris Ockenhouse[5], James McCarthy[1]

[1]Queensland Institute of Medical Research, Brisbane, Australia, [2]Qpharm Pty Ltd, Brisbane, Australia, [3]Imperial College London, London, United Kingdom, [4]Mahidol University, Bangkok, Thailand, [5]PATH, Malaria Vaccine Initiative, Washington, DC, United States

Interventions to interrupt transmission of malaria from humans to mosquitoes, including vaccines, represent appealing approaches to assist its elimination. A limitation has been the lack of a methodology to reliably test the efficacy of such interventions before proceeding to clinical efficacy trials in the field. Building on our work demonstrating the feasibility of induced blood stage *Plasmodium vivax* infection, we have undertaken a study to evaluate transmission to *Anopheles stephensi* mosquitoes. Study endpoints included the presence of the gametocyte-specific transcript *pvs25* in the blood of volunteers by qRT-PCR, and mosquito infection by midgut dissection for oocyst visualisation. The study design entailed 3 cohorts of 2 volunteers, each inoculated on Day 0 with approximately 100 viable *P. vivax*-infected human erythrocytes administered intravenously. On the three to four days up to the anticipated commencement of treatment (approximately day 11, 12, 13 and 14), transmission studies were undertaken by membrane feeding assays and direct feeds on volunteers with 30 mosquitoes per session. At the time of abstract submission, 2 of the 3 cohorts have been completed. No significant adverse events were observed. All subjects experienced mild to moderate symptoms of malaria consistent with previously published data. Direct mosquito feeding was well tolerated with all volunteers reporting mild to moderate local reactions and pruritis, easily controlled with symptomatic treatment. Elevated liver function tests were observed in 3 of the 4 volunteers in the form of asymptomatic elevations in both ALT and AST. However, this completely resolved in all subjects. Gametocytaemia detected by a positive pvs25 RT-PCR was observed in all subjects. Mosquito infection was detected by midgut dissection following both direct and indirect feeding assays. The demonstration of the feasibility of this system to test transmission-blocking interventions represents a promising development for future efficacy studies.

1489

SAFETY AND REPRODUCIBILITY OF AN INDUCED BLOOD STAGE MALARIA CHALLENGE FOR EXPEDITED TESTING OF ANTIMALARIAL TREATMENT AND VACCINES

Paul Griffin[1], Silvana Sekuloski[1], Suzanne Elliott[2], Cliodna NiBuachalla[2], Casey Rowe[2], David Khoury[3], Deborah Cromer[3], Miles Davenport[3], James McCarthy[1]

[1]Queensland Institute of Medical Research, Brisbane, Australia, [2]Qpharm Pty Ltd, Brisbane, Australia, [3]The University of New South Wales, Sydney, Australia

Despite recent progress in malaria control there is concern that momentum is slowing and new challenges are emerging, including the development of drug resistance. Thus, new interventions are required. Controlled Human Malaria Infection (CHMI) studies are assuming an increasing place in evaluation of interventions as they can lead to accrual of pivotal efficacy data in a faster and more cost-effective fashion than Phase IIa studies in endemic settings. An alternative to infection via sporozoite, either by

mosquito bite or injection of cryopreserved sporozoites, Induced Blood Stage Malaria (IBSM) infection represents a convenient approach where pre-erythrocytic stages are not being studied. Here we report on the safety and reproducibility data from 121 subjects in 12 trials from our centre, the largest report of IBSM. The majority of subjects (86%) experienced at least some symptoms of malaria infection. In total 755 adverse events were recorded however the majority (75%) were mild. No SAE's were attributed to malaria with 4 SAE's unrelated to trial protocol and 3 SAE's attributed to the investigational product. Despite the positive serostatus of the donor for CMV, and the inclusion of seronegative subjects, no CMV seroconversions were detected nor were any additional coinfections observed on extensive serological testing. Analysis of parasitaemia by sensitive qPCR demonstrates that the method is reliable and reproducible. Further analysis of the reproducibility and variability of parasite growth rates is currently underway and will be presented. These data illustrate the safety and reproducibility of an induced blood stage malaria model thus providing a valuable tool for assessing candidate drugs and vaccines for control of malaria.

1490

A PHASE IIA CLINICAL TRIAL TO CHARACTERIZE THE PHARMACOKINETIC-PHARMACODYNAMIC RELATIONSHIP OF PIPERAQUINE USING THE INDUCED BLOOD STAGE INFECTION MODEL

James S. McCarthy[1], Silvana Sekuloski[1], Paul Griffin[2], Suzanne Elliott[2], Louise Marquart[1], Joerg Moehrle[3], Mark Baker[3]

[1]QIMR Berghofer Medical Research Institute, Herston, Australia, [2]QPharm Pty Ltd, Herston, Australia, [3]Medicines for Malaria, Geneva, Switzerland

Piperaquine (PQP) is a 4-aminoquinoline antimalarial structurally related to chloroquine. It was widely used for malaria control in China in the 1970's and 1980's, and more recently has undergone renewed development as component of an ACT co-formulation with dihydroartemisinin (DHA-PQP). Although knowledge of the pharmacokinetic-pharmacodynamic (PK-PD) relationship of antimalarials is essential for dose selection, there is a paucity of such data for PQP. We therefore undertook an experimental dose de-escalation clinical trial of this drug in the induced blood stage infection malaria (IBSM) system, with simultaneous measurement of drug levels and parasitemia, the latter by qPCR. The trial was designed to include 3 single dose cohorts, each of 8 volunteers. The pharmacokinetic profile of PQP following single doses of 960 and 640 mg was linear with CL/f = 89 L/hr (95%CI: 75-101 L/hr), with measurable plasma levels (>1 ng/mL) out to 672 hrs following administration of 640 mg. Recrudescent parasitemia occurred after ≥144 hours in 4 of the 7 volunteers who received 640 mg PQP; each received rescue treatment with artemether/lumefantrine. The rich dataset accrued facilitated the fitting of a PK/PD model to the PK and parasitemia data. The concentration response relationship identified by analysis of data from the 960 and 640 mg cohorts was characterized by a PRR of 3.3 (95%CI: 3.0-3.6; t1/2: 4.4 hr), an IC50 of 9.2 ng/mL (95%CI: 7.1-11.9), an MPC of 14.3 ng/mL (95%CI: 11.0-18.5 ng/mL), and an MIC of 8.1 ng/mL (95%CI: 6.3-10.5 ng/mL). The model accurately predicted the parasitemia response observed in the 480 mg PQP cohort. The IBSM system demonstrated that the PK/PD relationship of an antimalarial can be determined from data obtained from just two cohorts of 8 volunteers. The compiled PK/PD model can then be linked with safety data to forecast optimal dosing, either as a single agent or in combination, whilst accounting for effects of age, DDI and parasitemia.

1491

DELAYED ANEMIA ASSESSMENT IN PATIENTS TREATED WITH ORAL ARTEMISININ DERIVATIVES FOR UNCOMPLICATED MALARIA: A POOLED ANALYSIS OF CLINICAL TRIALS DATA FROM MALI

Issaka Sagara[1], Renaud Piarroux[2], Abdoulaye Djimde[1], Roch Giorgi[2], Kassoum Kayentao[1], Ogobara K. Doumbo[1], Jean Gaudart[2]

[1]University of Bamako, Bamako, Mali, [2]Aix-Marseille University, Marseille, France

In sub-Saharan Africa, Artemisinin-based combination therapies (ACT) and injectable artesunate are the first line treatments for uncomplicated and severe *Plasmodium falciparum* malaria, respectively. However, recent studies suggest that delayed anemia is associated with these treatments in non-immune travelers. We aimed to assess the risk factors associated with delayed anemia after falciparum malaria treatment with artemisinin-containing drugs in malaria endemic populations. Pooled, individual malaria patient data were extracted from 13 clinical trials performed from 2002 to 2011 in various settings of Mali. Treatment regimens were Artemether-Lumefantrine, Artesunate plus Amodiaquine, Artesunate plus Sulfadoxine-Pyrimethamine, Artesunate plus Sulfamethoxypyrazine-pyrimethamine, Artesunate plus Mefloquine, Artesunate-Pyronaridine, Artesunate monotherapy, Chloroquine, Sulfadoxine-pyrimethamine, Amodiaquine and Sulfadoxine-pyrimethamine plus Amodiaquine. Univariate and multivariate analyses were performed using the generalized linear and latent mixed model procedures to assess risk factors associated with hemoglobin concentration evolution and anemia during the treatment follow-up. A total of 5990 participants were recruited and followed from Day 0 to Day 28. The participants' median age was 5 years, ranging from 3 months to 70 years. There was a decrease in hemoglobin level on day 7 in all treatments arms, but the magnitude varied across treatments. There was a significant risk of hemoglobin level decrease on day 7 in the artemisinin-based therapies compared to the non-artemisinin treatments. The risk of hemoglobin concentration drop was associated with age group < 5 years old (0.61 g/dL 95% CI [0.71 to 0.51], p<0.001), baseline high parasite density (0.43 g/dL 95% CI [0.51 to 0.35], p<0.001) and treatment failure (0.40 g/dL 95% CI [0.59 to 0.20], p=0.018), while high hemoglobin level at baseline was a protective factor [0.53 to 0.59] p<0.001). No association was found between artemisinin-based therapies and severe delayed anemia. Oral artemisinin derivative treatments for uncomplicated *P. falciparum* malaria are associated with a transient and clinically moderate hemoglobin decrease by day 7 but not associated with a delayed severe anemia.

1492

ARTEMISININ PARTNER'S DRUGS DAY 7 CONCENTRATION PROFILE AND ITS EFFECT ON RECURRENT EPISODES OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

Mamadou M. Tekete[1], Abdoulaye Djimde[2], Jürgen Burhenne[3], Issaka Sagara[2], Bakary Fofana[2], Omar Bila Traore[2], Souleymane Dama[2], Sekou Toure[2], Nianwalou Dara[2], Bouran Sidibe[2], Ogobara K. Doumbo[2], Walter Haefeli[3], Steffen Borrmann[1]

[1]Institute for Tropical Medicine, University of Tübingen, Tübingen, Germany, [2]University of Sciences, Techniques and Technologies of Bamako, Mali, Bamako, Mali, [3]Department of Clinical Pharmacology and Pharmacoepidemiology, University of Heidelberg, Heidelberg, Germany

Bougoula, Kolle, Sotuba are three sites in Mali participating in a large Phase III/VI trial of four ACTs (Artesunate-amodiaquine, artemether-lumefantrine, Dihydroartemisinin-piperaquine and Artesunate-pyronaridine). The role of the long half-life partner drugs is to mop up any remaining blood stage parasite biomass after treatment. Whether the long half-life may lead to accumulation when ACTs are used frequently in high transmission settings is currently not known but potentially important for drug safety and for the duration of post-treatment prophylactic. Patients with uncomplicated *Plasmodium falciparum* malaria, aged ≥6 months,

after inclusion in one of the treatment arms are followed up for two years, during which patients will receive the same treatment for any subsequent episode of malaria occurring at least 28 days after the start of the previous treatment. To date we have plasma concentrations in day 7 samples from 317 treatment episodes for desethyl-amodiaquine and 564 episodes for lumefantrine. Our first results show an increase of desethyl-amodiaquine concentrations from the first episode to consecutive episodes of malaria treatment with a median (quartile range) concentration of 70.6 ng/ml (58.8 to 89.1 ng/ml) (n=102) for the first, 90.8 ng/ml (69.0 to 111.0 ng/ml) (n=80) for the second; 80.2 ng/ml (61.8 to 100.3 ng/ml) (n=32) for the third and 97.7 ng/ml (82.2 to 1293.0 ng/ml) (n=23) for the fourth episode P<0.0001. For lumefantrine, there was no difference between the first and second episode 632.1 ng (405.7 to 948.6 ng/ml; n=343) and 697.15ng/ml (491.69 to 967.93ng/ml; n=135). There was, however, an increase between first and third episodes (789.3ng/ml; 574.3 to 1362.9 ng/ml; n=52; P = 0.002). All patients with day 7 concentration of lumefantrine below 100 ng/ml had recurrence of infections before day 42 of follow-up. These preliminary data show substantial accumulation of desethyl-amodiaquine in the study population exposed to frequent re-treatments in an area of intense seasonal malaria transmission. A larger dataset will be available at the meeting, including detailed analyses of laboratory parameters of safety and a survival analysis of time to recurrence corrected by transmission season, parasite genotypes (to distinguish recrudescent primary infections from new infections) and drug plasma concentrations.

1493

MEASURING THE EFFICACY OF FOUR ACT REGIMENS IN MALI USING QPCR-BASED ESTIMATES OF *PLASMODIUM FALCIPARUM* CLEARANCE TIME

Khalid B. Beshir[1], Nouhoum Diallo[2], Bakari Fofana[2], Aliou Traore[2], Aly Kodio[2], Amadou Togo[2], Nianwalou Dara[2], Moctar Coulibaly[2], Francois Dao[2], Issaka Sagara[2], Colin Sutherland[1], Abdoulaye Djimde[2]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Malaria Research and Training Centre, Department of Epidemiology of Parasitic Diseases, Faculty of Pharmacy, Bamako, Mali

The efficacy of artemisinin combination therapy remains high in sub-Saharan Africa, but the prolongation of parasite clearance times of artemisinin-treated *Plasmodium falciparum* infections in Cambodia and neighboring countries is a warning that careful monitoring of efficacy is required Worldwide. As an alternative to laborious frequently-spaced blood sampling and evaluation by standard light microscopy, we have developed a qPCR-based parasite clearance assay that utilizes daily finger-prick dried blood spots for the first 72 hours of treatment, and which underwent a successful proof-of-principle trial in western Kenya. We have now applied this approach to evaluate parasite clearance in over 200 falciparum malaria patients treated with artemisinin-combination therapy in two sites in Mali: Bougoula and Kolle. All patients were participants in efficacy evaluations by the WANECAM project, and were randomised to receive either artemether-lumefantrine, dihydroartemisinin-piperaquine, amodiaquine-artesunate or artesunate-pyronaridine for all malaria episodes during two years of follow-up. Parasite clearance estimates for 209 first malaria episodes and 186 second episodes across the two sites will be presented, and estimates of the parasite reduction ratio at 48 hours derived for each episode. These data will be analysed with reference to site, regimen received, patient age and, for second episodes, time elapsed since first episode.

1494

STUDY ON EFFICACY OF ARTESUNATE-MEFLOQUINE COMBINATION THERAPY FOR TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN THAILAND AS PART OF A DEPARTMENT OF DEFENSE MULTI-CENTER TRIAL

Krisada Jongsakul[1], Delia Bethell[2], Michele Spring[1], Ilin Chuang[1], Sha Law[3], Sabaithip Sriwichai[1], Worachet Kuntawunginn[1], Saowaluk Wongarunkochakorn[1], Alaina Thomas[4], Donna Tosh[5], David Saunders[1], Mark Fukuda[6], James Cummings[4]

[1]Armed Forces Research Institute of Medical and Sciences, Bangkok, Thailand, [2]Department of Paediatrics, Oxford Children's Hospital, Oxford, United Kingdom, [3]Kwai River Christian Hospital, Kanchanaburi, Thailand, [4]Armed Forces Health Surveillance Center, Silver Spring, MD, United States, [5]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [6]United States Agency for International Development, Bangkok, Thailand

Artemisinin-resistant *Plasmodium falciparum* threatens effectiveness of all artemisinin-based combination therapies. A multi-center artesunate-mefloquine (A+M) efficacy trial is ongoing in three DoD laboratories in Peru, Kenya, and Thailand, to compare parasite clearance rates for 72 hour after artesunate initiation and to conduct standardized microscopy, in-vitro drug-sensitivity testing and molecular testing across all three sites. Only initial data from Thailand will be presented. Patients aged 5-65 years with uncomplicated *P. falciparum* malaria, with asexual parasite density between 1,000 - 200,000/µL, no signs or symptoms of severe malaria, no other cause of febrile illness were enrolled starting in September 2013. Participants received 4 mg/kg artesunate at 0, 24, and 48 h, 15 mg/kg mefloquine at 72 h, and 10 mg/kg mefloquine at 84-96 h, with 0.5 mg/kg primaquine for transmission blocking, all under direct observation therapy. We assessed parasite density on thick/thin smears every 4 h during first 12 h after first artesunate dose and every 6 h for 72 h or until two consecutive negative smears. The parasite clearance half-life will be calculated from the parasite clearance curve. Efficacy outcome for 42 days will be assessed. Between Oct 31, 2013, and Feb 3, 2014, we assessed 52 persons suspected of malaria from four malaria clinics and hospitals in Sangkhlaburi district of Kanchanaburi province in western Thailand near Thai-Myanmar border. We screened 12 and enrolled 8 patients with *P. falciparum* malaria who met inclusion criteria. Forty cases could not be screened including 11 (27%) who previously took antimalarias including artemisinin monotherapy. Five cases had parasite clearance time more than 72 h and three cleared before 72 h [GeoMean=57.7 h (95% CI + 18)]. All eight subjects met adequate clinical and parasitological response endpoint and no recurrence was reported to date. Although no resistance to A+M detected among eight subjects in Thai-Myanmar border so far, more data to include up to 59 more subjects will be presented.

1495

PRELIMINARY RESULTS: PHASE 2 RANDOMIZED PROOF OF CONCEPT STUDY COMPARING AN INVESTIGATIONAL AMINOQUINOLINE ANTIMALARIAL (AQ-13) TO COARTEM IN ADULT MALIAN MALES WITH UNCOMPLICATED MALARIA

Ousmane A. Koita[1], Aliou Sissako[1], Sangare Lansana[1], Haiyan Deng[2], Trevor A. Thompson[2], Sahare Fongoro[1], Sounkalo Dao[1], Mamadou A. Diakite[1], Frances J. Mather[2], Boubakar Diallo[1], Asif Anwar[2], Joon Sup-Yeom[3], David M. Mushatt[2], Mark Sather[4], Donald J. Krogstad[2]

[1]University of Bamako, Bamako, Mali, [2]Tulane University Health Sciences Center, New Orleans, LA, United States, [3]Sungkyunkawan University, Seoul, Republic of Korea, [4]ScottCare, Cleveland, OH, United States

Although artemisinin-combination therapies (ACTs) are the recommended first-line treatment for uncomplicated *Plasmodium falciparum* malaria, there is increasing concern about artemisinin resistance because of prolonged parasite clearance times in southeast Asia. For this reason,

it would be helpful to have alternatives to the artemisinins that were effective against chloroquine(CQ)-resistant *P. falciparum*, were safe in human subjects and could be given orally. Our previous studies have shown aminoquinolines with modified side chains such as AQ-13 are active against CQ- and multi-resistant *P. falciparum in vitro* and in a squirrel monkey model of CQ-resistant human *P. falciparum* infection, are safe orally in human subjects and have pharmacokinetics similar to those of CQ. The preliminary results reported here are from a blinded study comparing the investigational antimalarial AQ-13 (1,750 mg over 3 days) to the current recommended first-line treatment (Coartem=artemether + lumefantrine; 480 and 2,880 mg over 3 days) for uncomplicated *P. falciparum* malaria in adult Malian males (≥18 years of age). Based on the first 33 subjects enrolled, there have been no differences in efficacy (asexual parasite clearance on or before day 7), clinical recovery (resolution of fever, chills and myalgias on or before day 3) or side effects (no serious or Grade 3 or Grade 4 adverse events) between treatment groups. Because the study is blinded, we do not know whether there are other differences between the AQ-13 and Coartem groups. However, because the second group of 33 subjects is now being enrolled, it should be possible to address those questions at the time of this presentation. The results available at this time suggest that AQ-13 alone may be as efficacious and safe as Coartem in adult Malian subjects with uncomplicated *P. falciparum* malaria.

1496

INDIVIDUAL AND HOUSEHOLD LEVEL FACTORS ASSOCIATED WITH ITN USE BETWEEN 2008 AND 2013 IN A LOW MALARIA TRANSMISSION SETTING OF SOUTHERN ZAMBIA

Jessie Pinchoff[1], Harry Hamapumbu[2], Tamaki Kobayashi[1], Jennifer Stevenson[1], Douglas E. Norris[1], Phil Thuma[2], Elizabeth Colantuoni[1], William J. Moss[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Macha Research Trust, Macha, Zambia

The prevalence of malaria has declined in parts of sub-Saharan Africa; as perceived risk decreases there are concerns that use of personal protective measures may also decrease. Determining factors that influence insecticide-treated net (ITN) ownership and use in these areas is critical to promote their continued use to achieve malaria elimination. Households in the Macha Hospital catchment area, Choma District, Southern Province, Zambia were enumerated and randomly selected using satellite imagery. Households were either visited once (cross-sectional) or every other month (longitudinal). Adults and caretakers of children were administered a survey regarding malaria-related beliefs and behaviors and a malaria rapid diagnostic test (RDT). Mosquitoes were collected in the households using light traps. Individual and household level factors associated with use among those who owned an ITN were assessed using longitudinal, multi-level regression models. Qualitative questions were tabulated to identify reasons for not owning or using an ITN. In a smaller sample of households, the association between total mosquitoes caught and ITN use was assessed to determine if culicine mosquitoes prompted use. ITN use was higher at follow-up visits (77.4%) as compared with first visits (62%) in the longitudinal cohort (p<0.0001). In the multi-level model, ITN use was 77% higher during the rainy season (OR=1.77 (95% confidence interval=1.46, 2.16)) and over twice as high after ITN distribution in June 2012 (OR=2.33 (1.21, 4.5)). Those that learned about malaria from a community health worker had 42% higher odds of using their net (OR=1.42 (1.09, 1.84)). Those that owned 3 or more nets were over twice as likely to use their ITN (OR=2.13 (1.35, 3.36)). Also, odds of ITN use was over twice as high if more than 10 culicine mosquitoes were caught in the house controlling for season and study design (OR=2.15 (1.27, 3.63)). ITN use can be sustained in low transmission settings with continued education and distributions, and may be driven in part by the presence of culicine mosquitoes.

1497

A PAN-AFRICAN HIGH-RESOLUTION SEASONAL MALARIA FORECASTING SYSTEM

Felipe J. Colón-González[1], Adrian M. Tompkins[1], Volker Ermert[2], Francesca Di Giussepe[3]

[1]The Abdus Salam International Centre for Theoretical Physics, Trieste, Italy, [2]Institute of Geophysics and Meteorology, Cologne, Germany, [3]European Centre for Medium Range Weather Forecasts, Reading, United Kingdom

Malaria transmission models are useful for understanding the epidemiology of the disease, and for the development early warning systems (EWS) in epidemic-prone regions. Dynamical models explicitly model the disease dynamics through a series of differential equations. Unlike statistical models, dynamical models are not confounded by sparse or short data records. Population density is key for determining disease occurrence, and should be incorporated in the models to effectively differentiate between urban, peri-urban, and rural malaria. Weather factors such as temperature and precipitation, are key determinants of disease niche, and should also be included in the modeling framework. Accurate predictions of weather conditions could provide useful information for targeting bespoke interventions in high-risk areas one or two months in advance. Here, we coupled a state-of-the-art dynamical malaria model that can be used at a fine spatial resolution of O(10) km, and applied over a continental scale, with two operational state-of-the-art weather prediction systems to develop a pilot malaria EWS for Africa. To our knowledge, this is the first attempt to developing a pan-African malaria EWS using state-of-the-art weather forecasts and dynamical malaria models. We determined the seasons and regions in which such a forecasting system be more valuable to decision-makers, and assessed the skill of the model. The EWS provides forecasts of malaria prevalence and intensity up to four months in advance with good skill across large regions. A further evaluation of the model using sentinel-site surveillance data demonstrates that the EWS is able to predict malaria dynamics in some target regions one to four months ahead. This findings show that the EWS could significantly help public health decision makers optimising resources, and making informed decisions about the areas and periods of high risk.

1498

SET-UP AND VALIDATION OF POST-SCREENING TOOLS FOR A NEW MALARIA TRANSMISSION-BLOCKING APPROACH BASED ON DEFORMABILITY

Papa Alioune Ndour[1], Julien Duez[1], John Holleran[2], Sylvestre Biligui[1], Liliane Cicéron[1], Nathalie Chartrel[3], Sylvie Garcia[4], Inês F. Amado[4], Harvey Tawfik[5], Bruno Le Pioufle[5], Olivier Français[5], Catherine Lavazec[6], Vicky Avery[2], Pierre A. Buffet[1]

[1]CIMI-PARIS, INSERM U1135, Paris, France, [2]Eskitis Institute, Griffith University, Brisbane, Australia, [3]AP-HP, Hôpital Pitié-Salpêtrière, Service de Parasitologie-Mycologie et Service des Maladies Infectieuses et Tropicales, Paris, France, [4]Institut Pasteur de Paris, Paris, France, [5]SATIE, CNRS UMR8029, Ecole Normale Supérieure de Cachan, Paris, France, [6]Cochin-Inserm, Paris, France

Gametocytes are the sexual forms of *Plasmodium falciparum* parasite and are essential for transmission from human to human. Unlike immature (stage I-IV) gametocytes that are stiff and sequestered, mature gametocytes (stage V) are deformable and circulate. A drug increasing their stiffness will induce their clearance by the spleen, thereby removing them from the transmission cycle. We are screening for active compounds based on their ability to induce the retention of mature gametocytes through an automated filtration process that mimics the mechanical sensing of RBC by the spleen. To validate the activity of selected compounds, we developed post-screening tools using a biomimetic microfluidic device, a simple mouse model and human spleens perfused ex-vivo. The common read-out of post-screening tools was the retention or enrichment rates of mature and immature gametocytes exposed or not to selected compounds. Typically, stage V gametocytes were pre-exposed to a

selected compound then co-infused in the microfluidic device, in mice or in the human spleens along with the same gametocyte population exposed to the solvent control. Normal RBCs, asexual ring-IRBCs and heated RBCs were used as controls. We first confirmed that unlike stage I-IV, stage V gametocytes from an *in vitro* culture were not markedly retained in microsphere-based microplate filters and in human spleen perfused ex-vivo, consistent with the hypothesis that deformability of gametocytes is a major determinant of their circulation in peripheral vessels. Using the microfluidic device, we showed that stage V exposed to a recently identified stiffening compound C were enriched to 74.9% (vs. 25.08% for unexposed controls, p=0.0001 paired t test) in narrow 2 µm-wide spaces mimicking inter-endothelial slits in the spleen. In macrophage-depleted C57 Bl/6 mice, immature gametocytes (10 mice) and heated RBCs (4 mice) were cleared by 86% or 75% in 3 hours, respectively. By contrast, a majority of mature gametocytes (5 mice) or normal RBCs (4 mice) were still circulating 3 hours after infusion (Retention rates: 44% and 30%, respectively (p=0.0058, p=0.0002). Similar results were observed in human spleens. Mature circulating gametocytes can be stiffened to induce their mechanical retention, thereby interrupting transmission. The stiffening effect can now be validated in a biomimetic microfluidic device and in a simple rodent model as a prerequisite before further development.

1499

THE USE OF RESPONDENT DRIVEN SAMPLING METHODS TO IDENTIFY MALARIA PREVENTION KNOWLEDGE AND BEHAVIORS BY MIGRANT AND MOBILE POPULATIONS IN WESTERN CAMBODIA

John Hustedt[1], Chandary Rang[1], Sophal Uth[1], Sara Canavati[1], John Macom[1], Po Ly[2], Siv Sovannaroth[2], Arantxa Roca-Feltrer[1], Tarekegn Abeku[1], Sylvia Meek[1]

[1]Malaria Consortium, Phnom Penh, Cambodia, [2]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia

Mobile and migrant populations (MMPs) along the Thai-Cambodian border are at high-risk for malaria infection and have been found with artermisinin resistant parasites. However, the mobile nature of this population makes it difficult to adequately measure malaria infection and risk behaviors, which is vital as we move to elimination in the region. Utilizing respondent driven sampling methods, MMPs residing within two villages in Palin province (Pang Rolim and Sala Krau) were recruited in two independent rounds of sampling (602 in 2013 and 604 in 2014). All responses were adjusted for network size and recruitment patterns allowing for calculation of population-adjusted statistics. While the prevalence of *Plasmodium vivax* is estimated to be 0.2% among the general population, this study found 2.0% and 1.3% of MMPs in these networks to be infected with *P. vivax* in 2013 and 2014 respectively, and an absence of *P. falciparum*. Most respondents from Pang Rolim, from both rounds, identified having seen malaria messages within the previous three months (99.7%, 95% CI: 97.6-100 in 2013 and 99.0%, 95% CI: 95.9-99.8 in 2014). However, in Sala Krau, the percentage of respondents answering similarly decreased from 97.0% (95% CI: 94.1-98.4) in 2013 to 59.1% (95% CI: 51.3-66.4) in 2014. While knowledge related to malaria transmission, symptoms and prevention increased noticeably in Pang Rolim, similar knowledge remained low in Sala Krau across both rounds. Furthermore, while the percentage of respondents from Pang Rolim who didn't use a net the previous night remained the same across both rounds (2.4%), there was a slight increase in non-users in Sala Krau from 6.1% (95% CI: 0.9-6.7) to 9.5% (95% CI: 5.3-16.4). These findings correlate with the fact that there were increased efforts on malaria prevention in Pang Rolim (eg. concerts and videos with prevention messaging) and not in Sala Krau; suggesting that as MMPs change frequently there is a need for sustained public health efforts to reach this population, especially within an elimination context.

1500

IMPLEMENTING ENHANCED HIGH-RESOLUTION SURVEILLANCE USING SPATIAL DECISION SUPPORT SYSTEMS TO GUIDE TARGETED RAPID RESPONSE IN MULTI-DRUG RESISTANT AREAS OF VIETNAM

Gerard C. Kelly[1], Colin Ohrt[1], Hieu M. Truong[2], Hien T. Nguyen[3], Nicholas Martin[4], Xa X. Nguyen[2], Quy Anh Nguyen[2], Roland Gosling[1], Jennifer Wegbreit[1], Duong T. Tran[2], Duong T. Tran[2], Thang D. Ngo[2]

[1]University of California San Francisco, San Francisco, CA, United States, [2]National Institute of Malariology, Parasitology and Entomology, Hanoi, Vietnam, [3]U.S. Embassy, Hanoi, Vietnam, [4]Naval Medical Research Center-Asia, Singapore, Singapore

Emerging artemisinin resistant malaria in the Greater Mekong Subregion (GMS) has important implications for public health. A project was established to research, develop and implement enhanced surveillance and targeted appropriate intervention measures to stop the spread of multi-drug resistant malaria through elimination of the disease in the region. The aims of this project are to pilot a spatial decision support system (SDSS) approach to conduct high-resolution surveillance to guide swift and targeted responses. Pilot sites were established in selected communes in Vietnam with associated customised SDSS developed. Publically available topographic geographic information system data were uploaded into the SDSS to provide baseline information. Household and forest transmission location data were located and enumerated through field-based geographical reconnaissance using handheld computers. Passively detected malaria cases were geo-referenced to the suspected transmission location sites upon diagnosis. Using case location data in the SDSS, active transmission foci were automatically classified and response areas-of-interest (AOI) generated. Supporting data (including population, location and number of sleeping locations within the AOI) were automatically produced in the SDSS and sent to village health workers and district level units to mobilize appropriate responses. Complete pilot data for presentation are expected in September 2014. This new approach utilizing novel geo-spatial tools to support targeted, appropriate and aggressive response measures to support malaria elimination in areas of global significance will be presented.

1501

RESTRATIFICATION OF MALARIA EPIDEMIOLOGY IN VIETNAM FOR MORE EFFECTIVE APPLICATION OF LIMITED RESOURCES

Thang D. Ngo[1], Hung X. Le[1], Hung M. Nguyen[1], Thieu Q. Nguyen[1], Trung D. Ho[1], Xa X. Nguyen[1], Anh Q. Nguyen[1], Ha S. Dinh[1], Roland Gosling[2], Bruce Lee[3], Hien T. Nguyen[4], Gerard C. Kelly[3], Nicholas Martin[5], Colin Ohrt[3], Duong T. Tran[1], The NIMPE Advisory Groups[1]

[1]National Institute of Malariology, Parasitology and Entomology, Hanoi, Viet Nam, [2]University of California San Francisco, San Francisco, CA, United States, [3]U.S. Embassy, Hanoi, Viet Nam, [4]Naval Medical Research Centre - Asia, Singapore, Singapore

The National Malaria Control Program in Vietnam is updating malaria epidemiology in order to more effectively apply limited malaria diagnosis, prevention and treatment resources. The most recent prior restratification was conducted in 2009. This on-going 2014 restratificaton effort (2009-2013 data) is using the same methods of 2009 to collect all malaria case data to the commune (county) level. Indicators for classification are based on the average number of confirmed cases per 1000 population over the 5 year period, the presence of at least one of the three malaria vectors, socioeconomic disadvantaged or border commune, poor health system, drug resistant parasites, chemically resistant mosquitoes, and migratory populations. Each indicator has a score, with the sum of the scores used to define the level of endemicity and priority for interventions. This score will be used to characterize each commune into one of five zones (no

malaria transmission, area at risk for reintroduction of malaria, low (>0-1/1000), medium (1-5/1000), or high (>5/1000)). The current levels of malaria endemicity using historical passive case detection data will be determined by September 2014. Using available data, greater precision of where malaria transmission is occurring and populations at risk will also be estimated. Enhanced methods to collect these data prospectively will be developed. Additionally, data on all prior malaria interventions for the last 5 years will also be collected and entered in to a database. These will be analyzed to estimate the impact of prior malaria control interventions in an operations research model to help select which methods should be continued or reassessed. Methods to prospectively assess the impact of new interventions in an on-going and iterative fashion will also be developed. We will present the new 2014 restratification data and compare and contrast it with the 2009 data. We will illustrate how these new data will be used to better target interventions. The plan to collect more precise prospective information, as well as the status of the analysis of intervention impact, will also be presented.

1502

LAMP AS DIAGNOSTIC TOOL FOR DETECTION OF SUB-PATENT ASYMPTOMATIC MALARIA INFECTIONS IN PRE-ELIMINATION SETTINGS IN NORTHERN NAMIBIA

Davis R. Mumbengegwi[1], Munyaradzi Tambo[1], Ronnie A. Bock[1], Roland D. Gosling[2], Hugh J. Sturrock[2]

[1]University of Namibia, Windhoek, Namibia, [2]University of California San Francisco, San Francisco, CA, United States

The global map of malaria is shrinking with 34 out of 99 malaria endemic countries embarking on eliminating the disease. Transmission in Namibia has declined dramatically from 477,786 cases in 2000 to 1546 in the 2013 malaria season. Namibia is now in the pre-elimination phase of malaria and is targeting elimination by 2020. A new challenge facing the elimination campaign is the detection of asymptomatic malaria cases with low levels of parasitaemia. These infections are difficult to detect as they are below the threshold of routinely used Rapid Diagnostic Test (RDT) kits, yet can result in onward transmission. Molecular tools based on DNA amplification such as PCR are sensitive and specific enough to detect low parasitaemia but their routine use requires expensive, highly technical equipment and expertise. Loop-mediated isothermal amplification (LAMP) is a tool based on DNA amplification and has the advantages of PCR yet is requires less expertise and equipment. This study was conducted to determine the usefulness of LAMP as a diagnostic tool to detect asymptomatic, sub-patent infections found during reactive case detection in Engela district in Northern Namibia. All RDT confirmed malaria cases reported in the Engela district and members of their households as well as occupants of the four surrounding households were recruited into the study. RDTs and dried blood spots (DBS) of all subjects were collected and DNA was extracted from both using the chelex method. LAMP was run using DNA extracted from all the collected samples and results detected as fluorescence under a UV light. Preliminary results from 416 RDTs and DBS collected during follow up of 11 index cases, showed 11 individuals positive by RDT and 18 positive by LAMP. Thus 7 additional secondary malaria cases associated with index cases (a 1.6 fold increase) were detected by LAMP over RDTs. This shows LAMP could be a useful tool to detect sub-patent asymptomatic malaria infections at low transmission and may be a suitable diagnostic tool for use in pre-elimination settings.

1503

BASELINE EPIDEMIOLOGICAL CHARACTERISTICS OF PARTICIPANTS ENROLLED IN A TRIAL OF INTERMITTENT MASS SCREENING AND TREATMENT FOR MALARIA IN WESTERN KENYA

Aaron M. Samuels[1], Norbert A. Odero[2], Wycliffe Onyango[2], Kephas Otieno[2], Peter Otieno[2], Ya Ping Shi[1], Mary J. Hamel[1], Kim Lindblade[1], Simon Kariuki[2], Meghna Desai[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya

The prevalence of malaria infection remains high in western Kenya despite over 30 years of control efforts. Community parasite prevalence in children <5 years of age was estimated at >80% in the early 1980s, and dropped to 26% by 2008. Prevalence rose to 43% in 2011. Individuals with asymptomatic parasitemia account for 90% of infections in specific age groups and may be sustaining the continued high level of transmission. Mathematical models suggest that strategies targeting the asymptomatic population could reduce malaria transmission. We have initiated a multi-year two-arm cluster randomized controlled trial to evaluate the impact of intermittent mass screen and treat (iMSaT) campaigns for malaria in Siaya County, western Kenya. We describe baseline cross-sectional survey results. Twenty compounds were randomly selected from each of 20 clusters during peak malaria transmission season in July-August, 2013. All consenting individuals within houses of selected compounds were asked demographic, behavioral and symptom-based questions by community health workers using personal digital assistants. Blood samples were tested for malaria using a combination HRP2/pLDH rapid diagnostic test (RDT), light microscopy (LM), and polymerase chain reaction (PCR). A total of 1,987 persons living in 605 households from 359 selected compounds were interviewed. Of these, 1,402 consented for both RDTs and LM. Baseline malaria infection prevalence was 47.1% (95% Confidence interval [CI] 43.9-50.2) and 36.6% (CI: 33.3-39.8) by RDT and LM, respectively. RDT positivity was strongly associated with age; 64.9% (CI: 57.6-72.2), 70.8% (CI: 65.1-76.4), and 27.8% (CI: 24.5-31.1) of persons aged <5 years, 5-15 years, and older than 15 years were RDT positive ($P < 0.0001$), respectively. Only 25% who were RDT positive reported a fever in the prior 24 hours, and 45% reported a fever in the previous two weeks. Overall, history of fever in the previous 2 weeks was not associated with RDT positivity, PR 0.94 (CI: 0.84-1.04). Of persons reporting a fever in the previous 2 weeks, RDT positive individuals were as likely to seek care as those who were not, PR 1.04 (CI: 0.93-1.16). PCR results and multivariable analyses are pending. The large proportion of infections that were not associated with fever or care-seeking behavior suggests that strategies targeting the asymptomatic population may be beneficial for reducing malaria transmission in western Kenya.

1504

COMMUNITY-LEVEL MALARIA SURVEILLANCE IN SOUTHERN PROVINCE, ZAMBIA - AN ANALYSIS OF PERCEPTIONS, PRACTICE AND PROGRESS IN AN AREA TARGETING ELIMINATION

Lynne Lohfeld[1], Tokozile Kangome-Ngwenya[2], Anna M. Winters[2], Zunda Chisha[2], Busiku Hamainza[3], Mulakwa Kamuliwo[3], John Miller[4], Matthew Burns[2], Daniel J. Bridges[2]

[1]McMaster University, Hamilton, ON, Canada, [2]Akros, Lusaka, Zambia, [3]Zambia National Malaria Control Center, Lusaka, Zambia, [4]PATH-MACEPA, Lusaka, Zambia

Since its inception in 2011, the training techniques and personnel associated with the program have evolved and matured using anecdotal feedback from community health workers (CHWs) trained to deliver the program, their supervisors and district-level staff. We have recently conducted a systematic qualitative program review to determine how malaria is perceived by the program implementers and beneficiaries, how the community received the surveillance platform, and solutions to key

operational challenges that could improve implementation. A criterion-based sampling framework based on training regime and performance level of facilities offering the program was used to select six rural health posts, three per district, and a representative from associated stakeholder groups (community health workers delivering the program, their clinic-based supervisors, regional-level staff, and community members). Individual interviews and focus group discussions were then held in these selected sites. Service providers, supervisors, program administrators and community members all credited the program with helping to reduce the number of malaria cases. Barriers to fuller implementation of the program included transportation (e.g. ensuring all CHWs had working bicycles), communication (e.g. providing CHWs with working cell phones and "talk time" to transmit data by phone) and supplies (e.g. ensuring adequate number of RDT kits to test for malaria in clinics and communities, artemether-lumefantrine to treat uncomplicated malaria cases, and antipyretics for malaria-negative patients to encourage future visits to rural health centers). Results from this review will be used when developing plans to scale-up the program for delivery in other parts of Zambia.

1505

REACHING MIGRANT AND MOBILE POPULATIONS THROUGH A PRIVATE SECTOR INITIATIVE: MALARIA BED NET LENDING SCHEME IN CAMBODIA

Soy Ty Kheang

University Research Co. LLC, Phnom Penh, Cambodia

While tremendous strides have been made toward eliminating malaria in Cambodia, pockets of risk persist in border areas that are remote and have high population mobility. Lacking knowledge of malaria, migrant and mobile populations (MMPs) are particularly at risk. The PMI/USAID Control and Prevention of Malaria (CAP-Malaria) Project in the Greater Mekong Sub-region works with private sector employers to increase access to and use of long-lasting insecticidal nets (LLINs) by their migrant workers. Employers range from small farm owners to large companies that manage rubber, cassava plantations, and hydroelectric dam construction. Employers receive a stock of LLINs and malaria educational materials for their employees. The employers then lend the LLINs to their workers, retrieving them prior to their departure for reuse with other migrant employees. An evaluation of the lending model was conducted during the harvesting season in late 2013. The study assessed access to and utilization of LLINs by migrant workers, and explored reasons for non-use. Interviews were conducted with 207 farm owners and 712 workers. Results showed that farm owners were generally satisfied with the LLIN lending model. Some employers (28%) ran out of nets. LLIN uptake among the workers was high, most (93%) had a bed net at their residence, and almost all (96%) reported sleeping under a bed net the previous night. Half of the workers (58%) had received an LLIN from their employer. The main barrier for not using a LLIN was that it was considered too stiff (29%). A fifth of respondents also said they were allergic to the insecticides. Half of the farm workers said they would be willing to pay a small amount for their own net, suggesting an opportunity for subsidized vouchers for LLINs.

1506

MODELING PHARMACOKINETICS AND PHARMACODYNAMICS OF ANTIMALARIAL DRUGS IN THE EPIDEMIOLOGICAL MODELING (EMOD) MODEL WITH IMPLICATIONS FOR TRANSMISSION REDUCTION

Jaline Gerardin, Edward Wenger, Philip Eckhoff

Intellectual Ventures, Bellevue, WA, United States

Modeling approaches can predict the effect of large-scale drug deployments on reducing morbidity over several years of campaigns, leading to better understanding of the role of antimalarial drugs in eradication. The Epidemiological Modeling (EMOD) simulation program includes individual-level modeling of vectors and within-host dynamics, providing an ideal environment in which to study the interplay of antimalarial drugs and host immunity in clearing both asexual parasites and transmission-stage gametocytes. We modeled the pharmacokinetics (PK) and pharmacodynamics of two artemisinin-based combination therapies (ACTs), artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP), and one gametocytocidal drug, primaquine, using age-based dosing and weight-dependent PK. We show that current dosing regimens, especially current fixed-dose recommendations for DP, significantly underdose children. We also find that asexual-stage immunity alone is insufficient to explain low gametocyte prevalence in populations with endemic malaria; host physiological responses are likely to modulate prevalence of the sexual stage. We identify a maximum EIR above which co-dosing ACTs with primaquine has little effect on reducing prevalence, and we demonstrate that a minimal level of individual compliance is necessary for mass drug treatments to impact transmission. Pharmacological modeling of antimalarials can guide community decisions in drug administrations and alert administrators to the most likely and deleterious modes of drug failure.

1507

SIMULATION OF MALARIA PARASITE RESERVOIR COVERAGE USING REACTIVE CASE DETECTION AND ACTIVE COMMUNITY FEVER SCREENING FROM CENSUS DATA IN SOUTHERN ZAMBIA

Joshua O. Yukich[1], Adam Bennett[1], Rudy K. Yukich[2], Busiku Hamainza[3], Kafula Silumbe[4], John M. Miller[4], Thomas P. Eisele[1]

[1]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Sensorstar, Inc., Ellicott City, MD, United States, [3]National Malaria Control Centre, Ministry of Health, Lusaka, Zambia, [4]PATH-MACEPA, Lusaka, Zambia

There is need for malaria elimination programs to determine when and where reactive case detection (RCD) is most effective and feasible. Geo-referenced census data on over 80,000 individuals from 6 rounds of a mass test and treatment (MTAT) intervention with rapid diagnostic tests (RDT) in southern Zambia (2012-2013) were analyzed using a Monte-Carlo simulation algorithm to assess the coverage, sensitivity and specificity of potential RCD systems. Data on household location and composition, fever history, treatment seeking, and RDT results for all individuals were included in MTAT census data. Simulations were conducted within 25 health facility catchments, and the following parameters were varied in sensitivity analysis: RCD search radius or number of households searched, sensitivity and specificity of diagnostics used to identify index cases, treatment seeking probability, household and individual RCD participation level, sensitivity and specificity of diagnostic used during RCD search. Results indicate that RCD and active community fever screening are potentially efficient ways of identifying the parasite reservoir. However, substantial resources are required before meaningful fractions of the parasite reservoir are found in a single search round. Treatment seeking for fevers and access to care are key limiting factors to the sensitivity of an RCD system for identifying the parasite reservoir in the community. A shift from RCD to active community fever screening would improve the fraction of the parasite reservoir identified, especially in areas with poor access to care. However, the fraction of the parasite reservoir identified remains small given feasible search criteria in both systems. Multiple RCD rounds may improve the fraction identified over a given period of time.

1508

MAPPING GLOBAL MALARIA CONNECTIVITY FOR STRATEGIC ELIMINATION PLANNING

Andres Garcia[1], Alessandro Sorichetta[2], David L. Smith[3], Deepa K. Pindolia[4], **Andrew Tatem**[2]

[1]Bill & Melinda Gates Foundation, Seattle, WA, United States, [2]University of Southampton, Southampton, United Kingdom, [3]Johns Hopkins School of Public Health, Baltimore, MD, United States, [4]Clinton Health Access Initiative, Boston, MA, United States

Calls for the eradication of malaria require the development of global and regional strategies based on a strong and consistent evidence base. Evidence from the previous global malaria eradication program and more recent transborder control campaigns have shown the importance of accounting for human movement in introducing infections to areas targeted for elimination. Here, (micro)census, survey and cellphone-based human movement data and models were analysed with network analysis tools to map globally the connectivity of both countries and subnational administrative units through population movements. These data were also combined with *Plasmodium falciparum* and *P. vivax* malaria transmission maps and a global population dataset to identify the likely principal sources and destinations of imported cases. Results indicate that certain groups of countries and regions within countries are much more strongly connected by high levels of population movement than others. The mapping here of both communities of subnational regions and countries linked by high levels of population exchange, and 'natural' migration boundaries that display reduced movement of people and infections between regions has practical utility. These inform the design of malaria elimination strategies by identifying regions afforded protection from re-colonisation by natural 'firebreak' regions of reduced connectivity. For more isolated areas, a regionally-focussed control or elimination program is likely to stand a better chance of success than those receiving high levels of visitors and migrants from high transmission regions. Moreover, we demonstrate how the mobility and malaria connectivity framework provides an evidence base for informing the design and simulation of malaria elimination strategies globally.

1509

PHASE 2A DOSE ESCALATION STUDY OF SAFETY AND EFFICACY OF LOW SINGLE-DOSE PRIMAQUINE FOR GAMETOCYTOCIDAL ACTIVITY AGAINST *P. FALCIPARUM* IN SUB-SAHARAN AFRICA

Halimatou Diawara[1], Joelle Brown[2], Ibrahima Baber[1], Almahamoudou Mahamar[1], Koualy Sanogo[1], Harouna Soumare[1], Fanta Koita[1], Eugenie Poirot[2], Jimee Hwang[2], Sekou Traore[1], Francois Nosten[3], Teun Bousema[4], **Alassane Dicko**[1], Roland Gosling[2]

[1]Malaria Research and Training Center, Bamako, Mali, [2]Global Health Group, University of California San Francisco, San Francisco, CA, United States, [3]Mahidol Oxford University Research Unit in Bangkok, Oxford, United Kingdom, [4]University of Nijmegen, Nijmegen, Netherlands

Primaquine is the only currently available drug with strong gametocytocidal properties against the more mature gametocytes and known to behighly effective in reducing gametocyte carriage and infectivity to mosquitoes. However its deployment hasbeen limited because of the safety concerns. To identify the lowest efficacious dose of PQ, we conducted a Phase 2a dose escalation study of safety and efficacy of low single-dose primaquine in non deficient G6PD male in Ouelessebougou Mali. The first 50 participants aged 5 to 50 years with *Plasmodium falciparum* gametocyte at blood smear, were randomly allocated to one of the following treatment groups with primaquine at 0, 0.125 mg/kg and 0.5 mg/kg. All participants received standard dose dihydroartemisinin-piperaquine. Subjects were seen at days 0, 1, 2, 3, 7, 14 and 28 for hemoglobin measurement and assessment adverse events. Mosquitoes were fed on blood meal using membrane feeding assay before administration of drug and 1, 2 and 7 days after. Infectivity to mosquitoes was measured by the presence of oocysts 7 days post infected feeding. There was no severe or serious adverse event. Preliminary analysis on the first 30 participants enrolled showed no differences among the three groups in mean change in hemoglobin following treatment on day 1 (p=0.89), day 2 (p=0.77), day 3 (p=0.10), day 7 (p=0.61), day 14 (p=0.66), and day 28 (p=0.81). The mean hemoglobin was 13.8 g/dL (range: 11.5, 16.2) at day 0, 13.9 g/dL (range: 11.3, 17.9) at day 7, and 14.0 g/dL (range: 11.6, 17.3) at day 28. In the control group, compared to day 0 there was no reduction in infectivity on day 2 or day 7, -22.8% (95% CI -100%,100%) and 40.0% (95% CI -86%, 100%), respectively. In the 0.125 mg/kg dose group, compared to day 0, there was 86.3% (95% CI 39.4%, 100%) reduction on day2 and 100% reduction in day 7. In the 0.5 mg/kg dose group, compared to day 0 there was a 100% reduction in day 2 and 90.1% (95% CI 64.7%, 100%) reduction on day 7. In summary our preliminary results indicate a higher reduction in infectivity in the 0.5 mg dose group (100% reduction at day 2) without safety concerns.

1510

GENOME-SCALE PROTEIN MICROARRAY ANALYSIS OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX* SYMPTOMATIC AND ASYMPTOMATIC INFECTIONS IN A REMOTE VILLAGE OF THE PERUVIAN AMAZON

Raul Chuquiyauri[1], Douglas Molina[2], Huw Davies[3], Hugo Rodriguez[4], Alejandro Llanos-Cuentas[5], Philip Felgner[3], Joseph M. Vinetz[1]

[1]University of California San Diego, San Diego, CA, United States, [2]Antigen Discovery Inc., Irvine, CA, United States, [3]Division of Infectious Diseases, Department of Medicine, University of California Irvine, Irvine, CA, United States, [4]Directorate of Health, Iquitos, Peru, [5]Tropical Medicine Institute "Alexander von Humboldt", Universidad Peruana Cayetano Heredia, Lima, Peru

The Peruvian Amazon is hypoendemic for *Plasmodium falciparum* (Pf) and *P. vivax* (Pv) malaria. To assess, at a population level, the proportion of people infected by the endemic malaria parasites, a prospective cohort study was done in Santa Emilia, a remote-rural village in the Peruvian Amazon in 2013 where the main seasonality for malaria relates to river height rather than rainfall. Light microscopy and an aldolase gene-specific qPCR assay that detects both Pv and Pf were used to assess the presence of parasitemia. Plasmas were obtained for genome-scale protein microarray analysis using a combined Pf500/Pv500 chip containing a down selected list of the 500 of the top most sero-reactive antigens for each Pf and Pv. At baseline, 33(22%) and 77(51%) of 151 subjects were positive by microscopy and q-PCR, respectively. On the last survey, 3(2%) and 8(5%) of 157 subjects were positive by microscopy and q-PCR, respectively. Asymptomatic parasitemia detected by microscopy ranged from 22% to 40%, while for q-PCR ranged from 41% to 60%. A significant proportion of infections detected by q-PCR, 39% and 47% for Pf and Pv, respectively, were undetected using microscopy. Comparison of proportions of negative subjects at each of the eleven surveys revealed there was a significant increase of negative subjects during September to December surveys in comparison to March surveys. Protein microarray analysis was done with 324 plasma samples: 132 matched paired samples from the two time points and 60 unpaired samples. The top 200 most sero-reactive antigens were selected for comparison. Seroreactivity increased with age and in response to documented malaria infection. Seroreactivity was lower in September than in March for all age groups, paralleling mosquito abundances (related to river height, not rainfall). While there was a gradual increase in seroreactivity associated with age, the differential seroreactivity between March (high) and September (low) was greatest in youngest and this difference decreased with age. Prior to embarking on an elimination strategy, monitoring changes in transmission intensity and identification of malaria foci is mandatory for best intervention efforts. This population-based study of a malaria-endemic population identified new serological markers of infection using

genome-scale protein microarray. This new tool has important potential for providing key control and elimination data for national surveillance programs.

1511

INVESTIGATING OPERATIONAL STRATEGIES FOR ANTIMALARIAL DRUG ADMINISTRATION IN ZAMBIA'S SOUTHERN PROVINCE: A SIMULATION STUDY

Erin M. Stuckey[1], John M. Miller[2], Megan Littrell[2], Nakul Chitnis[1], Rick W. Steketee[2]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]PATH Malaria Control and Evaluation Partnership (MACEPA), Seattle, WA, United States

Malaria elimination requires reducing both the potential of mosquitoes to transmit parasites and the infectious reservoir of parasites in humans, including asymptomatic infections. To achieve this goal in Southern Province, Zambia a mass test and treat (MTAT) campaign using artemether-lumefantrine was conducted from 2011-2013 to complement high coverage of long lasting insecticide-treated nets. In order to identify factors likely to increase campaign effectiveness, a modeling approach was applied to investigate the simulated effect of alternative operational strategies for MTAT in Southern Province. OpenMalaria, a discrete-time, individual-based stochastic model of malaria, was parameterized for the range of transmission intensities observed in the study area to simulate antimalarial administration for interruption of transmission. Simulations were run for scenarios with a range of artemisinin-combination therapies (ACTs), proportion of the population reached by the campaign, targeted age groups, frequency of campaign rounds, *Plasmodium falciparum* test protocols, and the addition of drugs aimed at preventing onward transmission. Scenarios were evaluated based on the reduction in all-age parasite prevalence during the peak transmission month following the campaign, compared to the currently-implemented strategy. Simulation results suggest that the most important determinant of success in reducing prevalence is the coverage of the population achieved in the campaign. However, even with high coverage with mass drug administration (MDA) in areas with a pre-intervention all-age parasite prevalence of less than 10%, simulations suggest that elimination would require more than one year of campaign implementation. Including single low-dose primaquine, which acts as a gametocide, to the drug regimen did not further reduce prevalence. The addition of an endectocide, such as ivermectin, resulted in a lower simulated parasite prevalence and warrants further investigation. Simulation results indicate a high proportion of low density infections were missed by rapid diagnostic tests that would be treated and cleared with MDA. The optimal implementation strategy for MTAT/MDA will vary by background level of prevalence and rate of infections imported to the area. Success of the campaign depends on continued coverage of vector control interventions to ensure sustained gains in reduction of disease burden.

1512

A FREE SURVEILLANCE APP FOR PLANNING MALARIA ELIMINATION INTERVENTIONS AND OUTBREAK RESPONSES AT THE COMMUNITY LEVEL IN MALARIA ENDEMIC COUNTRIES

Antonio M. Quispe[1], Josiah L. Kephart[2], Andres G. Lescano[1], Timothy M. Shields[2], Clive J. Shiff[2]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Planning malaria elimination interventions and outbreak responses at the community level represents an operational challenge in most malaria endemic countries. This happens primarily because most health systems report surveillance data at the regional and country levels only, despite data collection at the community level. Therefore, when planning interventions and responses at the community level, decision makers often need to perform their own data analysis, which is time-consuming and resource-intensive. In an attempt to address this problem, we have developed the "Free Surveillance App" (FREESAPP), an online application that converts time-series data from national surveillance systems into interactive graphs similar to those produced by the Gapminder Foundation. The applet can output burden distribution and decision-making trees for cost-effectiveness assessments as well as utilize satellite maps for background illustration. The overall goal of this application is to allow public health officers to efficiently and accurately use the surveillance data to guide their decision-making practice at each of the levels of the local health system hierarchy. To achieve this goal, FREESAPP allows users to plot the trends in malaria burden using a variety of interactive displays (i.e., bubble, bar, and line charts). FREESAP allows users to include and adjust for various covariates of interest (i.e., incident rate, population size, *P. falciparum* proportion, time, etc.) using different mathematical transformations. Users can contrast trends against epidemiological thresholds for each reporting level, which are automatically updated with each week of data entry. Furthermore, FREESAPP users are able to estimate costs of implementing interventions by using a cost-effectiveness algorithm that is adjustable by population size, distance, and coverage rates. In order to allow data managers to update the system without altering their current reporting protocol, FREESAPP was developed using a combination of four free tools: Motion Chart, Google Earth, Google maps, and R software. Given its open source free format, FREESAPP may contribute to enhancing the local readiness and response capacity at each of the levels of the Health System hierarchy in most malaria endemic countries. FREESAPP may also potentially be used for other reportable diseases, therefore facilitating improved public health decision-making.

1513

EVALUATING REACTIVE CASE DETECTION ACTIVITIES IN RANONG PROVINCE, THAILAND

Prayuth Sudathip[1], Jennifer Koonings[2], **Chris Cotter**[3], Roly Gosling[3]

[1]Bureau of Vector-Borne Diseases, Department of Disease Control, Bangkok, Thailand, [2]Global Health Sciences, University of California San Francisco, San Francisco, CA, United States, [3]Global Health Group at the University of California San Francisco, San Francisco, CA, United States

Active case detection (ACD) strategies are recommended by the World Health Organization and are implemented in many low transmission settings worldwide as a critical part of malaria elimination programs. ACD strategies include determining the origin of infection, case investigation, and responding to locally acquired cases of malaria, known as reactive case detection (RACD). Effectively implementing RACD requires substantial programmatic and human resources. Thailand is pursuing a spatially-progressive approach to eliminate malaria from 80 percent of the country by 2020. Between April and June 2014, the Thailand Bureau of Vector-Borne Diseases (BVBD) conducted an evaluation in Ranong Province to identify best practices and inform RACD efforts in the province and across Thailand. Using a standardized monitoring and evaluation (M&E) tool, five districts within the province were evaluated, and included a mix of high, medium and low transmission settings. Case investigation and RACD rates and reporting timeliness were analyzed through secondary data extraction from the national malaria information system and district-level malaria clinics and measured against defined indicators. Questionnaires were administered to 60 malaria clinic staff regarding RACD operations and procedures. A financial analysis of RACD-related expenditures was also collected and analyzed to determine the primary cost drivers and operating costs for RACD. Findings from the evaluation will inform the BVBD on program efficiency within Ranong Province, identify best practices and gaps in RACD activities, and will assist the BVBD in optimizing RACD program effectiveness.

1514

LAMP FOR THE DETECTION OF SUB-MICROSCOPIC MALARIA INFECTIONS IN THE CONTEXT OF MALARIA ELIMINATION IN CAMBODIA

Nimol Khim[1], Heidi Hopkins[2], Saorin Kim[1], Dany Dourng[1], Vincent Sluydts[3], Somony Heng[4], Lydie Canier[1], Sovanaroth Siv[4], Tho Sochantha[4], Lies Durnez[3], Marc Coosemans[3], Vincent Deubel[1], David Bell[5], Iveth J. Gonzalez[2], Didier Menard[1]

[1]Institut Pasteur in Cambodia, Phnom Penh, Cambodia, [2]Foundation for Innovative New Diagnostics, Geneva, Switzerland, [3]Institute of Tropical Medicine, Antwerp, Belgium, [4]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [5]Global Good/ Intellectual Ventures Lab, Seattle, WA, United States

In the context of government's commitment to eliminate malaria in Cambodia in 2025, additional efforts and new methodologies to detect malaria parasites in asymptomatic individuals are needed. Indeed, in very low transmission settings such as Cambodia, asymptomatic infections remain the major reservoir of malaria parasites contributing to maintain disease transmission. As a consequence, the detection and treatment of the asymptomatic carriers is a crucial step in progress towards malaria elimination. This represents a new challenge as the proportion of asymptomatic parasite carriers is unknown. To date, although PCR methods show lower detection threshold compared to microscopy, Nested or Real time PCR assays required fully equipped laboratory and trained technicians. These approaches are not suitable as point-of-care in the field, contrarily to LAMP, which can be done on a simple bench top in a clinic, with basic reagents and equipment, by personnel with only a few days' training in the technique. To assess the performance of this promising tool, we have conducted a retrospective study on 516 samples from asymptomatic individuals collected in Rattanakiri province, eastern Cambodia. Ten microliters of DNA extracted by Instagene matrix from dried blood spots were used for both LAMP (Pan detection) and Real time PCR. Positive specimens with Pan LAMP were screened for falciparum species (*P. falciparum* LAMP reaction). The results between the two techniques were compared to calculate the diagnostic accuracy. Based on the LAMP detection, the prevalence of malaria infection was 19.8%. Compared to the Real time PCR, the specificity and the sensitivity of the malaria LAMP kit was 93.3% (95% CI: 90.5% - 95.4%) and 86.4% (95% CI: 76.6% - 92.7%), respectively. We concluded that LAMP detection has similar performances with the Real time PCR. In addition, LAMP results are available just one hour after sample processing begins for 14 samples. We suggest that high throughput LAMP assay for a large-scale screening would be developed for a step forward to malaria eradication.

1515

STAGE 1 TRIALS OF THE CLINICAL DEVELOPMENT PLAN FOR PFSPZ VACCINE FOR GEOGRAPHICALLY FOCUSED MALARIA ELIMINATION CAMPAIGNS

Stephen L. Hoffman

International PfSPZ Vaccine Clinical Consortium; VRC, Laboratory of Malaria Immunology and Vaccinology, DMID of National Institute of Allergy and Infectious Diseases, UMB CVD, Bamako Malaria Research and Training Center, IHI Bagamoyo, Swiss Tropical and Public Health Institute, Naval Medical Research Center, Walter Reed Army Institute of Research, University of Tubingen, MoH Equatorial Guinea, CRESIB, Centers for Disease Control and Prevention, Sanaria, Rockville, MD, United States

A vaccine for geographically focused malaria elimination campaigns must provide sustained sterile protection against infection. The Sanaria® PfSPZ Vaccine was developed to address this need. It is composed of aseptic, purified, cryopreserved, vialed PfSPZ. In a recent clinical trial at the Vaccine Research Center (VRC), NIAID, NIH, PfSPZ Vaccine protected 6/6 (100%) subjects against controlled human malaria infection at the highest dosage regimen administered (5 IV doses of 1.35x105 PfSPZ), There was a dose response in regard to antibody and T cell responses, and the vaccine was safe and well tolerated. An international consortium was established to facilitate development of PfSPZ Vaccine for use in geographically focused Pf malaria elimination campaigns, and a 4-stage clinical development plan (CDP) delineated. In Stage 1, which is in progress, trials at 3 sites in the US, and in Mali, Tanzania, Equatorial Guinea, and Germany are assessing the reproducibility of the VRC 312 trial, and optimizing durability, heterologous protection, and dosage regimens. These trials are intended to establish: (1) the reproducibility of the findings from the study conducted at VRC; (2) protection against heterologous Pf, including naturally acquired Pf; (3) durability of protection; (4) protective efficacy of different dosage regimens of PfSPZ Vaccine - regimens of 5, 4, 3, 2 or 1 doses of 1.35x105 to 2.2x106 PfSPZ/dose by direct venous inoculation (DVI) or IM routes; (5) an assay/biomarker that predicts protection; and (6) optimal approaches for DVI administration. >400 doses of 2.7x105 to 2.2x106 PfSPZ/dose have been administered in the US and Africa, and the vaccine has been safe and exceptionally well tolerated. Stage 2 will include age de-escalation and escalation and regimen optimization trials; Stage 3 will be pivotal phase 3 trials; and Stage 4 will include mass administration campaigns to halt transmission and eliminate Pf malaria from populations of > 200,000 individuals. Progress and plans will be explained.

1516

SPECTRUM OF INFECTIOUS DISEASES IN RURAL CLINIC FOR REFUGEES AND DISPLACED POPULATION ON RWANDA-DR CONGO BORDER: ANALYSIS OF 10,051 PATIENTS

Katarina Molnarova[1], Dominik Hes[1], Gertruda Mikolasova[2], Lenka Michalikova[3], Jaroslava Brnova[3], Nada Kulkova[2], **Vladimir Krcmery**[2]

[1]Bl. Clémentine-Anuarita Healthcare Centre, Bigugu, Rwanda, [2]Laboratory of Molecular Microbiology, Bratislava, Slovakia, [3]Faculty of Health Sciences and Social Work, Trnava, Slovakia

Migrants and internally displaced in sub-Saharan Africa are subject of increasing threat of infectious diseases (IDs) due to contaminated water supplies (cholera, typhoid fever), food (salmonellosis, shigellosis), malnutrition (tuberculosis-TB, HIV) and absence of housing (pneumonia, upper respiratory tract infections - RTI). Cross sectional study in area close to DRC border (Sud-Kiwu) and Rwanda (Bisesero) in two clinics serving for 50 000 population (of them 25 000 internally displaced and refugees in UNHCR camps) was performed to asses occurrence of major ID in 2013. Bigugu clinic is located in altitude of 2350 m and Bisesero United Nations High Commissioner for Refugees (UNHCR) camp is in 1150 m above sea level.Of 10 051 patients, only 31 (0,3%) had malaria, and 26 of them (0,26%) had true highland malaria (without down country travelling history), confirmed both microscopically and with rapid diagnostic test (RDT). Commonest IDs were upper RTI representing (72-89%) of all visits, followed by diarrheal and gastro-enteric diseases (13-19%). Also, 26-77% of all children were infected by geohelmints. Only one case of neuroinfection was recorded. Urinary tract infections and sexually transmitted diseases were rare as well (1-4%).Among 10 051 outpatient visits in two rural clinics, serving for UNHCR registered refugees from DRC in Rwanda and internally displaced population near Sud-Kiwu Province. Malaria was extremely rare due to high altitude, and diarrheal and gastrointestinal infections were relatively rare, too. Of all ID, upper RTI were the commonest, while neuroinfections (such as bacterial or viral meningitis and sleeping sickness) were only exceptional. Very high proportion of RTI was associated with malnutrition and very low socio-economic status in areas of high altitudes above sea level with low temperature.

1517

SPECTRUM OF INFECTION DISEASES IN BURUNDIAN RURAL HOSPITAL IN GASURA IN DRY VERSUS RAINY SEASON - 2012/2013

M. Kolibab[1], Jana Trilisinska[1], Eva Smrekova[1], Tomas Razsony[1], Eliska Maradova[1], Alexandra Mamova[1], Miriam Balazova[1], George Benca[2], Bianca Voinescu[1], **Vladimir Krcmery**[2]

[1]Health Centre in Gasura, Burundi, Gasura, Burundi, [2]Slovak Tropical Institute, Bratislava, Slovakia

Seasonal cycles of infectious diseases have been variously attributed to changes in atmospheric conditions, the prevalence or virulence of the pathogen, or the behaviour of the host. Also malaria has been observed during last 10 years with increasing frequency in areas above sea level (highlands malaria). The aim of this research was to asses if rainy/dry seasons are related with seasonal variation of infectious diseases similar to Kenya or Uganda highlands (western Kenya and south-western Uganda). Monthly incidences of malaria versus respiratory infections in June to November (rainy seasons) versus January to April (dray seasons) have been compared in 2012/2013 in community hospital in Gasura located in 1283 m.a.s. (Burundi). This hospital had about 50 beds and outpatients department. Hospital staffs was composed of 2 doctors, 8 nurses, 2 lab technicians and pharmacist, with a patient flow of 40-120 patients daily in the outpatients department and 2-10 inpatients daily. Malaria diagnosis was made microscopically (according to WHO guidelines) and was confirmed with rapid diagnostic test (RDT; according to manufacturer's instructions). Malaria was responsible for approximately 42,5 - 48,8% of all admission or consultations in rainy seasons but only 12,5% -29,7% in dry seasons. In dry season, proportion of respiratory tract infections increased from 19% in June to 42% in November and replaced malaria. Both malaria and pneumonia showed significant seasonal variations in occurrence despite of attitude of community health care centre in Gasura (1283 m.a.s.). In Burundi highlands health care centre in Gasura seasonal variations despite of high attitude (1283 m.a.s.) was observed with increasing proportion of malaria from 29,7 to 42% during rainy season replaced respiratory tract infection increased from 19 to 42% in dry season vice versa. Malaria in the Burundi highlands represented growing problem with variations in prevalence in rainy versus dry seasons.

1518

SEVERE MALARIA AMONG 3,707 ADMISSIONS IN SOUTH SUDANESE HOSPITAL FOR INTERNALLY DISPLACED POPULATION

Alexandra Mamova[1], Miriam Balazova[1], Lenka Dibusova[1], Veronika Sladeckova[1], Marian Bartkovjak[1], Juraj Benca[1], John Mutuku Muli[1], Lenka Michalikova[2], Nada Kulkova[3], Jaroslava Brnova[2], Gertruda Mikolasova[3], Maria Raucinova[1], Jirina Kafkova[1], **Vladimir Krcmery**[4]

[1]St. Francesis of D´Assisi Mission Hospital, St. Elizabeth Tropical Program Marial Lou, Mapuordit, South Sudan, [2]Trnava University, Faculty of Health Sciences and Social Work, Department of Theoretical Disciplines and Laboratory Investigation Methods, Trnava, Slovakia, [3]Laboratory of Molecular Microbiology, St. Elisabeth University College of Health and Social Science, Bratislava, Slovakia, [4]St. Elisabeth University College of Health and Social Science, Bratislava, Slovakia

Severe malaria is responsible for 1.2 million deaths worldwide, 90% of them in Sub-Saharan Africa, mainly in children below 5 years of age. The aim of this study was to assess proportion of severe malaria among all admissions hospital in area with internally displaced population in south Sudan. Data on infectious diseases were analyzed at admission within 12 months 1.1. 2013 - 31.12.2013 at St. Francesis of D´Assisi mission Hospital located in Marial Lou, South Sudan, built for internally displaced refugees coming from north to South Sudan due to civil war (1982 - 2005) and Darfour Conflict (2002 - 2012)and for about 50 000 Dinka population. Diagnosis of severe malaria was done clinically, plus microscopically, plus rapid diagnostic testing (RDT) has been used since 2013. Seasonality of severe malaria was observed among majority of cases in period from April to November, with 113 to 221 cases per month with up to 9 deaths on severe malaria (monthly among 3707 admissions, in 2013). Altogether, 1438 patients (38.8 %) had severe malaria clinically confirmed as fever plus severe anemia, or respiratory distress syndrome, or cerebral malaria, or liver, or kidney failure, or severe hypoglycemia with acidosis. Of 1438 severe malaria cases, 76 died (5.3 %). Relatively low mortality may be explained with: (i) good access to the hospital, (ii) pre-referral administration of antimalarial drugs due to education campaign with the in 2010 - 2013, and (iii) use of artemisinin-based combination therapy (ACT) since 2010 in Marial Lou. Severe malaria in travelers returning to Europe is associated with up to 20 % mortality. But it can by moresuccessfully treated on site in tropics due to semi-immune population, early pre-referral administration of antimalarial drugs and early empiric intramuscular and venous administration of antimalarial drugs; resulting to 5 % mortality even in more severe cases.

1519

GENETIC DIVERSITY AND POPULATION STRUCTURE OF *PLASMODIUM VIVAX* INFECTIONS AFTER RADICAL TREATMENT IN A RURAL COMMUNITY OF CENTRAL VIETNAM

Nguyen Van Hong[1], Christopher Delgado-Ratto[2], Pham Vinh Thanh[1], Peter Van Den Eede[3], Nguyen Thi Huong Binh[1], Umberto D'Alessandro[3], Tran Thanh Duong[1], Jean – Pierre Van Geertruyden[2], Annette Erhart[3], Anna Rosanas-Urgell[3]

[1]National Institute of Malariology, Parasitology and Entomology, Hanoi, Vietnam, [2]Faculty of Medicine, University of Antwerp, Antwerp, Belgium, [3]Institute of Tropical Medicine, Antwerp, Belgium

In Vietnam the malaria burden has been drastically reduced over the past two decades but *Plasmodium vivax* is becoming increasingly important mainly due to its relapsing nature and the difficulty to radically cure dormant parasites from the liver. A two-year cohort study was conducted in Central Vietnam to assess the efficacy of the radical cure regimen based on a 10-day Primaquine (0.5mg/kg/d) in combination with the standard 3-day chloroquine (total 25mg/kg) regimen. We report the genetic diversity and population structure of *P. vivax* infections before and after radical treatment. All day 0 (n=247) and post treatment *P. vivax* infections (n=788) detected by microscopy and PCR during the 2-year monthly follow-up were genotyped using 16 previously described microsatellites. Genetic diversity, linkage disequilibrium, population structure and haplotype clustering were analyzed in post treatment samples and compared to Day 0 samples. All markers were highly polymorphic with 3 to 30 alleles per marker and heterozigosity (He) values ranging from 0.35 to 0.90. Overall He values were not significantly different between day 0 and post-treatment samples (He = 0.64 and 0.66 respectively). In addition, 71.0% of all infections were polyclonal (76.9% at day 0 vs. 69.2% post-treatment samples) and the average multiplicity of infection (MOI) was 1.9 parasites/person (MOI = 2.1 at D0, MOI =1.8 at recurrences). Genetic diversity of parasite population experimented significant changes when parasite population before treatment was compared with parasite population in the second year follow up (FST= 0.21). which may suggest a delayed effect of the intervention or may reflect the intense follow up (with treatment of all cases) study design. In order to estimate multilocus linkage disequilibrium (LD) changes between day 0 and post-treatment samples, we calculated the index of association IsA (which is zero for LD). We observed higher LD in post-treatment than day 0 parasite population (IsA = 0.093, P=0.0001 and IsA = 0.039, P=0.0001, respectively), suggesting inbreeding and a clonal population structure. Overall parasite population in the study is genetically diverse, and has a low effective recombination rate that contrasts with the high number of polyclonal infections.

1520

SEASONALITY IN MALARIA TRANSMISSION - IMPLICATIONS FOR CASE-MANAGEMENT WITH LONG-ACTING ARTEMISININ COMBINATION THERAPIES

Matt Cairns[1], Patrick Walker[2], Lucy Okell[2], Jamie Griffin[2], Tini Garske[2], Kwaku Poku Asante[3], Seth Owusu-Agyei[3], Diadier Diallo[4], Alassane Dicko[5], Badara Cisse[6], Brian Greenwood[1], Daniel Chandramohan[1], Azra Ghani[2], Paul Milligan[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]MRC Centre for Outbreak Analysis and Modelling, Imperial College London, London, United Kingdom, [3]Kintampo Health Research Centre, Kintampo, Ghana, [4]London School of Hygiene & Tropical Medicine, now at PATH-Malaria Vaccine Initiative, Dakar, Senegal, [5]Malaria Research and Training Center, Bamako, Mali, [6]Universite Cheikh Anta Diop, Dakar, Senegal

Seasonality in malaria transmission is a key driver of malaria epidemiology, and has important implications for the effectiveness of interventions. One aspect that is not well understood is how seasonality affects the number of repeat malaria episodes that occur soon after a previous clinical attack, and which might be prevented if a long-acting artemisinin combination therapy (LACT) was used to manage the initial episode. We estimated the separate and combined effects of transmission intensity and seasonality on the timing and concentration of repeat malaria episodes, using data from six cohort studies in West Africa, and then used an individual-based model of malaria transmission across sub-Saharan Africa to extrapolate these results across a range of settings. Seasonality was quantified using the Markham seasonality index (MSI), taking account of areas with bimodal seasonality patterns, and the concentration of malaria episodes in time was quantified using a modified version of the Gini index. We explored 10% intervals of the MSI, and simulated transmission intensity that equates to prevalence in 2-10 year olds ranging from 5-60%. In settings where prevalence is less than 10%, repeat malaria episodes constitute a small fraction of the total burden, and few repeat episodes occur within the window of protection provided by currently available drugs. However, in higher transmission settings, and particularly in highly seasonal settings, repeat malaria becomes increasingly important, with up to 20% of the total clinical burden in children estimated to be due to repeat episodes within four weeks of a prior attack. At a given level of transmission intensity and annual incidence, the concentration of repeat malaria episodes in time, and consequently the protection from LACTs, is always highest in the most seasonal areas. As a result, the degree of seasonality, in addition to the overall intensity of transmission, should be considered by policy makers when deciding between ACTs that differ in terms of the duration of post-treatment prophylaxis they provide.

1521

DETECTING FOCI OF MALARIA TRANSMISSION: IMPLICATIONS OF SAMPLE SIZE AND CHOICE OF MALARIA METRIC

Gillian H. Stresman[1], Emanuele Giorgi[2], Amrish Baidjoe[3], Philip Knight[4], Wycliffe Odongo[5], Chrispin Owaga[5], Shehu Shagari[5], Euniah Makori[5], Jennifer Stevenson[6], Chris Drakeley[1], Jonathan Cox[1], Peter Diggle[2], Teun Bousema[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Lancaster University, Lancaster, United Kingdom, [3]Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [4]University of the West of England, Bristol, United Kingdom, [5]KEMRI/Centers for Disease Control and Prevention, Kisumu, Kenya, [6]Johns Hopkins Bloomberg School of Public Health, Macha, Zambia

Identifying malaria foci in endemic settings can be challenging. Many pragmatic decisions must be made, including the sample size to employ and which malaria metric to use. However, it is not known how these decisions affect the ability to detect foci of infection, particularly the boundaries of foci identified. If control or elimination programs are targeted to foci of infection that do not accurately reflect the true nature of transmission in the community, ensuing interventions may incorrectly be perceived as being ineffective. To determine the impact of sample size and choice of malaria metric, data for 17,500 individuals residing in 3,200 compounds, approximately one third of the population, collected during a cross-sectional survey in the western Kenyan highlands were used to identify foci of transmission in the community. All structures in the area were digitized to provide a total census of the area and several structures can comprise a compound. Model-based geostatistical methods were used to analyze the spatial variation of parasite prevalence, as determined by polymerase chain reaction (PCR), a measure for current infection, and by seropositivity, a measure of malaria exposure. Informative thresholds of risk were defined in order to identify foci in the spatial distribution of the two outcomes. The impact of the sample size on both the accuracy of prevalence estimates and the ability of the model to identify foci was assessed through a simulation study. Preliminary findings suggest that foci defined by the two outcome measures were measures were only moderately correlated (r=0.43) with only 36% of structures identified by both outcome measures. Among 14 discrete foci identified as having increased risk by one or both outcomes 6 clusters were identified by both metrics, although only 3 had good overlap. Five clusters (592 structures) were identified based on PCR but missed using seroprevalence, and 3 clusters (1214 structures) were missed using PCR but identified using seroprevalence. In terms of sample size, initial findings indicate that halving the sample size would have a minimal impact on model efficiency for generating the predicted surface for both outcome measures.

1522

UTILITY OF PCR-BASED SURVEILLANCE METHODS RELATIVE TO AGE IN RAINY AND DRY SEASONS IN SOUTHERN MALAWI

Jenny A. Walldorf[1], Jenna E. Coalson[2], Andy Bauleni[3], Kondwani Nkanaunena[3], Clarissa Valim[4], Lauren M. Cohee[1], Nelson Chimbiya[3], Jacqueline Fiore[5], Atupele Kapito-Tembo[3], Karl Seydel[6], Don Mathanga[3], Terrie E. Taylor[6], Miriam K. Laufer[1]

[1]University of Maryland Baltimore, Baltimore, MD, United States, [2]University of Michigan School of Public Health, Ann Arbor, MI, United States, [3]Malaria Alert Center, Blantyre, Malawi, [4]Harvard School of Public Health, Boston, MA, United States, [5]College of Osteopathic Medicine, East Lansing, MI, United States, [6]Blantyre Malaria Project, Blantyre, Malawi

Routine malaria surveillance often relies on microscopic or rapid diagnostic test (RDT) parasite detection, which misses low density infections. We have previously shown in cross-sectional surveys that school age children are at high risk of PCR-detectable asymptomatic infections in dry and rainy seasons. Using the same survey data, we now assess risk factors for low density infection to determine the utility of PCR by age and transmission setting. Submicroscopic infection was defined as *Plasmodium falciparum* detected by PCR but with a negative malaria smear read by quality-controlled microscopy. Sub-RDT infection was defined as PCR detection with microscopy negative or <200 parasites per microliter. Among all PCR-detected infections, infections were more likely to be submicroscopic in the dry (56%,180/319) vs. rainy season (38%, 208/544, p<.0001). The proportion of infections that were submicroscopic among adults (≥16 years), school age (6-15 years), and young children (≤5 years), was 72% (69/96), 55% (93/168), and 33% (18/54) in the dry season and 56% (99/178), 30% (81/274), and 30% (27/91) in the rainy season, respectively (p<.0001 both seasons). In mixed modeling, relationships between age and low density infections varied by season. In the rainy season, adults had 3.0 [95% CI: 1.7, 5.2] increased odds of submicroscopic infection, while school age children did not differ from young children. In the same communities in the dry season, both adults (OR 5.3 [2.6, 11.0]) and school age children (OR 2.5 [1.3, 5.0]) had increased odds of submicroscopic infection relative to young children. Associations were independent of district, net use, house materials, and gender. The relationship between age and sub-RDT infections followed a similar pattern. Microscopy and RDTs have inconsistent performance across age groups and seasons. While

both microscopy and molecular detection have demonstrated the highest malaria burden among school age children, surveillance with microscopy or RDT alone fails to detect most infections among adults and half of the infections in school age children.

1523

ESTIMATING THE MALARIA ATTACK RATE OF A TANZANIAN MILITARY COHORT IN THE SEARCH FOR NON-IMMUNE POPULATIONS FOR MALARIA PROPHYLAXIS, VACCINE AND TREATMENT STUDIES

Charles E. Mwanziva[1], Shekalaghe Seif[2], Lucky Temu[3], Sarah Chiduo[4], George Amoo[5], David Schnabel[6], Lalaine Anova[6], Colin Ohrt[6], Eyako Wurapa[6], Akili Kalinga[7], Christopher Mswanya[1], Yadon M. Kohi[1], Adam S. Mwambulanga[1]

[1]Tanzania People's Defence Forces, Dar es Salaam, United Republic of Tanzania, [2]Ifakara Health Institute, Bagamoyo, United Republic of Tanzania, [3]Tanzania People's Defence Forces, Walter Reed Program - Tanzania, United Republic of Tanzania, [4]Walter Reed Program - Tanzania, Dar es Salaam, United Republic of Tanzania, [5]Amethyst Technologies LLC, Baltimore, MD, United States, [6]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [7]National Institute for Medical Research, Tukuyu, United Republic of Tanzania

In Tanzania, malaria is the leading cause of outpatient and inpatient health service attendance, and accounts for about 32% of hospital deaths. The disease is unevenly distributed in the country. Some areas of Tanzania have little to no malaria transmission as a result of anti-malarial interventions (e.g., use of treated bednets and ACT) as well as differences in climate and geography, while other areas are highly endemic for malaria. Exposure to the malaria parasite gives individuals the ability to develop immunity and asymptomatic infection. However, naive populations from low-risk areas have no protective immunity and are at high risk of developing the disease. This study aims at determining the malaria attack rate of proposed non-immune individuals from non-malarious areas when entering a training camp in a highly endemic area. 500 recruits from Tanzania People's Defence Forces (TPDF) from non-endemic areas were selected by multistage random sampling; consenting, eligible participants were followed for six months. Malaria smears were collected every fortnight by active and passive detection of infection at the camp health facility. Blood samples for PCR and serological tests were collected. Malaria diagnosis was confirmed by malaria microscopy. There was a high rate of study subject follow-up; 98.1% (491/500) individuals participated in all activities, while 8 withdrew their participation for personal reasons. 21% (102/491) were terminated after confirmed malaria infection by clinical laboratory test (study end point), and one participant died from a non-malarial infection. The malaria attack rate was found to be as low as 18% to above 24%. *Plasmodium falciparum* was the predominant species detected. This study revealed one of very few non-immune populations with sufficient malaria exposure to conduct malaria prevention studies of a reasonable size, made possible by the heterogenous disease distribution in the country. TPDF recruits from non-endemic areas may be an ideal non-immune population for future malaria prophylaxis, vaccine and treatment trials.

1524

MALARIA INCIDENCE IN A DISTRICT WITH THREE ECOLOGICAL ZONES IN SOUTHERN GHANA

Alberta A. Quartey

Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Information on malaria incidence,age patterns of morbidity,mortality and serological exposure can be combined with entomological data to help target enhanced malaria control. Although the Dangme West District in Ghana hosts a research centre and has conducted many malaria interventions,the last malaria transmission measurement was undertaken in1994.We have therefore undertaken a new detailed malaria transmission study stratified by ecological zone. We followed a cohort of 2145 participants of all ages(715 per zone, selected by multistage cluster sampling)once a month from April 2011 to March 2012.A history of fever within the previous 2weeks was elicited at each visit and further information obtained by questionnaire from those with a positive history, after which a finger-prick blood sample was taken for the preparation of blood smears. Data was analyzed in STATA12.We completed 77% of all planned visits;8% of participants reported fever,3% had used an artemisinin combination therapy (ACT) for treatment of perceived fever and 6% had used an insecticide treated bed-net (ITN)the night before visits.The incidence of slide confirmed malaria per 1000 person years was 85 in the Forest,41 in the Coastal and 13 in the Lakeside zones.Verified ITN use the night before visits in each of the zones was 3%,4% and 9% respectively.The absence of a ceiling in a room was associated with an excess risk of malaria of 15%. Malaria incidence per 1000 person years was 119 in those aged 0-4years,136 in those aged 5-9,50 in those aged 10-19, 9 in those aged 20-29,18 in those aged 30-39 and 24 in those over 40 years of age.Overall rates had decreased by 40% from the 1994 levels.The Lakeside zone had the lowest incidence despite vast irrigated fields and the lowest access to ACTs.The Forest zone with the lowest verified ITN use and ownership(25%) and highest access to ACTs bore the brunt of morbidity.The data suggest that in an area of declining malaria transmission,efficient surveillance is required to promptly determine levels and patterns of morbidity to identify remaining foci for targeted interventions

1525

CONTRIBUTIONS OF WOMEN WITH CHILDREN AND YOUTH WORKERS TO SPATIAL MALARIA TRANSMISSION IN SUB-SAHARAN AFRICA

John M. Marshall[1], Mahamoudou B. Touré[2], André Lin Ouédraogo[3], Micky Ndhlovu[4], Samson S. Kiware[5], Ashley Rezai[1], Emmy Nkhama[4], Jamie T. Griffin[1], T. Deirdre Hollingsworth[6], Seydou Doumbia[2], Nico Govella[5], Neil M. Ferguson[1], Azra C. Ghani[1]

[1]Imperial College London, London, United Kingdom, [2]Malaria Research and Training Center, Bamako, Mali, [3]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [4]Chainama College of Health Sciences, Lusaka, Zambia, [5]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [6]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Recent investment in global malaria control has led to malaria prevalence dropping in many parts of the world. As people play a dominant role in parasite dispersal, a quantitative understanding of human movement patterns is relevant to determining how best to maintain gains made through these efforts. We conducted a survey of human movement patterns in four countries throughout sub-Saharan Africa - Mali, Burkina Faso, Zambia and Tanzania - with additional questions on malaria risk factors and cell phone usage behavior, the latter to enable anonymous cell phone signal data to be better-correlated with movement patterns. A total of 4,352 individuals were interviewed and 6,141 trips recorded. A cluster analysis highlighted two distinct traveler groups of relevance to malaria transmission - women traveling with children (all four countries) and youth workers (Mali). Women with children were predominantly between the ages of 16 and 45 and were more likely to travel to areas of relatively high malaria prevalence in Mali ($p<0.001$) and Zambia ($p=0.035$) compared to other travelers. They were also more likely to own bed nets in Burkina Faso ($p=0.001$) and Zambia ($p<0.001$), to use bed nets in Zambia ($p<0.001$) and Tanzania ($p=0.046$), and to own a cell phone in Mali ($p<0.001$), Burkina Faso ($p<0.001$) and Zambia ($p<0.001$). Taking into account that children are especially receptive to malaria parasites, women with children were estimated to account for the majority of spatial malaria transmission in Mali, Burkina Faso and Zambia. Malian youth workers were predominantly between the ages of 16 and 29 and were more likely to travel to areas of relatively high malaria prevalence ($p<0.001$) and for longer durations ($p<0.001$) compared to other travelers. They were estimated to make a

significant contribution to spatial malaria transmission in Mali. Knowledge of the spatial patterns of malaria transmission and the contributions of key traveler groups to this spread will assist in the design of control and surveillance programs targeting "hot spots" of malaria transmission.

1526

EPIDEMIOLOGY OF MALARIA INFECTION AMONG SCHOOL-AGED CHILDREN IN KINTAMPO NORTH DISTRICT, GHANA: AN EVALUATION OF BEHAVIOR, NUTRITIONAL STATUS, HOOKWORM CO-INFECTION AND ANTIBODY RESPONSES

Jennifer Du Mond[1], Benjamin T. Simms[1], Dylan Davey[2], Joseph Otchere[3], Debbie Humphries[1], Josephine Quagraine[3], Samuel Newton[4], Lisa Harrison[2], Daniel Boakye[3], Michael Wilson[2], Michael Cappello[2]

[1]Yale University School of Public Health, New Haven, CT, United States, [2]Yale University School of Medicine, New Haven, CT, United States, [3]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [4]Kintampo Health Research Center, Brong Ahafo, Ghana

A cross-sectional study was conducted in June 2010 in the Kintampo North Municipality, Ghana. Children (n=286) from 16 schools were enrolled after screening (n=844) if they had HAZ Z-score below -1.80 or above -0.10, with no more than one child from each household. Serum, fecal samples, and household surveys were used to assess the associations between the presence of malaria parasites, malaria parasitemia, and individual and household risk factors including nutritional status, hookworm co-infection, household risk prevention behaviors, and serum measures of parasite-specific immunoglobulin G (IgG). The primary factors associated with reduced risk of malaria infection included spraying the house in the past year (OR=0.04, p<0.001), the child having a health care visit in the past year (OR=0.39, p<0.001), household malaria in the past year (OR=0.37, p=0.001), higher hookworm antibody levels, and geographic location, while greater household food insecurity was associated with reduced risk of high levels of parasitemia. Primary risk factors for elevated parasite density included the house being sprayed in the past year (OR=9.83, p<0.001), household bednet usage (higher proportion of use associated with greater parasitemia), household and child history of malaria in the past year (OR=2.80, p=0.039; OR=0.15, p<0.001, respectively), frequency of consumption of protein-rich food groups, and geographic location, while those with the highest hookworm antibody levels showed reduced parasite density. Hookworm infection was associated with increased risk of malaria infection (OR=2.65, p=.10) and higher density of malaria parasites among those infected (OR=2.81, p=0.001). These risk factors highlight areas of programmatic interest, particularly the elevated risks of malaria infection and higher density of parasites among those infected with hookworm. Further research should elucidate the mechanism of this interaction, and treatment measures should focus on reducing the burden of hookworm in malaria endemic areas.

1527

PREVALENCE OF TWO NEWLY RECOGNIZED HUMAN MALARIA SPECIES IN MALI: *PLASMODIUM OVALE CURTISI AND P. OVALE WALLIKERI*

Nouhoum Diallo[1], Sangare P. Cheick[1], Antoine Dara[1], Aboubacrine Haidara[1], Bakari Fofana[1], Bakari Sidibe[1], Souleymane Dama[1], Omar B. Traore[1], Aliou Traore[1], Sekou Toure[1], Amadou Togo[1], Niawalou Dara[1], Moctar Coulibaly[1], Issaka Sagara[1], Khalid Bechir[2], Colin Sutherland[2], Ogobara K. Doumbo[1], Abdoulaye Djimde[1]

[1]University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

Ovale malaria is caused by two sympatric *Plasmodium ovale* sub-species; their biology and morbidity need to be investigated. In the present study we determine the presence and the prevalence of these sub-species in Mali using molecular analysis of blood blotted onto filter papers. Between 2011 and 2013, 7044 volunteers were screened by light microscopy in the context of various clinical studies conducted in five sites of Mali with different malaria epidemiology (Bougoula-Hameau, Faladje, Kolle, Pongonon and Sotuba). Thick smears were made and read onsite by experienced microscopists. Genomic DNA was extracted using Qiagen kits. First, ssrRNA-based PCR methods detecting the *P. ovale* specy were performed. Second, nested PCR of *P. ovale tryptophan-rich antigen (potra)* gene designed to distinguish the sub-species *P. ovale. curtisi and P. ovale. wallikeri* were run. Overall, 84/7044 (1.2%) of slides were positive for P. ovale. To date, 483 dried blood spots were analyzed by PCR. ssrRNA analysis revealed 12 (2.5%) cases of *P. ovale*. Potra analysis showed that 6/12 (50%) were *P. ovale curtisi*, 5/12 (41.7%) were *P. ovale wallikeri* and 1 sample was not sub-typable. We show the two recently described *P. ovale* sub-species were both present in Mali.

1528

USING DHIS2 FOR ROUTINE MONITORING OF QUALITY OF HEALTH SERVICES IN THE PRIVATE SECTOR

Purity Njagi[1], Martin Dale[1], Victor Lara[1], Preston Izulla[2], Stephanie Dolan[1], Nikki Charman[1]

[1]Population Services International, Nairobi, Kenya, [2]PS Kenya, Nairobi, Kenya

District Health Information System (DHIS2) has increasingly become the preferred Health information system for effective management of health data in many countries, mostly for collection and management of routine service data at various levels of the health sector. However, DHIS2 capabilities can also be optimised for routine monitoring of quality of health services in the private sector: Population Services International (PSI) is implementing a multi-country project aiming to create a market for malaria Rapid Diagnostic Tests (mRDTs) to improve quality of care in the private health sector by enabling effective treatment based on diagnosis. This project is being implemented by PSI in Kenya, Madagascar and Tanzania. The use of DHIS2 for the purpose of tracking provider level case management will be presented, with specific focus the power of DHIS2 to convert routine data into decision-making. DHIS2-enabled tablets can be used to (i) undertake provider quality of care and service preparedness assessments, (ii) automatically score and benchmark the provider's performance on site, and (iii) provide effective on the spot feedback for continuous improvement. The power of DHIS2 dashboards to manage and give feedback to providers will be highlighted, bridging information on provider quality of service, productivity based on caseloads, and behaviour change based on the adoption stairway. In addition, an innovative adaptation of DHIS2 to allow effective allocation of resources through automated supervision planning, taking into account provider quality of service benchmarks will be discussed (frequency and scheduling of provider assessments in particular). The presentation will further demonstrate how DHIS2 can enable program managers to effectively track service provision and make informed decisions leading to program quality improvement through the use of tailored dashboards. Potential future links with national systems will also be discussed, given the widespread use of DHIS2 by governments in the countries we work.

1529

USE OF SATELLITE IMAGERY TO ESTABLISH A SAMPLING FRAME AND MEASURE HOUSEHOLD MOVEMENT IN SOUTHERN ZAMBIA BETWEEN 2007 AND 2011

Timothy M. Shields[1], Jessie Pinchoff[1], William Moss[1], Jailos Lubinda[2], Harry Hamapumbu[2], Frank Curriero[1]

[1]Johns Hopkins School of Public Health, Baltimore, MD, United States, [2]Johns Hopkins School of Public Health, Macha, Zambia

High-resolution satellite imagery can be used to establish a sampling frame for epidemiologic research and to describe patterns of household

distribution and movement. Assessing the frequency and geographic distribution of household movement by comparing satellite images taken over time may suggest a time period for satellite image accuracy and utility for epidemiological research. All households in a 575 km2 region of southern Zambia were enumerated based on satellite images taken in 2007 and in 2011. Movement of households in the study area was assessed by comparing the images to calculate the percentage of households that were built, removed or stayed the same. We created a spatial intensity map to identify geographic areas of household movement, and to describe the spatial variation in household movement. There were a total of 3,287 household enumerated in 2007 and 3,721 in 2011. 970 households were newly observed in 2011 and 536 were no longer present. Reporting a net change of 434 households occurring over the four year period does not adequately describe the population movement within this region. Spatial variation around key features, such as around the new sealed road, points to non-uniform dynamics in population movement. These population dynamics may have implications for field studies working in this area over this time period.

1530

REDEFINING THE URBAN-RURAL CONTINUUM FOR MALARIA RISK: NEW APPROACHES TO CHARACTERIZING PATTERNS IN MALAWI

Peter S. Larson[1], Joseph N. Eisenberg[1], Carl H. Campbell[2], Don P. Mathanga[3], Mark L. Wilson[1]

[1]University of Michigan School of Public Health, Ann Arbor, MI, United States, [2]University of Georgia, Athens, GA, United States, [3]University of Malawi College of Medicine, Blantyre, Malawi

Urban and rural setting is often included in the analysis of infectious disease risk, as it may be an important determinant of pathogen transmission. This dichotomous designation, however, is politically defined, and masks the true biological and social causal determinants of risk. To improve understanding of disease patterns and targeting of interventions, transmission-specific characterization of the urban-rural continuum is needed. We developed and tested a composite measure for malaria in Malawi that included features such as health facilities, roads, rivers, lakes and electricity, along with census-derived population density. Analysis was based on a household-level survey of infection and various risk factors among 6-59 month old children. Geographic distances to features were estimated and a principal component analysis (PCA)-based composite measure for each household was produced for all points on a fine-scale grid throughout Malawi. Statistical relationships of all factors were tested against *Plasmodium* parasitemia status using multivariate regression based methods, including potential household-level confounding factors such as treated net use and material wealth. Urban-like and rural-like areas existed throughout Malawi, even within areas classified as "urban" and "rural" by the Malawi Government. Individual factors associated with urban and rural divides, including proximity to health services and roads, as well as population density, were associated with *Plasmodium* infection. Community-level factors associated with human settlements and urban development were predictive of decreased malaria risk, even in the presence of more traditional household-level prevention methods such as ITN use. Infection probability was similar for most "rural" areas, but declined linearly after a breakpoint with increasing urbanicity, as measured by the PCA based composite. Dichotomized measures of urban and rural spaces fail to adequately characterize environments which might be associated with risks for infectious disease transmission. Politically determined destinations may ignore "urbanized" pockets within traditionally "rural" areas, and rural-like spaces within areas classified as "urban." A composite measure which analyzes many factors associated to varying degrees with spaces roughly defined as rural and urban presents an opportunity to refine places of disease risk.

1531

MALARIA ATTRIBUTABLE FRACTION TO FEVER IN A COHORT OF PAPUA NEW GUINEAN CHILDREN

Anna Rosanas-Urgell[1], Inoni Betuela[2], Benson Kiniboro[2], Danielle I. Stanisic[2], Lorna Samol[2], Leanne J. Robinson[2], Peter Siba[2], Ivo Mueller[3]

[1]Institute of Tropical Medicine, Antwerp, Belgium, [2]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [3]Walter and Eliza Hall Institute, Parkville, Australia

In areas of high malaria endemicity, a high proportion of individuals is asymptomatic, thus are infected with malaria parasites without developing clinical symptoms. In addition, those who develop symptoms in the presence of malaria parasites, may be ill due to non-malaria diseases. Therefore, a case definition able to distinguish between malaria and non-malaria morbidity is essential to correctly estimate the burden of disease, design adequate control strategies -including case management-, and measure the effect of malaria interventions. In a cohort of 500 Papua New Guinean children aged 1 to 5 years and followed up for 9 months, logistic regression methods were used to estimate the risk of fever by parasite density above specific cut-offs. *Plasmodium falciparum* and *P. vivax* attributable fraction (AF) and population attributable fraction (PAF) to fever were calculated from odds ratio estimates for each case definition. Sensitivity and specificity of case definitions were also evaluated. *P. falciparum* AF ranges from 79% to 94% when all parasite densities and densities higher than 50000 P/µL are used as cut-off values, respectively. Overall, the PAF of fever to *P. falciparum* was 16% when all parasite densities in the presence of fever were considered. On the other hand, *P. vivax* AF increased from 9% (all parasite densities) to 85% when parasites densities higher than 10000 P/µL were used. *P. vivax* PAF exhibit the highest values (5-7%) when a cut-off of >1500 P/µL was used. Estimates of the sensitivity and specificity of case definitions cut-off by parasite density show that a low *P. falciparum* cut-off (<2500 P/µL) achieves high sensitivity (80-100%), while when only using high parasite density cut-off values high specificity (90%) is obtained. 80% sensitivity and specificity is achieved with *P. vivax* cut-off value > 10000 P/µL.Approximately 80% to 94% of fevers with *P. falciparum* infections occurring in Papua New Guinean children aged 1 to 5 years are attributable to malaria regardless of parasite densities. On the other hand, only 9% of fevers occurring in the presence of *P. vivax* infections, at any parasite density, are attributable to malaria, suggesting that clinical tolerance against low density *P. vivax* infections is already acquired at this young age.

1532

PRIVATE SECTOR READINESS FOR MALARIA CASE MANAGEMENT AND MALARIA MARKET COMPOSITION BEFORE AND AFTER THE AFFORDABLE MEDICINES FACILITY - MALARIA (AMFM): RESULTS FROM THREE PILOT COUNTRIES

Megan Littrell[1], Julius Njogu[2], Raymond Sudoi[2], Kevin Duff[2], Hana Bilak[3], Vamsi Vasireddy[4], Kate O'Connell[5], Stephen Poyer[2]

[1]Population Services International, Washington, DC, United States, [2]Population Services International, Nairobi, Kenya, [3]Population Services International, Chevry, France, [4]Independent Consultant, Chicago, IL, United States, [5]Independent Consultant, Yangon, Myanmar

People living in malaria endemic countries often turn to the private sector for fever case management. However, private sector markets are typically characterized by low levels of readiness for appropriate malaria case management, particularly in comparison with public health facilities. Efforts to improve this have included the Affordable Medicines Facility - malaria (AMFm), which aimed to improve availability and affordability of quality assured Artemisinin Combination Therapy (ACT) in both public and private sectors. The pilot demonstrated favorable improvements in private sector readiness for malaria case management in most countries. The private sector comprises a diverse set of actors, including regulated pharmacies and health facilities, and unregulated drug shops and general

retailers. We examine the level of private sector readiness post-AFMm, and compare private sector market composition before, during, and after the pilot in Madagascar, Nigeria and Uganda. Trends in key readiness indicators are examined, including availability of blood testing and ACT, and provider knowledge. Favorable trends in private sector readiness may be driven by improvements across existing market actors, or may be an indirect effect of shifting market composition towards a market dominated by regulated market actors. In the context of varying degrees of improvement, we examine the private sector malaria market composition over time. Multiple nationally representative outlet surveys were conducted between 2009 and 2013 by the ACTwatch project in the 3 countries. During each survey, a census of all outlets with the potential to sell antimalarials was conducted, allowing examination of relative market composition and antimalarial market share between and within sectors. Private sector readiness improved over time in all 3 countries although to varying degrees across contexts and measures of readiness. We examine readiness trends over time in relation to shifts in market composition, and discuss implications for improving private sector readiness for malaria case management.

1533

TRENDS IN AVAILABILITY OF MALARIA MEDICINES AND DIAGNOSTICS IN KINSHASA, DR CONGO FROM 2009-2013

Julie Archer[1], Godéfroid Mpanya[2], Stephen Poyer[1], Antoinette Tshefu[3], Willy Onema[2], Jamie Ciesla[2], Joris Losimba Likwela[4], Vamsi Vasireddy[5], Megan Littrell[6]

[1]Population Services International, Nairobi, Kenya, [2]Association de Santé Familiale/PSI, Kinshasa, Democratic Republic of the Congo, [3]Ecole de Santé Public de Kinshasa, Kinshasa, Democratic Republic of the Congo, [4]National Malarial Control Program, Kinshasa, Democratic Republic of the Congo, [5]Independent Consultant, Chicago, IL, United States, [6]Population Services International, Washington, DC, United States

Malaria is a leading cause of illness and death in the Democratic Republic of the Congo (DRC) where the malaria disease burden is estimated to be the second highest in the world. Malaria is endemic throughout the country, including in the large urban agglomeration of Kinshasa. National policy has recommended diagnostic testing since 2006, and the national treatment protocol includes two artemisinin-based combination therapies (ACT) for first-line treatment. However, according to past surveys, access to and use of malaria diagnostics and ACT remains low. A 2009 survey conducted by ACTwatch found that 60% of antimalarial-stocking public and private sector outlets in Kinshasa had ACT in stock, but a 2010 ACTwatch household survey found that just 15% of children with fever had received a diagnostic test for malaria and only 4.5% of those with fever had received an ACT. In a late 2013 follow-up to the 2009 survey, ACTwatch conducted a representative cross-sectional survey of 3,654 public and private sector health facilities and retail outlets in Kinshasa to assess the availability, price and market share of antimalarials and malaria diagnostics. Findings from this survey and a trend analysis will be presented. Preliminary results show that availability of malaria diagnostics remains low and the antimalarial market is still dominated by quinine in the private sector. Results will be examined in the context of qualitative research findings from in-depth interviews conducted in 2014 in Kinshasa with private doctors, pharmacists, drug shop owners and clients. There are renewed efforts in DRC to increase the quality of fever case management following World Health Organization recommendations calling for universal parasitological confirmation before treatment. This survey of the landscape of availability of ACT and malaria diagnostics, as well as ACT market share will inform renewed case management efforts in Kinshasa that can then be scaled up throughout the country.

1534

EFFECTS OF AGE AND CONTROL INTERVENTIONS ON PREVALENCE OF *PLASMODIUM FALCIPARUM* GAMETOCYTEMIA DURING PEAK MALARIA TRANSMISSION SEASON IN WESTERN KENYA

Zhiyong Zhou[1], Rebecca M. Mitchell[1], Simon Kariuki[2], Christopher Odero[2], Peter Otieno[2], Kephas Otieno[2], Vincent Were[2], Mary Hamel[1], John Gimnig[1], Edward D. Walker[3], Meghna Desai[1], Ya Ping Shi[1]

[1]Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]Department of Microbiology and Molecular Genetics, East Lansing, MI, United States

Gametocytes are the sexual stage of *Plasmodium* parasites responsible for malaria transmission from human to mosquito host. However, risk factors for gametocytemia such as age and ameliorating effects of interventions (antimalarial and ITN use) are poorly understood. We measured the prevalence and density of *P. falciparum* gametocytes by pfs25 gametocyte mature stage V marker using real-time quantitative nucleic acid sequence-based amplification assay (QT-NASBA) and by pfg377 female gametocyte marker using qRT-PCR on samples from a cross sectional survey conducted at peak transmission season in 2012 in Siaya County of western Kenya. We also measured parasitemia using 18S QT-NASBA. Total of 446 samples (225 malaria smear-positive and 221 randomly selected smear-negative) from 832 individuals were used to determine gametocyte carriage. Overall, 18S-NASBA detected 354 positives, of which 129 infections were from smear-negative individuals. Pfs25 was detected in 78.7% of smear positive and 10.9% of smear negative samples while pfg377 was found in 55.1% of smear positive and 2.7% of smear negative samples. In multivariable analysis, children (<5 and 5-15 years old) were more likely positive with pfs25 and pfg377 than adults >15 years old (pfs25: OR 3.4, CI 2.0-6.0 and OR 4.0, CI 2.1-7.7; pfg377: OR 15.3, CI 6.3-37.1 and OR 7.6, CI 3.0-19.3, respectively). Children <5 years were more likely pfg377 positive than children 5-15 years old (OR 2.0, CI 1.2-3.4); however, gametocyte density detected by pfg377 or pfs25 did not differ between these two age groups of children. Anemia (Hb < 11 g/dl) was associated with higher 18S density (1.45x per log10, CI 1.18-1.80, p=0.0005). Importantly, anemia was also associated with pfs25 and pfg377 positive status (OR 2.1, CI 1.4-3.4 and OR 2.4, CI 1.4-3.9). The odds of being pfs25 positive were lower in individuals using ITNs (OR 0.41, CI 0.23-0.71). No differences were seen for pfs25 density between individuals with and without ITN use. Antimalarial use (90% artemether-lumefantrine) during the two weeks prior to the survey was associated with fewer pfs25 carriers (OR 0.32, CI 0.17-0.62), but not with pfs25 density. These results show that children provided the highest gametocyte reservoir compared to adults and anemia was associated with an increased risk of gametocytemia. ITN and antimalarial use decreased the gametocyte prevalence, but not the gametocyte density in the study population.

1535

IDENTIFYING FACTORS ASSOCIATED WITH MALARIA PARASITEMIA IN MOZAMBIQUE USING A GEOSTATISTICAL MODEL

James Colborn[1], Kathryn Colborn[2], Edward Wenger[2], Guidion Mathe[3], Graca Matsinhe[3]

[1]Centers for Disease Control and Prevention, Maputo, Mozambique, [2]Intellectual Ventures, Seattle, WA, United States, [3]National Malaria Control Program, Maputo, Mozambique

Malaria is among the largest contributors to child mortality in Mozambique. Donors such as the Global Fund and the US President's Malaria Initiative have been successful in controlling malaria by supporting national scale-up of interventions, though transmission remains high in many parts of the country. Malaria Indicator Surveys (MIS) and Demographic Health Surveys (DHS) are intermittent household surveys

aimed at collecting nationally representative demographic and health related data, including socio-economic status (SES), insecticide treated net (ITN) ownership and use, coverage with indoor residual spraying (IRS), and malaria parasitemia prevalence by rapid diagnostic test (RDT) for children 6-59 months of age. When examined independently, these surveys may not capture annual variation in malaria transmission. To overcome this, we combined both the 2007 MIS and the 2011 DHS data to capture the inherent spatial and temporal variation in environmental factors and malaria transmission. Using these data, we estimated the associations between relevant factors and interventions and cluster-level parasitemia prevalence using a geostatistical model. This model included a fixed factor for survey year, IRS coverage, ITN use, age, SES (wealth quintiles), environmental factors, and a spatial random effect. Our results suggested that higher SES (OR=0.79; 95% CI 0.73, 0.85) and coverage of IRS up to 40% (OR=0.93; 95% CI 0.88, 1.00) were associated with decreased odds of parasitemia, and higher monthly average enhanced vegetation index (increased greenness; OR=5.10; 95% CI 1.83, 16.91) was associated with increased odds of parasitemia; ITN use was not associated with parasitemia, although this could be related to the low levels of ITN use in both survey years (cluster medians=0% and 33%, respectively). These findings suggest that socioeconomic status and vegetation are important factors to consider for understanding malaria transmission. Additionally, the lack of evidence using this analytic method for additional protection by IRS coverage above 40% bears further investigation.

1536

ASSESSING THE IMPACT OF CLINICAL MENTORSHIP ON MALARIA DIAGNOSIS AND TREATMENT PRACTICES IN UGANDA

Deepa K. Pindolia[1], Kathleen Maloney[1], Alex Ogwal[2], Chelsea Solmo[1], Vicky Abenakyo[2], Albert P. Okui[3], Anthony Mbonye[3], Bosco B. Agaba[3], Lindsay Bryson[1], Caitlin Dolkart[1], Justin M. Cohen[1], Arnaud Le Menach[1]

[1]Clinton Health Access Initiative, Boston, MA, United States, [2]Clinton Health Access Initiative, Kampala, Uganda, [3]Ministry of Health, Kampala, Uganda

To address problems associated with malaria misdiagnosis and inappropriate treatment, Uganda has implemented nationwide case management trainings, and procured Rapid Diagnostic Tests (RDTs) widely in the public sector since 2012. Despite these efforts, only 60% of suspected malaria cases were tested and 35% of negative tests were provided with anti-malarials in early 2014. In this study, a clinical mentorship for health workers, as a potential tool to overcome barriers of testing and adherence, and to improve overall fever case management is evaluated. This is a cluster randomized control trial, with one control and two intervention arms: one with district-selected peer mentors and one with facility in-charges as mentors. Mentorships occur on a monthly basis over 6 months, starting in April 2014 in 150 public health facilities distributed in 17 pilot medium endemicity districts. Diagnosis and treatment patient-level data are collected at monthly intervals in all study facilities. Multivariate logistic regressions will be performed to assess the impact of clinical mentorship on confirmatory diagnosis and on adherence to test results adjusted for covariates such as age, health facility level and district locations. At baseline, there were no significant differences in clinical diagnosis and adherence between any of study arms. Over 6 months, it is expected that clinical mentorship improves confirmatory diagnosis and adherence to test results in both intervention arms, and that differences of at least 10% are identified pre and post intervention and between each intervention arm and the control group. Clinical mentorship can work as an effective method to increase confirmatory diagnosis and adherence to test results, and improve overall fever case management, providing countries with a novel and effective tool to meet their national malaria case management targets.

1537

COLLABORATIVE DATA MANAGEMENT FRAMEWORK FOR BORDER MALARIA RESEARCH IN SOUTHEAST ASIA ICEMR CENTER

Ming-Chieh Lee[1], Ying Wang[2], Kirakorn Kiattibutr[3], Guofa Zhou[1], Jeeraphat Sirichaisinthop[4], Jetsumon Prachumsri[3], Liwang Cui[5], Guiyun Yan[1]

[1]University of California Irvine, Irvine, CA, United States, [2]Third Military Medical University, Chongqing, China, [3]Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [4]Vector Borne Disease Training Center, Saraburi, Thailand, [5]Pennsylvania State University, University Park, PA, United States

Document and data archiving for the research activities are the tedious but important tasks through the whole scientific research project. It is a challenge for the scientist to maintain a solid, flawless and well organized data structure and data entry procedures especially for the project included international collaboration within multi-cultures and languages. Southeast Asia ICEMR center is focused on bolder malaria transmission between several Southeast Asia countries. We collaborated with several Universities, government agencies, local hospital and clinics, and field research labs in the remote border area of China, Myanmar, and Thailand. How to standardize the field survey and data entry procedures and semi-real-time to share research results is the goal for data manager and coordinators to conquer. Therefore, we design multi-language survey forms and data entry system as the tools to provide consistent interface and user experience for all research staffs. In order to expedite the data sharing and secured data services, we utilized several open source applications and cloud computing services to sustain our database system. To minimize the catastrophe of computer hardware/software collapse or network traffic congestion, the failover and load balancing features were setup in main server and several mirror servers and offsite remote backup scheme were implemented. To maintain the maximum data quality and structure, the standard operating procedures (SOPs) for field survey, data entry, data QA/QC were developed and documented. Currently the database system is online since July 2012.

1538

MALARIA PREVALENCE AND SPOROZOITIC INDEX IN SUBURBAN AREA IN KINSHASA BEFORE ITN MASS DISTRIBUTION, DR CONGO

Aimée Kahindo, Betricia Mavuzi, Freddy Kabasele, Serge M. Kayembe, Tommy M. Nseka, Solange E. Umesumbu, Thierry L. Bobanga

University of Kinshasa, Kinshasa, Democratic Republic of the Congo

Malaria is a parasitic disease due to *Plasmodium* transmitted by a female mosquito of *Anopheles* genus. It is a public health problem which causes a high mortality in less 5 years children. Estimated deaths by malaria word report are around 60000. This study interest is to update data on malaria prevalence in Kinshasa suburb area, in order to provide data for the stratification and control of the disease. The objectives of the study were to assess malaria prevalence into sub urban Kinshasa area population, to determine the most concerned population group, to identify plasmodial species and to determine sporozoitic index. An analytical cross-sectional survey was conducted in the village of LUZIZILA to 329 people whose age ranged between 6 months and 76 years for the period from August 10 to 25, 2013 Blood sample for a thick, a thin smear was conducted to determine the prevalence of malaria and determining plasmodial species. The sporozoitic index was determined by ELISA Results The overall prevalence of positive thick smears was 53.2%. In the age group under 5 years, prevalence was higher with 68.8%. Between 6 and 15 years it was 55% beyond 15 years the prevalence stood at 37.3%. *P. falciparum* was found in 97.7% of cases in thin smear slides The sporozoitic index was 11%. In conclusion, the prevalence found place Luzizila in an hyperendemic area. After the distribution we can expect a reduction of these indices

1539

MALARIA INFECTION IS ASSOCIATED WITH PREGNANCY LOSS IN OUELESSEBOUGOU, MALI

Almahamoudou Mahamar[1], Naissem Andemel[2], Youssoufa Sidibe[1], Adama Dembele[1], Moussa Traore[1], Oumar Attaher[1], Moctar Tounkara[1], Djibrilla Issiaka[1], Kadidia B. Cisse[1], Bakary S. Diarra[1], Moussa B. Kanoute[1], Amadou Barry[1], Patrick E. Duffy[2], Alassane Dicko[1], Michal Fried[2]

[1]Malaria Research and Training Center, Faculty of Medicine, Pharmacy and Dentistry, University of Sciences Techniques and Technologies of Bamako, Bamako, Mali, [2]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

In malaria endemic areas, pregnant women are more susceptible to malaria infection compared to their non-pregnant counterparts. While the relationships between pregnancy malaria (PM) and outcomes such as severe maternal anemia and low birth weight are well established, there have been limited studies on the relationship between PM and pregnancy loss particularly in areas of high malaria transmission. We evaluated the relationship of fetal loss to malaria infection among pregnant women in Ouelessebougou Mali from November 2010 to January 2014. Pregnant women were enrolled during the antenatal consultation visits and followed up to delivery. Malaria infection in peripheral blood was detected by bloodsmear, and submicroscopic infection by PCR when the BS was negative. The proportion of women with submicroscopic malaria infection at delivery was 25.5% and pregnancy loss occurred in 5.8% of the cohort (80/ 1,377). Submicroscopic infection at delivery was associated with increased odds of fetal loss (unadjusted OR = 3.26, 95% confidence interval (CI) 1.35 - 7.89; and adjusted OR = 3.35, 1.37 - 8.16). A recent positive bloodsmear also increased the odds of fetal loss. In summary, preliminary analysis indicates that a submicroscopic malaria infection is associated with four times increase in odds of the pregnancy loss.

1540

ASYMPTOMATIC INFECTIONS AND MALARIA TRANSMITTED BY BLOOD TRANSFUSION: AN INVISIBLE RISK

Giselle F. Lima[1], José E. Levi[1], Maria E. Carvalho[2], Juliana Inoue[1], Angélica D. Hristov[1], Luis G. Nascimento[2], Arianni R. Sanchez[1], Eduardo M. Ramos-Sanchez[1], Maria J. Costa-Nascimento[2], Silvia M. Di Santi[1], Maria Carmen A. Sanchez[1]

[1]São Paulo University, São Paulo, Brazil, [2]SUCEN, São Paulo, Brazil

Transfusion transmitted malaria represents a major challenge, essentially due to the occurrence of asymptomatic infections. The vector transmission in Brazil mainly occurs in the Amazon Region, where 166,864 cases were notified in 2013. Outside the endemic region sporadic cases of autochthonous malaria are reported, including asymptomatic carriers of *Plasmodium*. In the state of São Paulo, transfusional cases were detected, due to asymptomatic donors harboring *P. malariae*, one of them leading to the death of the recipient. The occurrence of parasitemia without clinical symptoms in addition to the fact that *Plasmodium* can survive in stored red blood cells between 2 and 6º C for up to three weeks, increases the risk of transmission. In order to minimize the possibility of transfusional cases, the use of platforms including molecular and serological tests might point out donors suspected of harboring *Plasmodium*. We tested samples from 56 candidates for blood donation living in an area of São Paulo State where asymptomatic infections are reported. Thick blood smear, PCR, ELISA with recombinant *P. vivax* MSP119 antigen and SD Bioline Malaria Pf/Pv immunochromatographic test were used. Two samples (3.5%) (0.98 -12.1) were positive by thick blood film for *Plasmodium*, in a very low parasitemia. One of them was also positive by PCR, indicating the presence of *P. malariae*. ELISA detected 53.6% (40.7- 65.9) of samples reagent for *P. vivax*, with Reactivity Index ≥1.0. SD Bioline detected antibodies against *P. vivax* MSP and CSP recombinant antigens in 48.2% (35.6 - 60.9) of the samples. The frequency of positive samples in the serological tests pointed out to the risk of transfusional malaria, even in areas of low endemicity, since asymptomatic donors could be accepted based on clinical screening. Moreover, the lack of knowledge about this silent malaria outside the Amazon Region increases the risk of transmission. The use of platforms with different approaches could minimize this invisible risk.

1541

SUB PATENT INFECTION OF *PLASMODIUM FALCIPARUM* IN NORTHWESTERN PERU

Juan Jose Contreras-Mancilla[1], Dionicia Gamboa[2], Angel Rosas-Aguirre[1], Alejandro Llanos-Cuentas[1], Socrates Herrera[3]

[1]Instituto de Medicina Tropical Alexander von Humboldt, Lima, Peru, [2]Instituto de Medicina Tropical Alexander von Humboldt, Departamento de Ciencias Celulares y Moleculares, Facultad de Ciencias, Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Latin American Center for Malaria Research, Cali, Colombia

The North-western region in Peru, is an area categorized as low endemicity for malaria after the El Niño Southern Oscillation (ENSO) phenomenon that increased the number of malaria cases to a peak with more than 200 000 cases in 1998 for both species, *Plasmodium vivax* and *P. falciparum*. The new treatment scheme implement in 2000 in this area decreased the number of *P. falciparum* cases in this area and since 2008, no cases have been reported by Ministry of Health. In Peru, microscopy is the diagnostic test used as routine by the Ministry of Health; but there are other technics as the Polymerase Chain Reaction (PCR), which is more sensitive to detect and identified correctly the species of *Plasmodium*, under the microscopy detection limit. In June of 2013, a total of 750 individuals from 3 urban areas were enrolled in a surveillance study in Piura, a malaria endemic region North-western of Peru: 350 from Bellavista, 329 from Obrero and 71 from Querecotillo. From each individual, a blood sample was taken to prepare 2 slides for microscopy and a filter paper for PCR diagnostic. Microscopy diagnosis was performed twice, one a local level and the second one by an expert microscopist as quality control. The DNA extraction from the filter paper was done by the Chelex-100 method and the PCR was based in a Real time protocol using specific probes to detect *P. falciparum* and/or *P. vivax*. No malaria cases was detected by microscopy; but PCR detected two positive cases for *P. falciparum* only, one case was located in Obrero and the other one in Bellavista. The parasitaemia level in both cases was lower than 450 parasites/µL and no symptom was present at moment when the sample was taken. These results showed the presences of *P. falciparum* in the North-western region of Peru and stress out the need to implement more sensitive tools for malaria diagnostic in areas of low endemicity where microscopy cannot detect if the country aims to improve control measures looking into malaria elimination.

1542

IMPLICATIONS AND EFFECTS OF DIVERSE *PLASMODIUM VIVAX* RELAPSE DISTRIBUTIONS IN SIMULATIONS OF VARYING TRANSMISSION SETTINGS

Philip A. Eckhoff, Caitlin A. Bever, Jaline Gerardin, Edward A. Wenger

Institute for Disease Modeling, Bellevue, WA, United States

Control and elimination of *Plasmodium vivax* is complicated by recurring relapses from hypnozoites in the liver of infected individuals. Different strains of vivax exhibit different patterns of relapse, ranging from the Chesson strain with an initial infection followed by early relapses, to strains with mixes of short and longer relapses, to North Korean strains that have infrequent early infections and longer latencies to relapse. Which strains predominate in a given geographic region depends on the local transmission setting, and earlier observational and modeling studies by various groups have allowed classification of different malaria zones. We present a new model for *P. vivax* transmission, host interactions, and relapse distributions and incorporate it into the EMOD model for malaria transmission. The broad diversity of relapse patterns is recreated with

a simple set of biological and immunological mechanisms, providing a mechanistic mathematical framework for comparing different strains. The fitness and population-level effects of different relapse patterns are then explored for a variety of transmission settings, with reference to earlier work by others on classification of malaria zones. Finally, implications of different relapse patterns for control and elimination efforts scaling up in the Solomon Islands and other settings are explored in simulation. Interventions simulated include primaquine and chloroquine combinations and vector control.

1543

NONINVASIVE SURVEILLANCE OF ZOONOTIC MALARIA

Josephine Siregar[1], Christina Faust[2], Lydia Murdiyarso[1], Andrew Dobson[2]

[1]Eijkman Institute of Molecular Biology, Jakarta, Indonesia, [2]Princeton University, Princeton, NJ, United States

The fifth human malaria parasite, *Plasmodium knowlesi*, is a novel public health threat in Southeast Asia. The parasite is primarily found in macaques, but within the last decade it has been increasingly recorded in humans, particularly on the island of Borneo. Human malaria treatment is effective for the parasite in humans, but to prevent transmission in the first place a better understanding of prevalence in its natural hosts is necessary. The objectives of this research are to develop and optimize noninvasive sampling methods for macaque malaria in wild populations. By using naturally infected macaques, we will compare blood and fecal samples to determine if noninvasive samples offer a logistical solution to widespread surveillance of macaque malarias. Results of this project will be applied to field collected specimens and inform experimental designs for surveillance of this pathogen in Borneo and elsewhere. Results from this work are essential for understanding malaria prevalence in macaque hosts and controlling emergence of the pathogen in new human populations.

1544

A COMPARATIVE CASE CONTROL STUDY OF THE DETERMINANTS OF CLINICAL MALARIA IN THE GAMBIA

Kalifa A. Bojang[1], Julia Mwesigwa[1], Eugene L. Kama Kama[2], Serign J. Ceesay[1], Serign J. Ceesay[1], Fanta Njie[1]

[1]MRC Unit, Banjul, Gambia, [2]National Institute of Public Health, Conakry, Guinea

The massive deployment of life saving malaria interventions has not only resulted in a decline in disease burden but a change in the risk of infection and disease. The study reassesses the importance of known risk factors and reviews socio-demographic determinants of malaria risk in the population. We conducted a case-control study involving 150 children aged 6 months to 12 years with slide-confirmed malaria recruited from the outpatient clinics of three health facilities (cases) in the Greater Banjul area, The Gambia. One hundred and fifty controls were matched on age, residence, and were negative for malaria. We collected information on the use of long lasting insecticidal nets, occupation of parents, housing structure, knowledge of malaria and socio-demographic factors. The mean age of study participants was 6.8 (SD 3.3) years with 147 (49%) being males. Significant determinants of malaria risk were parent's occupation: mother as trader (OR 0.18, 95% CI 0.04 - 0.73, p = 0.017), father as trader (OR 0.02, 95% CI 0.002- 0.193, p = 0.001), civil servants (OR 0.04, 95% CI 0.008- 0.257, p =0.001) or handyman (OR 0.03, 95% CI 0.005- 0.182, p < 0.001). Children sleeping in rooms with windowpanes had a 76% reduction in their odds of malaria (OR 0.24, 95%CI 0.07-0.82, p = <0.022. Household socio-economic status plays an important role in management of illnesses. The ability of mothers to engage in an occupation increases household resources to access healthcare and on time. The balance between the type of mother's occupation and her time available to supervise the child is an interesting emerging issue that needs further investigation.

1545

ANTIBODIES TO *PLASMODIUM VIVAX* MSP1-19 RECOMBINANT ANTIGEN IN BLOOD DONORS FROM BRAZILIAN LOW ENDEMIC AREAS

Arianni R. Sanchez[1], Eduardo M. Ramos-Sanchez[1], Silvano Wendel[2], Silvia M. Di Santi[1], Hiro Goto[1], Maria Carmen A. Sanchez[1]

[1]São Paulo University, São Paulo, Brazil, [2]Sírio Libanês Hospital, São Paulo, Brazil

In non-endemic and low endemic areas, transfusion-transmitted malaria (TTM) is a rarely reported event. However, four TTM were detected in São Paulo State, in Southeastern Brazil, including one death. Infected donors were identified as asymptomatic carriers with displacements to the Atlantic forest biome in São Paulo State. Due to the immune status of these donors, the parasite densities are low and undetectable in the thick blood smear or rapid diagnostic tests requiring the use of other methods for detection in blood banks outside the endemic areas. In this study, since *Plasmodim vivax* is the most prevalent species in Brazil, we assessed the prevalence of anti- *P. vivax* MSP1-19 IgG antibodies among blood donors from Southeastern Brazil. Initially, for validation, ELISA-PvMSP1-19 was assayed with 197 sera from patients with positive thick-blood smear for *P. vivax* yielding 96.95% sensitivity. A specificity of 100.0% was achieved in serum specimens from 101 normal individuals and 98.21% in 168 serum specimens from other diseases patients. After validation, 1,974 blood bank serum samples were tested: 1,309 from São Paulo and 665 from Rio de Janeiro. These samples were collected after the donors had been screened by clinical parameters, provided they were considered fit to donate and had signed the informed consent form. Regarding samples from São Paulo, 1.15% (N=15) positivity was achieved. In Rio de Janeiro samples, the positivity was 1.65% (N=11). The reactivity index (RI) of the positive samples ranged from 8.98 to 1.16 (Sao Paulo) and 13.03 to 1.08 (Rio de Janeiro). The detection of specific antibodies is not necessarily a marker of parasitemia or disease, but the detection of anti- *P. vivax* IgG antibodies in blood bank donors in non-endemic areas constitutes an alert that impel us to review the adopted criteria for screening of the donors aiming to reduce the risk of TTM in these areas without losing donations.

1546

HOUSEHOLD-LEVEL SOCIAL AND ENVIRONMENTAL FACTORS ASSOCIATED WITH BED NET OWNERSHIP AND DIFFERING MALARIA PREVALENCE IN SOUTHERN MALAWI

Lindsay R. Townes[1], Dyson Mwandama[2], Don P. Mathanga[2], Mark L. Wilson[1]

[1]University of Michigan, Ann Arbor, MI, United States, [2]Malaria Alert Centre, Blantyre, Malawi

Since 2002, Malawi's primary malaria prevention program has been a nationwide, health facility-based distribution of insecticide-treated nets (ITNs). Despite these efforts, ITN ownership in households with under-5 children remains sub-optimal. Knowing what characterizes such low-ownership households may improve targeting of ITN distribution. From June to August 2011, data on household ITN ownership, child malaria status, house location, building materials, and nearby environmental characteristics were collected by cross-sectional survey from 398 households in two rural Traditional Authorities (TAs) of southern Malawi, Sitola and Nsamala, which were in the catchment area of Machinga District Hospital (MDH). The proportion of households in Sitola reporting bed net ownership was significantly lower (OR 0.59, 95% CI 0.39-0.89), and the prevalence of malaria among under-5s significantly higher (OR 4.20, 95% CI 2.74-6.46) than in Nsamala, this despite higher bed net use among owners in Sitola (OR 2.56, 95% CI 1.00-6.57). Households in Sitola were also much more likely to be located within 50 m of active agriculture (OR 9.60, 95% CI 4.93-18.68), of brick-making sites (OR 4.59, 95% CI 2.34-9.01), and of water sources (OR 11.87, 95% CI 3.49-40.40). Households in the two TAs did not differ with respect to housing materials,

recent or current maternal pregnancy, number of children, or number of household residents. Among those with a bed net, there was no difference in whether they had received their bed net at MDH or whether the net was insecticide-treated. Curiously, within the higher malaria prevalence context of Sitola, ITN ownership was not significantly associated with child malaria status, land use/land cover, quality of housing materials, nor recent maternal pregnancy; however, current maternal pregnancy was inversely associated with net ownership (OR 0.35, 95% CI 0.13-0.95). In contrast, current maternal pregnancy had no association with net ownership in Nsamala (OR 0.69, 95% CI 0.31-1.50), while houses built with higher quality materials were more likely to own at least one bed net (OR 3.31, 95% CI 1.20-9.12). These results suggest a geographical disparity in ITN distribution between Nsamala and Sitola, which may be reduced through improved targeting of pregnant women in Sitola.

1547

MALARIA TRANSMISSION IN BOLENGE HEALTH ZONE, EQUATORIAL SETTING, DR CONGO

Thierry L. Bobanga[1], Billy S. Kunyu[2], Solange E. Umesumbu[3], Olivier A. Fataki[1], Prosper Abanda[4], Dieudonné N. Mumba[1]

[1]University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [2]National Institute of Biomedical Research, Kinshasa, Democratic Republic of the Congo, [3]National Malaria Control Program, Kinshasa, Democratic Republic of the Congo, [4]Bolenge Health Zone, Mbandaka, Democratic Republic of the Congo

Malaria constitutes a public health major problem in Democratic Republic of Congo (DRC). For malaria control , one of adopted approach is Insecticide treated Net(ITN). In order to evaluate the malaria transmission, level in a area , different parameters are therefore used. One of them is the parasitic parameter which includes plasmodic index (PI), gametocytic index (GI), parasitemia density (PD) and the plasmodial species. Another parameter is the sporozoitic index (SI). This study aimed to assess the level of transmission in a stable Heath Zone where ITN have been partially distributed. A transversal study has been conducted from 11th of October to 17th of November 2011 in Bolenge Heath Zone where in, there are 3 Health Areas(Bolenge, Wendji Secli and Bongonde) separated between themselves by the distance of at least 10 kilometers, have been selected. Thick blood smear and thin blood smear have been done in all members of the households which remount to 185 in total, which include by the way 1066 subjects. *Anopheles* were captured in household for determining SI Results The global PI in Bolenge Health zone was of 41.8 %. The rate of mosquisto bednet utilization was 95 %, 13 % and 23 %, respectively in Bolenge health area, Bongonde and wendji-Secli and in the same way, the PI was of 32.7 %, 50.4 % and 42.2 %; p<0.01. The global average parasitemia of 3 Heath areas was of 2213±354 trophozoites/ µl (2326.±54; 3182±603 and 965.±194 respectively in Bolenge, Bongonde and Wendji-Secli health areas and in the same way, GI was of 3.7 %, 10.4 % and 4.4 % SI was respectively 5, 7 et 10 in Bolenge , Wendji secli and Bongonde and..*Plasmodium falciparum* was found at 99.9 %. All anopheles were An.gambiae s.s M molecular form. In conclusion, transmission was high in Bolenge Health Zone, it was very raised in Bongonde Health Area, where the rate of the ITN use was low.

1548

DETERMINANTS OF RISK OF MALARIA PARASITEMIA IN BUNKPURUGU-YUNYOO DISTRICT, NORTHERN GHANA, INCORPORATING REMOTE SENSING AND SURVEY DATA

Paul J. Psychas[1], Brooke Eckman[1], Benjamin Abuaku[2], Collins Ahorlu[2], Kwadwo Koram[2]

[1]University of Florida Emerging Pathogens Institute, Gainesville, FL, United States, [2]Noguchi Memorial Institute of Medical Research, University of Ghana, Legon, Ghana

Ghana's malaria control strategy prioritizes the northern savannah regions due to persistent hyperendemicity. In Bunkpurugu-Yunyoo district, previously reported anemia and parasitemia surveys were conducted serially in 3 rainy seasons (RS) and 3 dry seasons (DS) in 2010-13, covering 11,945 children under five from 179 communities. In spite of high coverage for insecticide-treated bed nets (>75% use in each RS) and indoor residual spraying (IRS) with pyrethroid pesticides in years 2 and 3 (>98% households sprayed), investigators found unexpectedly high and geographically heterogeneous malaria prevalence. To better define and explain this heterogeneity, this study enhanced the survey dataset with remotely sensed data, then analyzed by ecologic zones, delineated as urban (Zone 1, n=1131); rocky uplands (Zone 2, n=5234, >750 ft altitude); transition (Zone 3, n=3456), and riverine plains (Zone 4, n=2124, <550 ft). The RS odds ratios for microscopic malaria parasitemia in children living in Zones 2, 3, and 4, as compared with Zone 1, were respectively 3.9 (95% CI: 2.8-5.4), 7.6 (95% CI:5.7-10.3), and 11.1 (95% CI: 8.0-15.6; all p values here and in the following <0.0001). Zone 4 parasitemia prevalence across the 3 years was 65.6-72.1% in the RS and 39.8-54.9% in DS. Among 17 variables with statistically significant odds ratios (OR) for malaria risk, 12 exhibited a zonal gradient favoring reduced risk in Zone 1 vs. Zone 4, with Zone 2 intermediate. In the RS these included lower wealth quintile (OR=3.6; 95% CI:2.7-4.7), caregiver's lack of education (OR = 2.7; CI: 2.1-3.3), ethnicity (OR = 3.9; CI: 3.2-4.8), lack of health insurance coverage (OR 3.0; CI=2.4-3.6), higher vegetation index (OR=1.6; CI: 1.1-2.3), lower human influence index (OR 5.2; CI:3.8-7.1); and >3 km distance to nearest health facility (OR=2.4; CI:1.9-3.1), among others. DS findings were similar. No consistent zonal gradient was found for the malaria control measures (ITN use, ACT use, IRS). Findings suggest that, in spite of high coverage with ITNs and pyrethroid-based IRS, high malaria prevalence in northern Ghana may be found in locations where reduced socioeconomic status and isolation coincide with low-lying terrain. Such areas may require additional and/or modified methods for vector and parasite control.

1549

DEVELOPING A PASSIVE MALARIA CASE DETECTION STRATEGY IN TANZANIAN MILITARY HEALTH FACILITIES AND MALARIA EPIDEMIOLOGY DATA COLLECTED TO DATE

Akili Kalinga[1], Christopher Mswanya[2], George Amoo[3], Charles Mwanziva[4], Sarah Chiduo[5], Lucky Temu[5], Lalaine Anova[6], Colin Ohrt[6]

[1]National Institute of Medical Research, Dar es Salaam, United Republic of Tanzania, [2]Tanzania Peoples Defense Forces, Dar es Salaam, United Republic of Tanzania, [3]Amethyst Technologies LLC, Baltimore, MD, United States, [4]Tanzania Peoples Defense Force, Dar es Salaam, United Republic of Tanzania, [5]Walter Reed Malaria Programme-Tanzania, Dar es Salaam, United Republic of Tanzania, [6]Walter Reed Army Institute of Research, Silver Spring, MD, United States

The capability to accurately track malaria incidence is essential to measuring the true impact and efficacy of malaria control interventions or field evaluations of malaria therapeutics, vaccines, etc. However, gathering reliable malaria epidemiology data in rural and resource-challenged settings is often a daunting and difficult endeavor. In Tanzania, malaria persists as a major cause of morbidity and mortality. The US Army has partnered with the Tanzania People's Defence Forces (TPDF) and the Tanzania National Service Program (JKT) to support the TPDF's efforts to improve malaria management in a number of TPDF and JKT camps. The TPDF is an important provider of health services for both military and civilian populations, especially in remote areas where TPDF camps are based. Our initial efforts to perform passive malaria case detection relied on collecting microscopy slides from sites for cross-checking. These efforts were resource intensive and resulted in dubious success and questionable data. This led us to transition our focus to the use of malaria rapid diagnostic tests (RDTs) with RDT readers. In our approach, we deployed the Deki Reader, a rugged, mobile *in vitro* diagnostic device which interprets commercially available RDTs. Several advantages provided by the Deki system include real-time quality control measures, the ability for remote quality assurance (QA), and the automatic organization of

the data in a centralized web-based portal. After deploying devices to each site, training is provided to site staff regarding use of RDTs and Deki Readers and sites are allowed a period of practice. After the sites become active, QA monitors review the mRDT database and conduct QA quarterly visits for trouble-shooting, to cross-check mRDT results against laboratory, physician, and pharmacy records, and technician compliance and accuracy for data transmission. In our first year of implementation, our approach has undergone several adjustments to adapt to a number of challenges, however we have made great strides improving the reliability of the data. We will present the challenges and successes experienced with implementation of our approach and the data collected to date.

1550

PRECISE GENOTYPING TOOLS FOR INVESTIGATING TRANSMISSION DYNAMICS OF *PLASMODIUM FALCIPARUM* GAMETOCYTES

Rahel Wampfler[1], Lincoln Timinao[2], Hans-Peter Beck[1], Issiaka Soulama[3], Alfred B. Tiono[3], Peter Siba[2], Ivo Mueller[4], Ingrid Felger[1]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea, [3]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [4]Walter and Eliza Hall Institute, Parkville, Australia

Differentiation between gametocyte-producing *Plasmodium falciparum* clones depends on high stage-specific expression and high genetic diversity of a genotyping marker in the study area. High-resolution typing methods are crucial for longitudinal tracking of gametocyte producing clones in multiple infections. Pfs230 and pfg377 are classical length-polymorphic markers for differentiation of gametocytes. We have evaluated capillary electrophoresis-based differentiation of 6 length-polymorphic gametocyte genes. These assays were applied to asexual parasites by targeting genomic DNA from field samples and in parallel to gametocytes from the same blood samples by targeting gametocyte-specific RNA. Highest diversity was found for pfs230 with 18 alleles and for pfg377 with 15 alleles in 111 samples from PNG. When assays were performed in parallel on RNA and DNA from 46 samples from Burkina Faso, 85.7% of all pfs230 samples and 59.5% of all pfg377 samples contained at least one matching genotype in DNA and RNA. Out of the 93 PCR fragments amplified from DNA of all samples by pfs230, 41 (44.1%) were not observed in the corresponding RNA sample. Vice versa we found that 42.9% (39/91) of pfs230 fragments detected in RNA failed to be amplified from the corresponding DNA samples. The imperfect detection in both, DNA and RNA, was identified as major limitation for investigating transmission dynamics, owing primarily to the volume of blood processed and the incomplete representation of all clones in the sample tested. This finding emphasises the importance of expression levels of gametocyte-specific markers as well as optimal sampling and preservation of DNA and RNA. Larger volumes may improve clone detectability of abundant low-density gametocyte carriers and of initially sequestered gametocyte clones in follow-up samples. Application of these methods to samples from cohort studies will help to explain additional factors influencing detectability of gametocyte clones, e.g. the dynamics of gametocytogenesis of a specific parasite clone over the duration of its infection.

1551

CAN HEALTH MANAGEMENT INFORMATION SYSTEM (HMIS) DATA BE USED TO MAKE INDOOR RESIDUAL SPRAY POLICY DECISIONS IN BENIN?

Evelyne S. Alyko Chaffa[1], Elana Fiekowsky[2], Joel Sodjinou[1], Benjamin Johns[2], Allison Belemvire[3], Kristen George[3], Christen Fornadel[3]

[1]Abt Associates, Cotonou, Benin, [2]Abt Associates, Bethesda, MD, United States, [3]United States Agency for International Development, Washington, DC, United States

The President's Malaria Initiative (PMI) has supported Indoor Residual Spraying (IRS) in Benin as part of its malaria control strategy. The PMI/USAID-funded Africa Indoor Residual Spraying (AIRS) Project assessed the feasibility of using health facility data to inform targeted spray decision-making, and assess IRS's effect on malaria caseloads. AIRS accessed routinely HMIS data from nine IRS intervention districts and five neighboring comparison districts, covering the period 2006 - 2013, including the period of IRS implementation from 2011-2013. We then assessed the association between IRS and the number of malaria cases reported by health facilities for both the total population and children under five years of age. Two models were developed for this assessment. In the first model, we first assessed, for a subset of areas with available data, the association of IRS on the Entomological Inoculation Rate (EIR), we then analyzed the association between EIR and the number of malaria cases as reported in the HMIS data. Finally, we combined these two steps to evaluate the association of IRS on reported malaria cases through the EIR. The second model measured the association of IRS directly on reported malaria cases in the HMIS data. The sample size for the first model was small, yet it serves to assess the validity of the second model. For the analyses, we used a difference-in-difference approach that controls for rainfall, other malaria and health interventions, and time trends. We assessed the internal validity of the data assessing the association of other malaria interventions on health facility utilization and putting the treatment on a year before IRS took place to measure the association of a "false" treatment in the second model. We assessed the association separately for two classes of insecticides, carbamates and organophosphates. As estimated against the comparison group, initial results show a 20 to 30 percent decrease in the number of confirmed cases per person per month associated with carbamates and a 5 to 20 percent decrease per person per month associated with organophosphates. The sensitivity tests and the falsification tests make us question the internal validity of the second model; the falsification test showed a statistically significant effect in a year that IRS did not take place. Based on this research, we cannot recommend using HMIS data to make IRS targeting policy decisions in Benin without further data validation.

1552

INVESTIGATING THE SPECIFICITY AND KINETICS OF *PLASMODIUM FALCIPARUM*-SPECIFIC IGG RESPONSES THAT ASSOCIATE WITH PROTECTION FROM MALARIA: A LONGITUDINAL STUDY IN MALI

Eugene Liu[1], Jeff Skinner[1], Tuan M. Tran[1], Aissata Ongoiba[2], Shanping Li[1], Safiatou Doumbo[2], Didier Doumtabe[2], Younoussou Kone[2], Aboudramane Bathily[2], Seydou Dia[2], Moussa Niangaly[2], Jules Sangala[2], Chiung-Yu Huang[3], Huw Davies[4], Aarti Jain[4], Arlo Randall[5], Ogobara K. Doumbo[2], Kassoum Kayentao[2], Boubacar Traore[2], Philip L. Felgner[4], Peter D. Crompton[1]

[1]National Institutes of Health, Rockville, MD, United States, [2]Mali International Center of Excellence in Research, University of Sciences, Technique and Technology of Bamako, Bamako, Mali, [3]Research Program in Quantitative Sciences, Division of Oncology Biostatistics/Bioinformatics, The Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD, United States, [4]University of California Irvine, Irvine, CA, United States, [5]Antigen Discovery Inc., Irvine, CA, United States

Antibodies play a key role in malaria immunity, but antibody-mediated protection is only acquired after years of *Plasmodium falciparum* exposure, leaving children vulnerable to repeated bouts of symptomatic malaria. Our previous work in Mali suggests *P. falciparum* protein microarrays can be used in population-based studies to investigate the antigen specificity of protective antibodies and the kinetics of their acquisition; however, our prior work was limited by small samples size, a lack of active surveillance for clinical malaria and *P. falciparum* infection, and few time points. To address these limitations we conducted an independent two year cohort study in the rural village of Kalifabougou, Mali where intense malaria transmission occurs from June to December. Active surveillance for clinical malaria and *P. falciparum* infection was done weekly and bi-weekly, respectively. Of the 695 enrollees in the cohort study (aged 2 months to 25 years), we focused the present analysis on the 268 subjects who were *P. falciparum* PCR negative at enrollment before the malaria season. A microarray with 1024 *P. falciparum* proteins was probed with plasma collected from these 268 subjects at four time points: before the six-month malaria season, during the first episode of febrile malaria of the ensuing malaria season (if it did occur), after the malaria season, and after the subsequent 6-month dry season. In ongoing analyses that we expect to complete by mid 2014, we are comparing antibody profiles of children who were prospectively classified as clinically immune (documented infection not followed by fever) or susceptible to malaria, as well as individuals who showed evidence of sterile protection. We are also modeling the breadth, magnitude and kinetics of *P. falciparum*-specific antibody responses from 2 months to 25 years--the age range over which clinical immunity to malaria is acquired in this population. This rich dataset is shedding light on fundamental properties of the human antibody response to malaria and may help identify novel malaria vaccine targets.

1553

CIRCULATING IMMUNOGLOBULIN (IGG) AGAINST MSP1-$_{42}$ AND *PF*EMP-1 ARE NOT ASSOCIATED WITH PEDIATRIC MALARIA SEVERITY IN WESTERN KENYA

Evans Raballah[1], Prakasha Kempaiah[2], Samuel B. Anyona[1], Zachary Karim[2], Mark Webale[1], George Orinda[3], Michael F. Otieno[4], James B. Hittner[5], Douglas J. Perkins[2], John M. Ong'echa[1]

[1]University of New Mexico Laboratories of Parasitic and Viral Diseases, Kisumu, Kenya, [2]Center for Global Health, Albuquerque, NM, United States, [3]Department of Biochemistry and Biotechnology, Nairobi, Kenya, [4]Department of Medical Laboratory Sciences, Nairobi, Kenya, [5]Department of Psychology, Charleston, SC, United States

Malaria remains a major cause of morbidity and mortality among naïve immune children and pregnant women of Africa. The majority of cases are caused by *Plasmodium falciparum*. In holoendemic regions such as western Kenya, severe malaria cases in children under the age of five years manifests as severe malarial anemia [Hemoglobin (Hb) <6.0g/dL; any density parasitemia]. High levels of Immunoglobulin (Ig)-G to a number of surface proteins and invasion ligands have been associated with protection from malaria. Morever, recent studies suggest that MSP1-$_{42}$ interacts with heparin-like molecules on the RBC. Adhesion is mediated by the *P. falciparum* erythrocyte membrane protein 1 (*Pf*EMP-1), expressed at the surface of infected erythrocytes and is linked to both antigenic variation and cytoadherence. The role of antibodies against these antigens in the pathogenesis of SMA remains largely unknown. We therefore sought to elucidate the role of these antibodies by measuring circulating IgG levels against these antigens in children (n=117) presenting with acute malaria at Siaya County Hospital, western Kenya. Complete hematological measures were obtained with a Beckman Coulter Counter®, and Giemsa-stained slides were used to determine parasitemia. Participants were stratified based on Hb status as non-SMA (n=91), Hb≥6.0 g/dL and SMA (n=26), Hb<6.0 g/dL. Results presented here show that circulating IgG against MSP1-$_{42}$ were comparable between non-SMA and SMA groups [Median (Interquartile range) non-SMA 128.83 (295) and SMA 151.19 (321); *P*=0.963]. In addition, the IgG levels against MSP1-$_{42}$ did not correlate with parasite density (ρ=0.065; *P*=0.487). Similarly, circulating IgG against *Pf*EMP-1 was comparable between the groups [Median (Interquartile range) non-SMA 420.0 (590) and SMA 377.97 (710); *P*=0.632] and was not associated with parasite density (ρ=-0.006; *P*=0.952). These results suggest that the levels of circulating IgG against MSP1-$_{42}$ and *Pf*EMP-1 are not correlated with malaria disease severity in acutely infected children from this holoendemic region.

1554

THE ROLE OF THE INHIBITORY FC RECEPTOR, FCΓRIIB IN HOST IMMUNE RESPONSE AND SUSCEPTIBILITY TO MALARIA IN PAPUA NEW GUINEA

Alexander M. Barron[1], **Gloria Tavera**[1], Arlene E. Dent[1], Peter M. Siba[2], James W. Kazura[1], Christopher L. King[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Papua New Guinea Instititute of Medical Research, Goroka, Papua New Guinea

Binding of human Fragment crystalizable gamma receptor 2b (FcγRIIb) on B cells to antigen (Ag)-containing immune complexes (ICs) mediates a critical inhibition of host immune responses. Homozygosity for a FcγRIIb missense mutation (p.Ile232Thr) that reduces inhibition is associated with protection against severe malaria in Kenyan children. Protection against severe malaria in p.232Thr/Thr (TT) Kenyan children may result from a more robust humoral immune response and/or enhanced phagocytosis of malaria-infected erythrocytes (iRBC), which may select for this mutation. This hypothesis is supported by observations that TT frequency is high in malaria endemic areas (5-11%) and low elsewhere (~1-3%). In Papua New Guinea, the frequency of I232T phenotypes did not significantly differ between groups living in different malaria endemic versus non-endemic areas (p = 0.3794), however preliminary analysis suggests that individuals with TT phenotype were less likely to be infected with *P. vivax* malaria (p = 0.0319, N = 544). Studies are underway to correlate TT phenotype with risk of malaria disease. To understand how the TT phenotype may mediate protection from malaria infection and disease, we examined malaria-specific antibody (Ab) levels among different FCGR2B genotypes since TT polymorphism reduces repression of B cell activating pathways. Unexpectedly, TT Papuans had smaller repertoires and lower levels of *P. falciparum*- and *P. vivax*-specific Ab than II Papuans (i.e. p = 0.0045 for Duffy Binding Protein). Studies are underway to determine the role of FcγRIIb in modulating phagocytosis, further elucidating its role in regulating host immune responses and susceptibility to malaria.

1555

UNRAVELING THE HUMAN IMMUNE RESPONSE TO SYMPTOMATIC AND ASYMPTOMATIC *PLASMODIUM VIVAX* INFECTIONS THROUGH SYSTEMS IMMUNOLOGY

Vitor R. Mendonca[1], Andrea Kuhn[2], Marcus V. Lacerda[2], Steve Porcella[3], Manoel Barral-Netto[4], Peter D. Crompton[1]

[1]National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [2]FMT-HVD, Manaus, Brazil, [3]National Institutes of Health, Hamilton, MT, United States, [4]FIOCRUZ-BA, Salvador, Brazil

Approximately 2-3 billion people are at risk of *Plasmodium vivax* infection worldwide. The nature of the immune response during symptomatic and asymptomatic *P. vivax* infection remains elusive. We are applying systems biology tools such as genome-wide expression profiling by RNA-seq and multi-parameter flow cytometry to gain insight into host immune responses that associate with protection from disease during *P. vivax* infection. In the Brazilian Amazon we enrolled three groups: 1) *P. vivax*-infected adults with fever (n=19), 2) *P. vivax*-infected adults with no fever or symptoms for 30 days despite persistent parasitemia (n=17), and 3) age-matched uninfected controls (n=17). Blood samples were collected at enrollment from the first and third groups, whereas blood was collected from the second group after the 30-day period without fever or symptoms. Standard hematology and chemistry labs were done; and plasma, peripheral blood mononuclear cells (PBMCs) and RNA were isolated from whole blood. Analysis of the hematologic data showed that symptomatic subjects had a higher percentage of neutrophils (median 74.1%, p=0.0002) and a lower percentage of lymphocytes (median 21.0%, p=0.0007) compared to asymptomatic subjects. Symptomatic subjects also had higher levels of total bilirubin, creatinine and glucose (p<0.0001 for each comparison) versus asymptomatic subjects. Purified RNA was converted to cDNA and sequenced by next generation sequencing. In ongoing analysis of the RNA-seq data, differentially expressed pathways and gene sets in immune and susceptible individuals will be confirmed at the protein level and functionally using contemporaneous PBMCs and plasma samples. Molecular and cellular signatures that correlate with protection from malaria fever may yield new hypotheses regarding the biological mechanisms by which malaria immunity is induced by *P. vivax* infection. The resulting datasets may be of considerable value in the urgent effort to develop a malaria vaccine.

1556

EVALUATING CONTROLLED HUMAN MALARIA INFECTION IN KENYAN ADULTS WITH VARYING DEGREES OF PRIOR EXPOSURE TO *PLASMODIUM FALCIPARUM* USING SPOROZOITES ADMINISTERED BY INTRAMUSCULAR INJECTION

Susanne H. Hodgson[1], Elizabeth A. Juma[2], Amina Salim[3], Charles Magiri[2], Domtila Kimani[3], Daniel Njenga[2], Alfred Muia[2], Andrew O. Cole[2], Caroline Ogwang[3], Ken Awuondo[3], Brett Lowe[3], Marianne Munene[3], Peter F. Billingsley[4], Eric R. James[4], Anusha Gunasekera[4], B. K. Sim[4], Patricia Njuguna[3], Thomas Rampling[1], Adam Richman[4], Yonas Abebe[4], Gathoni Kamuyu[3], Michelle Muthui[3], Sean C. Elias[1], Sassy Molyneux[3], Stephen Gerry[5], Alex Macharia[3], Thomas N. Williams[3], Peter C. Bull[3], Adrian V. Hill[1], Faith H. Osier[3], Simon J. Draper[1], Philip Bejon[3], Stephen L. Hoffman[4], Bernhards Ogutu[3], Kevin Marsh[3]

[1]University of Oxford, The Jenner Institute, Oxford, United Kingdom, [2]Centre for Clinical Research, Kenya Medical Research Institute, Nairobi, Kenya, [3]Kenya Medical Research Institute - Wellcome Trust, Centre for Geographical Medical Research (Coast), Kilifi, Kenya, [4]Sanaria Inc., Rockville, MD, United States, [5]University of Oxford, Centre for Statistics in Medicine, Oxford, United Kingdom

Controlled human malaria infection (CHMI) studies, where healthy volunteers are infected with *Plasmodium falciparum* have become a vital tool to accelerate vaccine and drug development. As CHMI trials are carried out in a controlled environment, they allow unprecedented, detailed evaluation of parasite growth dynamics and immunological responses to infection. However, to date CHMI studies have not been used to investigate mechanisms of naturally-acquired immunity (NAI) to *P. falciparum* infection. We conducted an open label, randomized CHMI study using aseptic, cryopreserved *P. falciparum* sporozoites (PfSPZ Challenge) administered intramuscularly to evaluate infectivity and parasite growth dynamics in healthy Kenyan adults (n=28) with varying degrees of prior exposure to *P. falciparum*. All participants developed blood-stage infection, however one volunteer remained asymptomatic and blood film negative until day 21 post injection of PfSPZ Challenge, despite developing confirmed blood-stage infection by quantitative polymerase chain reaction (qPCR). A significant correlation was seen between parasite multiplication rate (PMR) and anti-schizont ELISA OD at screening (p=0.044; r=-0.384). Our study has shown that CHMI using PfSPZ Challenge is safe in African adults who have varying degrees of prior exposure to malaria and that NAI can impact on PMR post-CHMI, providing a novel method to investigate the dynamics and mechanisms of blood-stage immunity.

1557

CYTOKINE RESPONSES TO THE VAR2CSA VACCINE CANDIDATE IN PREGNANT BENINESE

Komi Gbedande[1], Gilles Cottrell[2], Samad Ibitokou[1], Bertin Vianou[1], Carine Agbowaï[1], Marita Troye-Blomberg[3], Thor Theander[4], Ali Salanti[4], Achille Massougbodji[1], Kabirou Moutairou[5], Nicaise Tuikue Ndam[2], Philippe Deloron[2], Adrian Luty[2], Nadine Fievet[2]

[1]Centre d'Etude et de Recherche sur le Paludisme Associé à la Grossesse et à l'Enfance, Cotonou, Benin, [2]Institut de Recherche pour le Développement, UMR D216 Mère et enfant face aux infections tropicales, Paris, France, [3]Department of Immunology, Wenner-Gren Institute, Stockholm University, Stockholm, Sweden, [4]Department of International Health, Institute of International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark, [5]Faculté des sciences et techniques, Université d'Abomey-Calavi, Cotonou, Benin

The STOPPAM consortium conducted 2 longitudinal cohort studies in pregnant women in Benin and Tanzania in order to evaluate the immunopathological consequences of infections with *Plasmodium falciparum* during pregnancy. In this context, parasite antigen-specific cellular responses, in particular to the vaccine candidate antigen VAR2CSA, have received little attention. Here we evaluated both, cytokine (IL10, IL13, IL17, IFN- γ, TNF-α) responses and the T cells IFN-γ specific responses to the DBL5 domain of VAR2CSA. In Come, southwestern Benin, we conducted a longitudinal prospective study of ~1000 pregnant women. Women at ≤24 weeks of pregnancy were enrolled and followed at each antenatal visit until delivery. Peripheral blood mononuclear cellular (PBMC) responses to VAR2CSA-DBL5 *in vitro* were determined in subgroups of 150 women at inclusion and 100 at delivery. In each subgroup those harbouring *P. falciparum* infections were matched by gravidity and gestational age with mothers with no infection at inclusion and those with no history of infection earlier in the pregnancy. The amounts of IL10, IL13, IL17, IFN-γ and TNF-α produced in response to mitogen (PHA) and to VAR2CSA-DBL5 were quantified in supernatants of stimulated PBMC. The *ex vivo* frequencies of IFN-γ secreting CD4 and CD8 T cells in response to PHA and VAR2CSA domains were evaluated in the same maternal PBMC groups. At the time of writing, all data have been collected, cytokine concentrations have been evaluated and multivariate analyses are under way. Results will be discussed firstly in the context of cytokine profiles that reflect the acquisition of a specific cellular memory response to the vaccine candidate according to gravidity or to previous *P. falciparum* infection. Secondly, we will discuss cytokine profiles as potential markers of protection in the context of infection, anemia and birth weight.

1558

MFERA (MALAWI) COHORT STUDY: COMMUNITY-BASED LONGITUDINAL STUDY OF MALARIA IMMUNOLOGY, EPIDEMIOLOGY AND GENOMICS

Esther N. Gondwe

College of Medicine, Malawi, Blantyre, Malawi

Malaria is endemic in Malawi, with climatic factors supporting transmission in most of the country except at high elevation. Individuals at risk of malaria infection are mostly young children and pregnant women. The risk of severe malarial disease and clinical symptoms declines in children residing in endemic regions when they experience continuing exposure to infectious mosquito bites. Thus older children sustain only mild symptoms or are asymptomatic. To understand host responses that develop with repeated exposures and are associated with clinical disease immunity, we undertook a longitudinal cohort study at Mfera Health Centre, Chikhwawa district in Malawi. 120 subjects with uncomplicated malaria are being followed for two years to capture host responses during repeated infections. We will also examine host responses in three age groups (1-5 years, 6-12 years and 13-50 years), as age serves as a proxy for clinical immunity. We will present data by age group to include clinical symptoms, rate of recurrence, host response profiling and parasite genotypes. We will also analyse these features in individuals who have repeated infections to identify changes over time using repeated measures design. A primary focus of the host response studies is the role of type I IFN in the development of clinical immunity. The role of type I IFN to confer protection or susceptibility to clinical malaria remains controversial and thus we will focus on type I IFN responses (cytokine, interferon responsive genes, type I IFN receptor genetic variants, immunophenotyping of relevant cells) in association with disease markers and age. The data generated will provide an unprecedented opportunity to understand how residents of malaria endemic areas develop less severe clinical disease with repeated parasite exposures and these data may inform vaccine strategies.

1559

PD1 EXPRESSION ON NEONATAL VΔ2 T CELLS MODULATES FUNCTIONAL RESPONSES TO MICROBIAL ANTIGENS

Sarah E. Boudova[1], Suchita Chaudhry[2], Christopher Harman[1], Miriam Laufer[1], David Pauza[2], **Cristiana Cairo**[2]

[1]University of Maryland, School of Medicine, Baltimore, MD, United States, [2]University of Maryland, Institute of Human Virology, Baltimore, MD, United States

In utero exposure to microbial antigens primes the fetal immune system, often with negative consequences for infant immunity and responses to pediatric vaccines. VΔ2 T cells, a subset of γΔ T cells, play important roles in antimicrobial immunity and participate in responses to Bacille Calmette-Guérin (BCG) vaccination. We observed that prenatal exposure to plasmodium antigens primes fetal VΔ2 cells, potentially causing dysregulation of infant VΔ2 cells. We are defining the impact of prenatal *Plasmodium falciparum* exposure on neonatal and infant immunity by measuring changes in VΔ2 cells. In this context, we want to identify inhibitory and activating receptors that modulate VΔ2 responses and differ in expression on fetal versus adult cells. These differences in regulation are important for normal immune function and may be altered by maternal infections. PD1 is a key negative regulator of immune responses and a marker of T cell functional exhaustion during chronic viral infections and malaria. We compared PD1 expression on healthy North American adult and neonatal VΔ2 cells after stimulation. For adult VΔ2 cells, PD1 expression peaked by day 4 and in most individuals returned to baseline by day 14. For neonatal (cord blood) VΔ2 cells, PD1 expression peaked between days 4 and 7, and in most subjects was still elevated at day 14, yielding a PD1+ fraction significantly larger than in adults (43.6% versus 8.5%, $p<0.0001$). PD1 expression on neonatal VΔ2 cells remained stable up to day 35. The ability of neonatal VΔ2 cells to produce the pro-inflammatory cytokine TNFα and mobilize cytotoxic granules in response to immobilized anti-T Cell Receptor antibody was inhibited by immobilized PD1-ligand in a dose-dependent manner. Our results suggest that VΔ2 cell function in the fetus is regulated by PD1 in order to limit inflammatory responses. Prenatal VΔ2 cell stimulation caused by maternal infection may induce long-term PD1 up-regulation, and hinder VΔ2 cell responses to pathogens and to BCG vaccination, affecting infant immunity.

1560

STATISTICAL MODELING METHODS REVEAL VARIABLE IMPORTANCE OF ANTI-MALARIAL ANTIBODIES IN KENYAN CHILDREN

Arlene E. Dent[1], Denise Babineau[1], Peter Sumba Odada[2], John Vulule[3], Ann Moormann[4], James Kazura[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]Centers for Disease Control and Prevention, Kisumu, Kenya, [4]University of Massachusetts, Worcester, MA, United States

Naturally acquired immunity (NAI) to *Plasmodium falciparum* is characterized by age-related control of parasitemia and protection from clinical malaria. With the goal of advancing knowledge of how the magnitude and breadth of anti-malaria IgG antibodies contribute to NAI, we used plasma from 97 children (1-14 years) who participated in a treatment time to infection study in western Kenya. IgG antibodies to 24 recombinant merozoite and pre-erythrocytic proteins were measured by multiplex microsphere assay. A global test had a p value of 0.0601 indicating that there is evidence that antibodies against at least one of the antigens is associated with delayed time to infection. Traditionally we have used only Kaplan-Meier analysis to examine the relationship between antibody responses and time to infection. Here we developed and compared 6 prediction models: 1) Kaplan-Meier, 2) Univariate Screening, 3) Backward Elimination, 4) Penalized Regression Models--Least Absolute Shrinkage and Selection Operator (lasso) 5) Penalized Regression Model--Elastic net, and 6) Random Survival Forests. Each method has benefits and limitations. By comparing the results of all analyses and evaluating the performance of each using the time-dependent Brier score, several promising antigen targets that appear to contribute to protection from infection such as AMA1(FVO), MSP3, MSP1 (3D7), and EBA181 would have been missed if we only used Kaplan-Meier analysis. We conclude that high level statistical models offer insights into targets of NAI and identify potential candidates for inclusion in multi-antigen malaria vaccines.

1561

TRACKING THE DEVELOPMENT OF IMMUNITY TO *PLASMODIUM FALCIPARUM* AFTER IMMUNIZATION WITH IRRADIATED SPOROZOITES IN A MALARIA ENDEMIC SETTING

Irfan Zaidi[1], Hama Diallo[2], Yeya Dit S. Sarro[3], Bourahima Kone[3], Abdoulaye Katile[2], Amadou Kone[3], Ousmane Koita[3], Freda Omaswa[4], Sharon Wong-Madden[1], Mahamdou S. Sissoko[2], Stephen L. Hoffman[4], Ogobara Doumbo[2], Sara A. Healy[1], Michael Walther[1], Patrick Duffy[1]

[1]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [2]Malaria Research and Training Center, Mali-NIAID ICER, University of Science, Techniques and Technologies of Bamako, Bamako, Mali, [3]SEREFO Core Immunology Laboratory, ICER, University of Science, Techniques and Technologies of Bamako, Bamako, Mali, [4]Sanaria Inc., Rockville, MD, United States

Vaccination with irradiated *Plasmodium falciparum* (Pf) sporozoites (SPZ) has been shown to induce sterilizing protection against malaria infection in naïve volunteers, but as yet this has not been tested in subjects living in malaria endemic regions who would have pre-existing immunity. In collaboration with Sanaria Inc. and the Malaria Research Training Centre (MRTC), the first double-blinded randomized phase 1b trial of the Sanaria®

PfSPZ Vaccine (radiation attenuated, aseptic, purified PfSPZ) in a malaria endemic region is being conducted in Mali. Ninety-three volunteers were randomized to receive five vaccinations of $2.7x10^5$ PfSPZ or normal saline placebo. In addition twelve volunteers received two vaccinations to ascertain safety of the vaccine, and nine of these volunteers will receive 5 immunizations. Vaccinations began in January 2014, and will be completed in July 2014. CD8 T cells may be the key mediators of protection against liver stages of *P. falciparum*, but other cellular subsets may also play a significant role. In earlier studies, CD38 and CD11a on CD8 T cells have been used to measure the development of immunity against malaria antigens after vaccination in mice and humans. Whole blood samples are being collected at baseline, 3, 7 and 27 days after each vaccination, and used to measure the percentages of CD8, CD4, T and NK cells expressing CD38 and CD11a using flow cytometry. The results after each of the 5 vaccine doses will be reported, and may be useful in discriminating the roles of various immune subsets in conferring protection after PfSPZ vaccination.

1562

ANTIBODY PROFILING BY PROTEIN MICROARRAY IN NAÏVE AND SEMI-IMMUNE INDIVIDUALS IN COLOMBIA AFTER EXPERIMENTAL CHALLENGE WITH *PLASMODIUM VIVAX*

Huw Davies[1], Myriam Arévalo-Herrera[2], Mary López-Pérez[2], Aarti Jain[1], Kelly Rubiano[2], José Millán Oñate[3], Philip L. Felgner[1], Socrates Herrera[2]

[1]University of California Irvine, Irvine, CA, United States, [2]Caucaseco Scientific Research Center, Cali, Colombia, [3]Centro Médico Imbanaco, Cali, Colombia

Acquisition of naturally acquired immunity to malaria in low transmission intensity regions is often accomplished from relatively few exposures and can occur at any age. This stands in contrast to high transmission areas where protection is acquired from repeated exposures during the first two decades of life. In this study we have evaluated serological differences in response to live *Plasmodium vivax* challenge in two groups of individuals from Colombia. These comprised 7 individuals with no history of malaria ("naïve"), and 9 individuals naturally exposed to *P. vivax* previously ("semi-immune"). Each individual was infected with *P. vivax* sporozoites via mosquito bites. As a result, 6 naïve (86%) and 6 semi-immune (67%) individuals developed clinical symptoms in the second week post-challenge. Fever and headache were significantly more frequent or severe in naïve compared to semi-immune individuals, while blood alanine aminotransferase, aspartate aminotransferase (markers of liver function) and C-reactive protein were also significantly higher in naïve. Overall, the clinical data indicated previous exposure to *P. vivax* is associated with protection against clinical symptoms in response to *P. vivax* challenge. To test whether protection might also be associated with IgG profiles, serum from d0, d5, d11, 3 weeks, and 4 months post-challenge were probed on a protein microarray displaying 500 *P. vivax* and 500 *P. falciparum* sero-reactive exon products. The array did not reveal strong serological differences between naïve and semi-immune individuals at the time of challenge. However, a difference was observed in the response to challenge with *P. vivax* sporozoites. In both groups, the response peaked at week 3 and declined thereafter, with the response by the naïve group being noticeably stronger and broader in comparison with the semi-immune group. Interestingly, the bulk of the serological response seen in the semi-immune group was also associated with those individuals with fever or headache, while those that were asymptomatic had an attenuated response. Thus the association between previous exposure and protection against clinical malaria is also associated with lower serological reactivity as measured by protein array, possibly reflecting activity of the memory pool in previously-exposed individuals.

1563

KIR3DS1 HOST GENOTYPE, IL17 SERUM CONCENTRATION AND *PLASMODIUM VIVAX* CSP GENOTYPES MODULATION OF VIVAX MALARIA PARASITEMIA

Giselle M. Viana[1], Andréa Luciana da Silva[2], Erian de Almeida Santos[3], Danielle Regina Barbosa[1], Gustavo Capatti Cassiano[4], Priscila Vianna[5], Nadine Glesse[5], José Artur Chies[5], Valéria Suenya Leal[2], Lilian de Araújo Mendes[2], Janaina Mota de Vasconcelos[3], Ricardo Luiz Machado[1], João Farias Guerreiro[2], Marinete Marins Póvoa[1], Eduardo José dos Santos[2]

[1]Instituto Evandro Chagas - IEC/SVS/MS, Ananindeua, Brazil, [2]Universidade Federal do Pará - UFPA, Belém, Brazil, [3]Universidade Federal do Pará - UFPA, Instituto de Ciências Biológicas, Belém, Brazil, [4]Faculdade de Medicina de São José do Rio Preto - FAMERP, São José do Rio Preto, Brazil, [5]Universidade Federal do Rio Grande do Sul - UFRGS, Porto Alegre, Brazil

Malaria is the most important arthropod borne disease in Brazil, occurring mainly in North region, being a serious Public Health concern, in terms of its high morbidity and mortality rates. Both host and parasite genetic features, as well as host immune profile, modulate resistance to infection and heterogeneity of clinical/laboratorial manifestations. This study approached: i) the *Plasmodium vivax* genotypes in Circumsporozoite Protein (CSP) region; ii) host KIR genes polymorphisms and; iii) immune profile during the infection (concentration of six circulating cytokines: IL-17, INF-g, TNF-a, IL-10, IL-6, IL-4, IL-2). Furthermore, we evaluated how these factors modulate the parasitemia and how host KIR polymorphism influences the number of CSP genotypes in infected individuals. Fourteen KIR genes and their ligands were genotyped by PCR-SSP on 62 *P. vivax*-infected individuals living in the town of Goianesia do Pará (Pará, Brazil). Cytokine levels were quantified using a Becton Dickinson cytometric bead array. CSP genotypes (Vk210, Vk247 and *P.vivax*-like) were determined by PCR-RFLP. Among the 14 KIR genes only KIR3DS1 presence was associated with higher parasitemia (Mann-Whitney test; p=0.01). Moreover, KIR3DS1 presence associates with *P. vivax* CSP multiple genotypes (Fisher Exact test; 0.0084). Interestingly, individuals presenting multiple genotypes of *P. vivax* CSP showed also higher parasitemia (Mann-Whitney test; p=0.028). Fulfilling this scenario IL-17 concentration correlated negatively with parasitemia (r=-0.6702; p=0.024), suggesting a protective role in the parasitemia control. Noteworthy, KIR3DS1 is a key stimulatory receptor of Natural Killer cells that produces many cytokines related to immune response to *P.vivax* infection being the results suggestive of a role of this gene in control of the number of different circulating CSP genotypes as well as parasitemia and highlights KIR3DS1 role's in malaria immune response in an Amazonian population.

1564

INDUCTION OF HOST CELL AUTOPHAGY PROMOTES THE DEVELOPMENT OF MALARIA LIVER STAGE

Wenyue Xu, Chenghao Zhao, Taiping Liu, Taoli Zhou

Third Military Medical University, Chongqing, China

Host cell autophagy has been reported to be involved in the restriction of the growth of a variety of microorganisms, but its role in the development of malaria pre-erythrocytic stage is still unknown. Here, we found that sporozoite infection induced LC3 containing vesicle to surround the exo-erythrocytic forms in hepatocyte, indicating the hepatocyte autophagy of the malaria parasite. Rapamycin treatment significantly increased the number of the autophagy of exo-erythrocytic form by hepatocyte, and promoted the fusion of autophagy containing malaria parasite with lysosome. However, host cell autophagy induced by rapamycin could significantly promote the development of exo-erythrocytic form *in vitro*. Further study showed that parasites inside the autophagosome could still survive and replicate normally as same as those in the parasitphorous vacuole, and the acidification of autolysosome was greatly inhibited. Therefore, we firstly provide evidence that sporozoite infection could induce host cell autophagy of malaria parasite, and the

induction of hepatocyte autophagy promoted the development of pre-erythrocytic stage, which might be associated with its ability to suppress the acidification of autolysosome. This data indicated the induction of autophagy as a novel escape strategy of exo-erythrocytic stage, and shed new light on the prophylatic therapy against liver stage.

1565

TRANSCRIPTOME ANALYSIS OF ATYPICAL MEMORY B CELLS IN THE SETTING OF NATURAL *PLASMODIUM FALCIPARUM* INFECTION

Richard T. Sullivan[1], Mary Fontana[1], Isaac Ssewanyana[2], Harriet Mayanja[3], Prasanna Jagannathan[1], Margaret Feeney[1], Charlie Kim[1], Bryan Greenhouse[1]

[1]University of California San Francisco, San Francsico, CA, United States, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Makerere University Medical School, Kampala, Uganda

Higher frequencies of an "atypical" phenotype of memory B cells have been associated with chronic exposure to *Plasmodium falciparum* and other pathogens, but the function of these cells in malaria pathogenesis and the development of immunity is not understood. Atypical memory B cells have been hypothesized to be dysfunctional based on their poor production of antibodies *in vitro*, while others have hypothesized that these cells actively produce antibodies. However, antibody secretion is not the sole function of B cells. To better understand the functional nature of atypical memory B cells, we performed a systematic evaluation of the differences between atypical and classical memory B cells using transcriptome analysis of both subsets in the presence of naturally occurring *P. falciparum* infection. B cells subsets were isolated from 6 parasitemic but asymptomatic children aged 8-10 years old and analyzed on whole genome microarrays. Expression of select genes was confirmed by qPCR and/or flow cytometry. Consistent with previously hypothesized atypical memory B cell dysfunction, we found a number of inhibitory genes elevated compared to classical memory B cells. However, atypical memory B cells were not dormant, but clearly metabolically active. In addition to elevated inhibitory genes, atypical memory B cells also upregulated multiple genes associated with activation, migration, and secretion pathways. Upregulation of genes in these pathways suggests a more complex function for atypical memory B cells beyond antibody secretion. Indeed, we performed functional assays confirming that these cells did not spontaneously secrete IgG *ex vivo*. We are currently performing additional functional assays to help define other roles atypical memory B cells have in modulating cellular responses. In summary, the transcriptome data suggest a functional role for atypical B cells, which, while as yet unknown, may be independent of antibody production.

1566

AGE-SPECIFIC MALARIA SERO-CONVERSION RATES IN HAITI: AN ANALYSIS OF MALARIA TRANSMISSION IN THE OUEST AND SUD-EST DEPARTMENTS OF HAITI

Michael E. von Fricken[1], Brandon Lam[1], Thomas A. Weppelmann[1], Will Eaton[1], Laura Schick[2], Roseline Masse[3], Madsen V. Beau De Rochars[1], Benjamin Anderson[1], Bernard A. Okech[1]

[1]University of Florida, Gainesville, FL, United States, [2]Community Coalition for Haiti, Jacmel, Haiti, [3]Christianville Foundation, Inc., Gressier, Haiti

Malaria transmission continues to occur in Haiti, with 32,000 confirmed cases of *Plasmodium falciparum* reported in 2011. As rates of malaria decrease, passive surveillance measures become less sensitive for capturing transmission intensity. By implementing highly sensitive antibody detection methods we aimed to quantifying malaria transmission intensity over time. A total of 770 serum samples were screened for malaria antibodies using indirect enzyme-linked immunosorbant assay (ELISA) coated with vaccine candidates, apical membrane antigen (AMA-1) and merozoite surface protein-11-19 (MSP-1). The "exposed" cut off value was established based on three standard deviations above the normal distribution of our negative serum absorbances (OD of 0.37 and 0.48 for AMA-1 and MSP-1 respectively). Between February and May 2013, sample collection occurred at four different sites; a rural community, two schools and a clinic in the Ouest and Sud-Est departments of Haiti. Of the 770 samples screened, 170 (22.1%) had been exposed to malaria at one point in their life time. Age was highly associated with the likelihood of having been exposed (p-value <0.001). After adjusting for age, the sero-conversion rate calculations indicated that the annual malaria transmission in the Ouest and Sud-Est department is roughly 1.03%. This data suggests that despite the absence of sustained malaria control efforts in Haiti, transmission has remained relatively low over multiple decades. Our results are further supported by passive hospital based surveillance conducted by the Haiti Health Surveillance system, which found low country-wide transmission (<1%). These findings provide valuable information that can be used to make a case for the elimination of malaria on the Island of Hispaniola.

1567

TOWARDS FULL *PLASMODIUM FALCIPARUM* PROTEOME AND REACTOME ANTIBODY SCREENING ASSAYS USING REPRODUCIBLE HIGH-THROUGHPUT PROTEIN ARRAYS

Joseph J. Campo[1], Douglas M. Molina[1], Chris Hung[1], Arlo Z. Randall[1], Li Liang[2], Arti Jain[2], D. Huw Davies[2], Elizabeth Baum[2], Peter D. Crompton[3], Robert W. Sauerwein[4], Robert Seder[5], Stephen L. Hoffman[6], Denise L. Doolan[7], John J. Aponte[8], Carlota Dobano[8], Angela Yee[1], Xiaowu Liang[1], Philip L. Felgner[2]

[1]Antigen Discovery, Inc., Irvine, CA, United States, [2]University of California Irvine, Irvine, CA, United States, [3]Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [4]Department of Medical Microbiology, Nijmegen, Netherlands, [5]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [6]Sanaria, Inc., Rockville, MD, United States, [7]QIMR Berghofer Medical Research Institute, Herston, Australia, [8]Barcelona Centre for International Health Research (CRESIB), Barcelona, Spain

High throughput (HT) technologies have rapidly advanced discovery of novel targets for disease diagnostic tests and vaccines and is especially useful for pathogens with large, complex genomes, such as *Plasmodium* spp. We have developed a full proteome antibody screening assay for *P. falciparum* proteins expressed via an HT *in vitro* transcription and translation (IVTT) system and printed onto nitrocellulose-coated microarray chips. Here, we demonstrate the reproducibility of this protein array platform, the design and reactogenicity of the full proteome array, and the development of a down-selected *P. falciparum* "reactome" array. Comparisons included variation in assay dates, sample position on microarray chips and print batches. Agreement between replicate protein array assays was good. Mean difference and limits of agreement, demonstrated graphically with Bland-Altman dotplots, was 0.03 +/- 0.6 normalized intensity (range -6 to 8 on log scale). Correlation between replicate measurements of antibody breadth and magnitude were 0.91 and 0.95, respectively. Classification of antigen reactivity showed high concordance (Cohen's kappa: 0.95, p<0.01). The final down-selection and fabrication of a 1,000-feature "Pf1000 reactome" chip is underway and expected completion is in summer of 2014. We are down-selecting based on a tiered antigen reactivity scoring algorithm using antibody responses from serum/plasma samples of highly exposed individuals from sub-Saharan Africa and Papua New Guinea and samples from experimental models of pre-erythrocytic immunity. Reactive antigens are cross-referenced for confirmation with previously published data from other regions, including South America and Southeast Asia. The latest generation of down-selected chips will be a useful tool for seroepidemiological studies and screening for antibody immune correlates of protection, and full proteome chips can be used for identifying novel targets of monoclonal therapeutic antibodies or potential vaccine target antigens.

1568

IMMUNE MECHANISMS OF CROSS-STAGE PROTECTION BY VACCINATION WITH A LATE LIVER STAGE-ARRESTING GENETICALLY ATTENUATED PARASITE

Brandon K. Sack, Gladys Keitany, Jessica Miller, Ruobing Wang, Ashley Vaughan, Stefan Kappe

Seattle Biomedical Research Institute, Seattle, WA, United States

Immunizations targeting the pre-erythrocytic stages of *Plasmodium* have demonstrated sterile protection against malaria by blocking the sporozoite stages and/or eliminating liver stage parasites. Strategies such as irradiated sporozoite immunizations however face a formidable challenge in that they only confer stage-specific protection, and in consequence, less than complete protection can result in full-blown blood stage infection. However, we have demonstrated that immunization with sporozoites of *P. yoelii* genetically attenuated parasites (GAP), which arrest late in the liver stage development and do not progress to blood stage infection, engenders protective cross-stage immunity against a direct lethal blood stage challenge. Here, we demonstrate that immune mechanisms conferring cross-stage immunity are diverse. In C57BL/6 mice, antibodies are both sufficient and necessary for this protection as GAP-immunized mice depleted of T cells completely control blood stage parasitemia whereas immunized mice lacking antibodies succumb to uncontrolled blood stage infection. Conversely, BALB/c mice depend on T cells for cross-stage protection. C57BL/6 antibodies recognize antigens in late liver stages as well as on the surface of blood-stage merozoites but are not specific for the well-characterized merozoite surface protein (MSP)-1. In contrast, immunization of BALB/c mice engenders anti-blood stage antibodies but they are lower in quantity and do not recognize the surface of merozoites. Cross-stage protection is unique to late-liver stage arresting GAP as animals immunized with an early liver stage-arresting GAP are not protected from a lethal BS challenge and fail to generate antibodies which recognize late-liver stage/blood stage antigens. Therefore, immunization with a late liver stage-arresting GAP induces T and B cell responses capable of protecting against multiple stages of *Plasmodium* infection and thus constitutes the most potent among vaccination strategies. This unique system also offers an opportunity to identify novel protective antigens, which are shared with between both the liver stage and blood stage parasites.

1569

PERIPHERAL BLOOD FOXP3+ REGULATORY CD4 T CELLS DECLINE WITH INCREASING MALARIA EPISODES IN YOUNG CHILDREN

Michelle Boyle[1], Samuel Wamala[2], Prasanna Jagannathan[1], Iejoma Eccles-James[1], Katherine Bowen[1], Tara McIntyre[1], Lila Farrington[1], Felistas Nankya[2], Charles Ebusu[2], Mayimuna Nalubega[2], Kate Naluwu[2], Esther Sikomyu[2], Victor Bigira[2], James Kapisi[2], Jordan Tappero[3], Mary Kakuru[2], Emmanuel Arinaitwe[2], Charlie Kim[1], Grant Dorsey[1], Moses Kamya[2], Margaret Feeney[1]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Infectious Diseases Research Collaboration, Kampala, Uganda, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

Plasmodium falciparum infection has been reported to induce immunoregulatory cell populations such as FoxP3+ CD4 regulatory T cells (T_{regs}). Studies of malaria-naïve adults and low exposure regions have indicated that T_{regs} expand during acute malaria infection and can be induced by malaria *in vitro*, however in children the relationship is less clear. T_{reg} expansion may be associated with the slow acquisition of immunity seen in children from malaria endemic areas. Indeed, other chronic diseases have been shown to induce T_{regs} which contributes to ongoing infection. We investigated the frequency and function of T_{regs} in well characterized cohorts of two-year-old (n=79) and four-year-old (n=72) children in Tororo, Uganda. All prior malaria episodes from age 6 months were documented and children were followed for 1 year following sampling. In both 2 and 4 year old children, we found that higher prior malaria incidence was strongly associated with lower frequencies of T_{regs} (2 yo cohort rho = -0.28, p=0.011; 4yo cohort rho = -0.35, p=0.005) However, there was no difference in frequencies of T_{regs} seen in children with or without current or recent parasitemia. Functional differences between T_{regs} from children with high or low prior incidences were investigated in *ex vivo* microarray analysis, specific and global suppression assays, and during the time course of acute infection. Our data suggests that repeated infection results in a loss of functionally suppressive T_{regs} from the peripheral blood. These data may indicate that although T_{regs} are induced following malaria infection in naïve individuals, this process becomes blunted after chronic repeated malaria exposure, which may have implications for the development of effective immune responses.

1570

INTERLEUKIN 8 AND TRANSFORMING GROWTH FACTOR-BETA (TGFB) AMONG MALARIA PATIENTS IN LAGOS, NIGERIA

Wellington A. Oyibo, Uche Igbasi, Ikechuckwu A. Ofili

College of Medicine of the University of Lagos, Nigeria, Lagos, Nigeria

The interaction between pro- and anti-inflammatory cytokines such as interleukin-8 (IL-8) and transforming growth factor beta (TGF-β) plays an important role in malaria pathogenesis and outcome.TGFβ, produced by a wide range of cells, has a pivotal role in the control of the transition between pro-inflammatory (Th1-type) and anti-inflammatory (Th2-type) response during the acute and resolving phases of malaria infection. The role of IL-8 in *Plasmodium falciparum* malaria is unknown although studies indicate it's likely use as a biomarker of intensity of malaria.The aim of this study was to measure the plasma levels of IL-8 and TGF- β in 136 individuals with malaria and correlate the production of these cytokines with the severity of the disease. IL-8 and TGF- βlevels were determined using enzyme-linked immunosorbent assay. The severity of malaria was established by parasitemia, clinical symptoms and haematological parameters. The level of IL-8 was found to be substantially elevated (508.8±755.1pg/ml) in malaria infected individuals and its value was significant in parasitemia levels (43200.0, $p<0.05$). In contrast TGF-β levels were found to be lower in malaria patients (23,672±30,703.8pg/ml) compared to non-malaria patients, the mean difference in levels of IL-8 between malaria positive and malaria negative individuals was statistically significant ($p<0.05$).The relationship between TGF-β levels and packed cell volume was negatively correlated ($r = -0.27$) . These findings suggest that fine mechanisms regulate the interaction between TGF-β andIL-8 in the immune response to *Plasmodium falciparum* infection, seemingly directing*in vivo* modulations in red cell population, and indicating a likely role in susceptibility to malaria

1571

IMPACT OF ACUTE MALARIA ON PRE-EXISTING ANTIBODY LEVELS TO COMMON CHILDHOOD VACCINES

Jill Coursen[1], Silvia Portugal[1], Aissata Ongoiba[2], Boubacar Traore[2], John T. Harty[3], Peter D. Crompton[1]

[1]National Institutes of Health, Rockville, MD, United States, [2]University of Sciences, Technique and Technology of Bamako, Bamako, Mali, [3]University of Iowa, Iowa City, IA, United States

Vaccine-induced protective immunity against many pathogens relies on long-lived plasma cells that maintain basal levels of antibody. Recent evidence from animal models suggests that *Plasmodium* infection mediates a transient drop in antibody titers induced by prior influenza virus infection, raising the possible public health concern that malaria may be detrimental to previously generated vaccine responses. Prior studies in humans have assessed the impact of concurrent *P. falciparum* infection on responses to standard childhood vaccines, but to our knowledge the impact of acute *P. falciparum* infection on previously induced vaccine responses is unknown. To address this question we conducted a

longitudinal analysis of IgG titers specific for common viral (measles, polio, Hepatitis B) and bacterial (*Haemophilus influenzae type b*, meningococcus, tetanus) vaccine antigens in 54 children living in an area of Mali where the 6-month malaria and dry seasons are sharply demarcated. Vaccine-specific IgG titers were measured for each subject at five time points over an 18 month period: before and after the first dry season, during and 10 days after the first episode of febrile malaria of the ensuing malaria season, and at the end of the second dry season. Preliminary analyses suggest that average IgG decay rates are not significantly accelerated by acute *P. falciparum* infection; however, at the individual level a minority of children exhibited accelerated IgG decay rates following *P. falciparum* infection compared to decay rates over the dry season. In addition, preliminary comparisons with studies of non-malaria exposed populations suggest that overall decay rates of vaccine-induced IgG responses may be higher in the population of children who experienced febrile malaria. These data highlight the need for additional studies to understand the factors underlying variability in vaccine-specific antibody decay rates in individuals residing in malaria endemic areas.

1572

THE IMPACT OF *SCHISTOSOMA HAEMATOBIUM* INFECTION ON *PLASMODIUM FALCIPARUM*-INDUCED IMMUNE RESPONSES

Safiatou N. Doumbo[1], Jules Sangala[1], Jacqueline Moebius[2], Silvia Portugal[2], Tuan M. Tran[2], Aboudramane Bathily[1], Aissata Ongoiba[1], Peter D. Crompton[2], Boubacar Traore[1], Ogobara K. Doumbo[1]

[1]University of Sciences, Technique and Technology of Bamako, Bamako, Mali, [2]National Institutes of Health, Rockville, MD, United States

Plasmodium falciparum and *Schistosoma haematobium* often overlap in tropical and subtropical countries and impose tremendous disease burdens. Evidence suggests that *S. haematobium* modifies the risk of febrile malaria; however, the nature of this putative immunomodulation remains unclear. To investigate this question we analyzed *S. haematobium*-infected individuals (n=15) and uninfected controls (n=24) within a longitudinal cohort study in Mali. Before the malaria season, peripheral blood mononuclear cells (PBMCs) were collected from *S. haematobium*-infected and uninfected individuals, all of whom were negative for *P. falciparum* by PCR. PBMCs were analyzed before and after *in vitro* stimulation with lysate of *P. falciparum* (3D7) infected red blood cells (iRBCs) and analyzed by flow cytometry with intracellular cytokine staining; and supernatants of stimulated PBMCs were analyzed by a multiplex cytokine assay. Compared to uninfected controls, *S. haematobium*-infected individuals had a higher baseline percentage of dendritic cells, but no differences were observed in the proportion of B cells, T cells, NK cells or their respective subsets. Stimulation with iRBCs showed a modest increase in IFN-γ producing $CD4^+$ T cells in *S. haematobium*-infected individuals. These preliminary data suggest that *S. haematobium* modulates the innate and adaptive immune response to *P. falciparum* infection. In an ongoing longitudinal study we are studying the impact of concurrent or recent *S. haematobium* infection on the host immune response to subsequent *P. falciparum* infection.

1573

AVIDITY OF NATURALLY ACQUIRED ANTI-*PLASMODIUM VIVAX* MSP1-19 ANTIBODIES IN INDIVIDUALS PRESENTING DIFFERENT CLINICAL EXPRESSIONS OF MALARIA

Maria Carmen A. Sanchez, Adeiana G. Lima, Arianni R. Sanchez, Eduardo M. Ramos-Sanchez, Silvia M. Di Santi, Marina P. Dornelas, Sandra L. Moraes, Hiro Goto

São Paulo University, São Paulo, Brazil

Plasmodium vivax is the most prevalent species in Brazil accounting for around 85% of clinical cases. *P. vivax* infections cause non-severe malaria in most cases, but may also be asymptomatic or cause severe disease. C-terminal region of merozoite surface protein 1 of *P. vivax* (PvMSP1-19) is highly immunogenic conserved region that plays a major role in the protective immunity against asexual blood stages of malaria parasites. Since this protective immunity has been shown to correlate with levels of anti-MSP1-19 antibodies, this study aimed to evaluate the humoral immune response against PvMSP1-19 of individuals naturally exposed to malaria, from endemic areas of Brazil, in order to assess the IgG and IgM profile, the avidity of IgG antibody (functional affinity) and their association with different malaria clinical presentations. Serum samples from four groups of individuals were studied: severe malaria (N=18), asymptomatic infection (N=17), non-severe symptomatic malaria undergoing their first malaria episode (N=104) and non-severe symptomatic malaria with previous malaria episodes (N=102). All were positive for *P. vivax* by thick blood smear and/or PCR and for IgG and/or IgM antibodies by ELISA-PvMSP1-19. High avidity (>50%) IgG antibodies were observed in 92.9% of patients who had previous malaria episodes (median reactivity index: IgG=8.7 and IgM=1.1) and in 88.0% of asymptomatic individuals (median reactivity index: IgG=2.4 and IgM=0.3). Low/moderate avidity (≤50%) was seen in 89.1% of patients undergoing their first malaria episode (median reactivity index: IgG=6.7 and IgM=3.0) and in 94.0% of severe malaria patients (median reactivity index: IgG=7.7 and IgM=6.7). Predominance of high-avidity antibody in individuals with non-severe malaria that had multiple episodes of malaria and in asymptomatic infections corroborates the protective role of humoral immunity. It likely reduces the risk to develop mild and severe malaria. Our results show that protective immunity not only correlate with levels of anti-MSP1-19 antibodies but also with the quality of these antibodies.

1574

GENERATION OF "FULLY HUMAN" MONOCLONAL ANTIBODIES AGAINST THE CIRCUMSPOROZOITE PROTEIN (CSP) OF *PLASMODIUM FALCIPARUM* USING HUMANIZED HLA MICE

Sai P. Majji[1], Wathsala Wijayalath[1], Soumya Shashikumar[1], Sandra Inoue[1], Andrea Akyeampong[1], Thomas L. Richie[1], Eileen Villasante[1], Teodor-Doru Brumeanu[2], Sofia Casares[1]

[1]Naval Medical Research Center/Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Uniformed Services University of Health Sciences, Bethesda, MD, United States

"Fully human" monoclonal antibodies (hmAb) are a novel approach to treat infectious diseases. Fully human mAbs are devoid of the complications associated to the use of mAbs derived from mouse or humanized antibodies. We have generated humanized HLA mice in NOD.RagKO.IL2RgcKO background that develop a functional human immune system and respond to vaccination. Using human B cells from humanized HLA mice immunized with irradiation-attenuated *Plasmodium falciparum* sporozoites we have generated a panel of hmAbs against *P. falciparum* CSP. Herein we present data on anti-CSP hmAb immunocharacterization and *in vitro* and *in vivo* anti-parasitic activity.

1575

MONITORING AND EVALUATION OF EFFECTIVENESS OF IFAKARA HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEMS (HDSS) IN QUANTIFYING IMPACT OF MALARIA CONTROL STRATEGIES AND INTERVENTIONS

Mwajabu M. Mwanaupanga

Ifakara Health Institute, Dar es salaam, United Republic of Tanzania

Health and Demographic Surveillance Systems (HDSS) have been set up in various sites in Africa and Asia. In the absence of effective vital registration and information on mortality, these health and demographic surveillance systems have well developed structures and standard operating procedures established to periodically monitor vital events like births, deaths and migrations in well defined areas to produce population health information. In Tanzania Ifakara DSS remain as an instrumental infrastructure of

producing key demographic indicators that are useful for planning and resource allocation to the community. The aim of my study is to determine the effectiveness of Ifakara HDSS in quantifying the impact of malaria control strategies and interventions. A cross sectional study design will be used to assess HDSS documentation mechanism on the impact of different malaria control strategies and interventions performed in the area, and to determine if the project effectively quantifies the impact of those interventions and strategies. Systematic sampling will be used to obtain number and types of documents that will be reviewed to fulfill the aim of our study. Collection and analysis of data will be both qualitative and quantities approach. Findings will be explained based on: 1) extent the knowledge and skills of the project staff in content of the HDSS malaria forms and data quality checking has increased from the pre to the post intervention periods of the project, 2) extent to which the malaria information management has improved, 3) ways in which the project was able to promote quality malaria data generation, 4) extents to which costs were able to reach project goal of documenting impact of malaria control strategies and interventions, and 4) how the HDSS understood and cared about the importance of resources at work and if the managers supported them with training, supervision and needed resources. The conclusion will be based upon findings that will be obtained during the data collection.

1576

BUILDING A CRITICAL MASS OF HEALTH WORKFORCE TO FIGHT MALARIA IN NIGERIA

Muhammad K. Salihu[1], Bolatito Aiyenigba[1], Veronica Momoh[2], Abba Z. Umar[1]

[1]FHI360, Abuja, Nigeria, [2]Health Partners International, Abuja, Nigeria

The Presidential Malaria Initiative (PMI) through the Malaria Action Program for States (MAPS) in Nigeria currently supports seven states to implement effective malaria control, prevention, diagnosis and treatment activities. Nigeria health workforces are diverse and cut across administrative, programmatic and clinical areas including community health services. Effective and sustainable malaria program will need to build capacity in all these areas. This study documents the PMI achievement in Nigeria. In-service training using adult learning principles were conducted for all cadres of staff relevant to malaria prevention and control. Administrative and programmatic staffs were trained on malaria program management; clinical staff were trained on prevention of malaria in pregnancy (MIP); malaria diagnosis using rapid test and microscopy; malaria case management including severe malaria. Other trainings conducted were focused on strengthening health information management; behavior change communication and community acceptance of interventions. From October 2011 to September 2013, 2770 health workers had been trained on MIP; over 9400 health workers and Community Care Givers had been trained on case management and 3380 trained on malaria diagnosis. About 2498 health managers were also trained on malaria program management. Over 4504 health workers were trained on health information management; 3344 health educators and journalists trained on malaria BCC. Programmatic results recorded include increase in fever cases tested from 45.9% in April 2013 to 70.2% in March 2014; and referral of over 40,000 pregnant women and children under 5 for ANC and treatment of fever. The critical mass of health workers trained will contribute to the implementation of a sustainable, efficient, integrated malaria program at state and local government levels because many of these workers offer informal services in their communities. Pre-service training is needed to sustain malaria control program.

1577

QUALITY OF CARE FOR CHILDREN UNDER FIVE YEARS WITH UNCOMPLICATED MALARIA AT PRIVATE CLINICS IN MAKINDYE DIVISION, KAMPALA DISTRICT

Ronald Mulebeke[1], Achillies Katamba[2], Aggrey Mukose[1], Pauline Byakika[2], Ellizeus Rutebemberwa[1], Phyllis Awor[1], Jane Achan[2], Adoke Yeka[1]

[1]Makerere University School of Public Health, Kampala, Uganda, [2]Makerere University College of Health Sciences, Kampala, Uganda

Over 60% of the population in developing countries seeks for care from private health facilities. In Uganda over 80% of the Population access health care from small drug shops, private clinics and private-not-for profit providers because they are closer to the communities and are perceived to be affordable. However, health providers in private clinics more frequently violate accepted medical standards and guideline because there primary goal is making profit. Quality of care for children with uncomplicated malaria in such settings is generally sub-optimal with low adherence to treatment guidelines. We are conducting a study to assess the quality of care provided to children under five years with uncomplicated malaria at private clinics in Makindye division, Kampala district, Uganda. The study is a cross-sectional cluster survey conducted in 30 private clinics. Data was collected using patient exit interviews, questionnaires for health workers, observation of health workers during consultation, health facility audits and Key Informant Interviews. A total of 180 exit interviews were conducted with caretakers of children and 30 healthcare workers were observed while treating patients and were interviewed thereafter. A total of 8 Key informant interviews were conducted with heads of private clinics. Data is being analyzed to determine predictors of quality of care for children with uncomplicated malaria at private clinics. A composite index will be utilized to assess overall quality of care.

1578

FACTORS AFFECTING TREATMENT SEEKING FOR FEVER BEFORE AND AFTER INTERVENTIONS TO IMPROVE ACCESS AND TARGETING OF ARTEMISININ COMBINATION THERAPIES

Charles Festo[1], Rebecca Thomson[2], Boniface Johanes[1], Admirabilis Kalolella[1], Happy Nchimbi[1], Katia Bruxvoort[2], Matthew Cairns[2], Mark Taylor[2], Immo Kleinschmidt[2], S. Patrick Kachur[3], Catherine Goodman[2]

[1]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

Artemisinin combination therapies (ACTs) were introduced as the first line antimalarial in Tanzania in 2006, but access and targeting were poor. The main sources of treatment for fever included public and private health facilities, drug stores, pharmacies and general shops, but the probability of receiving both parasitological diagnosis and an ACT varied considerably across these outlet types. It is therefore essential to understand factors that determine choice of provider. We assessed this using data from large scale household surveys conducted before (2010) and after (2012) national rollout of rapid diagnostic tests in public facilities, and ACT subsidies under the Affordable Medicines Facility-malaria. We visited a representative random sample of households in each of 3 regions (Mwanza, Mbeya, Mtwara) with varying malaria transmission and access to health care. 5,423 households at baseline and 5,511 at endline were sampled using a multi-stage design. All household members reporting fever were asked about treatment sought. There was no significant change in the percentage of people with fever seeking care between baseline and endline (69.5% and 73.6%, p=0.07). However, there were changes in treatment source, with an increase in the percentage using drug stores (41.3% to 54.1%, p<0.001), and a fall in use of public facilities (25.3% to 16.8% p<0.001). Overall, children <5 years old were more likely to be taken to a public facility at baseline and endline. We will present results

of multivariable analysis assessing the adjusted odds of seeking care, and of seeking care at specific provider types at baseline and endline. Relevant exposures include age, sex, region, urban/rural location, education of the household head, time to the nearest treatment source, enrolment in health insurance scheme and local ACT stockout levels in public facilities. Key factors affecting treatment seeking decisions will be discussed, including how these have changed following implementation of two key interventions affecting malaria treatment provision, and the implications for future policy to enhance access and targeting of ACTs.

1579

AN ASSESSMENT OF THE MALARIA-RELATED KNOWLEDGE AND PRACTICES OF TANZANIA'S DRUG RETAILERS: EXPLORING THE IMPACT OF DRUG STORE ACCREDITATION

Rebecca Thomson[1], Boniface Johanes[2], Charles Festo[2], Admirabilis Kalolella[2], Mark Taylor[1], Sarah Tougher[1], Yazoume Ye[3], Andrea Mann[1], Ruilin Ren[3], Katia Bruxvoort[1], Barbara Willey[1], Fred Arnold[3], Kara Hanson[1], Catherine Goodman[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [3]ICF International, Calverton, MD, United States

Since 2005 Tanzania has been upgrading its approximately 7,000 drug stores to Accredited Drug Dispensing Outlets (ADDOs), involving dispenser training, introduction of record keeping and enhanced regulation. ADDOs are permitted to stock 49 prescription-only medicines, including artemisinin combination therapies. Prior to accreditation drug stores could officially stock over-the-counter medicines only, though many stocked prescription-only antimalarials. Oral artemisinin monotherapies and injectables were not allowed in any drug stores. By late 2011 ADDO conversion was complete in 14 of 21 regions. We explored variation in malaria-related knowledge and practices of drug retailers in ADDO and non-ADDO regions. We excluded Dar es Salaam where market conditions were not comparable to other regions. Data were collected as part of the Affordable Medicines Facility-malaria Independent Evaluation, involving a nationally representative survey of antimalarial retailer in October-December 2011. We randomly selected 49 wards, and interviewed all drug stores stocking antimalarials. Interviews were conducted in 148 drug stores in ADDO regions and 127 in non-ADDO regions. Drug stores in ADDO and non-ADDO regions were similar in terms of employing staff with health-related qualifications (96.1% and 96.2%, p=0.99); stocking the first line antimalarial (59.5% and 60.7%, p=0.89); and stocking artemisinin monotherapy (0.9% and 0.0%, p=0.43). Drug stores in ADDO regions performed better on knowledge of the first line antimalarial (99.5% and 91.5%, p=0.001). There was weak evidence of a lower price and higher market share of the first line antimalarial in ADDO regions. However, drug stores in non-ADDO regions were less likely to stock injectables (21.5% and 3.6%, p=0.003). ADDO conversion is frequently cited as a model for improving retail sector drug provision. Drug stores in ADDO performed better on some but not all indicators, possibly indicating weaknesses in ADDO regulation and high staff turnover. More evidence is needed on the value-added and value for money of ADDO roll out to inform retail policy in Tanzania and elsewhere.

1580

AUDITING VILLAGE HEALTH TEAMS' CAPACITY FOR MANAGEMENT OF MALARIA: RESULTS OF THE 2013 ACTWATCH UGANDA OUTLET SURVEY

Julius Njogu[1], John H. Rogers[2], Henry Kaula[3], Vamsi Vasireddy[4], Megan Littrell[5]

[1]Population Services International, Nairobi, Kenya, [2]Centers for Disease Control and Prevention, Dar es salaam, United Republic of Tanzania, [3]PACE, Kampala, Uganda, [4]Independent Consultant, Chicago, IL, United States, [5]Population Services International, Washington, DC, United States

Malaria is endemic across 95% of Uganda. The Ugandan government aims at 85% of malaria cases receiving prompt and recommended treatment by the end of 2014. To achieve this goal, integrated Community Case Management (iCCM) initiatives have been implemented in 34 districts by building capacity of Village Health Teams (VHTs) to manage febrile children. Within these initiatives, all suspected malaria cases are tested and confirmed cases are treated with Artemether-Lumefantrine (AL), and severe cases are referred for rectal Artesunate treatment. To enumerate VHT malaria capacity under iCCM, a census of VHTs was conducted in 16 iCCM sub-counties using ACTwatch methodology. A questionnaire was administered to assess knowledge, availability of antimalarials and rapid diagnostic tests (RDTs). We present descriptive results on availability and knowledge. We surveyed a total of 1,862 VHTs. Of these, 33.7% (628) stocked AL, 10.0% (187) rectal artesunate and 21.6% (403) stocked malaria RDTs during the survey visit. Notably, when AL was available, 56.5% (355/628) of VHTs stocked malaria RDTs. Of those with antimalarials, 91.6% (606/660) correctly stated recommended first-line medicine for uncomplicated malaria (AL) and 83.3% knew its dosing regimen. Among RDT stockists, 93% (375/403) stated they would never dispense antimalarial following a negative RDT result. The iCCM presents an important channel for increasing access to integrated case-management in Uganda. According to these results, VHTs are highly knowledgeable but lack antimalarial and RDT stocks, which may undermine iCCM goals. Enhancing stable and reliable supply of first-line medicines and RDTs may sustain increased access to prompt and correct malaria-case management.

1581

ASSESSING THE COST OF PRIVATE SECTOR ACT SUBSIDIES - THE FINANCIAL AND ECONOMIC COSTS OF THE AFFORDABLE MEDICINES FACILITY - MALARIA (AMFM) IN THREE AFRICAN COUNTRIES

Catherine Goodman[1], Fiona Cianci[1], Daniel Cobos[2], Sarah Tougher[1], Kara Hanson[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Swiss Tropical and Public Health Institute, Basel, Switzerland

AMFm was designed to improve uptake of quality-assured artemisinin combination therapies (ACTs). Hosted by the Global Fund to Fight HIV/AIDS, Tuberculosis and Malaria, it operated in eight national scale pilots, involving: (i) price negotiations with ACT manufacturers; (ii) a subsidy for ACTs at the top of the global supply chain; and (iii) supporting interventions, such as communications campaigns and provider training. An Independent Evaluation found that in most but not all settings AMFm improved the availability, affordability and market share of quality-assured ACTs. However, no data were available on AMFm costs. This study aimed to address this gap. We collected data on the costs of implementing AMFm in the private for-profit sector only in Nigeria, Kenya and Madagascar, representing a range of performance on AMFm indicators. Costs were included for all AMFm-related activities at both country level and the Global Fund headquarters. We adopted a "funders perspective", covering all resources contributed by external funding agencies, NGOs and national governments, but excluding costs to commercial actors and households. Results are presented in terms of financial costs (actual expenditure) and economic costs (which include an annualised component

of start-up costs based on their expected useful life). All costs were converted to 2012 USD. The number of subsidised ACTs delivered for the private for-profit sector by the end of 2012 was 91,4 million (mn) in Nigeria and 28,3mn in Kenya, but only 2,1mn in Madagascar, where imports were much lower reflecting the very limited communications campaign, the predominance in the market of outlets not permitted to sell ACT, and political and economic disorder. Total financial costs for 2009-12 were $110,2mn in Nigeria, $39,9mn in Kenya and $4,9mn in Madagascar. Annual economic costs for 2011 were $57,2mn in Nigeria, $17,7mn in Kenya and $2,2mn in Madagascar, implying respectively an economic cost per capita of $0.35, $0.42 and $0.10, and per ACT dose delivered of $1.30, $1.51 and $1.91. The ACT subsidy itself accounted for 89% of economic costs in Nigeria, 80% in Kenya, and 48% in Madagascar. Sensitivity analysis will be presented to explore the impact of varying key assumptions and to estimate the costs of replication in other settings. The implications for the potential value for money of strategies to expand ACT use through the private sector will be discussed.

1582

INVESTIGATING THE DETERMINANTS OF DEMAND FOR ANTIMALARIAL MEDICINES IN BENIN, NIGERIA, UGANDA AND ZAMBIA

Benjamin Palafox[1], Kara Hanson[1], Catherine Goodman[1], Aurélia Lépine[1], Virginia Wiseman[1], Marcos Vera Hernandez[2]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]University College London, London, United Kingdom

Artemisinin-based combination therapy (ACT) is an essential health systems input in malaria endemic countries. By 2008, all of these countries had adopted ACT as the recommended first-line treatment for falciparum malaria. Although ACT may be available free of charge through public facilities, use of non-ACTs is common. This is particularly true for children under 5 years of age who may be treated presumptively where diagnostic capacity is limited. Much of these non-ACTs are purchased easily from private sector retailers who may also sell ACTs, but at much higher prices. Therefore, ensuring that children receive appropriate and quality treatment for malaria requires an understanding of how responsive household demand is to antimalarial prices and other determinants. We estimated econometric demand models for antimalarials to examine the determinants of antimalarial choice in Benin, Nigeria, Uganda and Zambia. In each country, data were collected through nationally representative surveys of households that experienced a recent paediatric febrile episode. This was complemented by survey data from all possible public and private sources of antimalarial medicines in the vicinity of these households. Treatment choices included ACTs, oral artemisinin monotherapies, non-artemisinin therapies, and no treatment. The range of determinants studied included various treatment price components (e.g. antimalarials, diagnostics, travel), and characteristics of the provider, household and caregiver. Our findings will focus on the most significant determinants of which antimalarial households obtain and from where, and examine how responsive antimalarial demand is to changes in antimalarial prices and household income. Given the considerable resources directed toward improving access to appropriate malaria treatment, we will also discuss how these findings may be applied to optimise the equitable impact of these investments in pluralistic health systems settings.

1583

DETERMINANTS OF STOCKING AND PRICING OF SUBSIDIZED ANTIMALARIAL TREATMENTS BY RETAILERS IN THE PRIVATE FOR-PROFIT SECTOR: EVIDENCE FROM NATIONAL SUBSIDY PROGRAMS IN KENYA, NIGERIA AND UGANDA

Sarah Tougher, Catherine Goodman, Kara Hanson

London School of Hygiene & Tropical Medicine, London, United Kingdom

There is increased interest in improving quality of care in the private for-profit sector in low and middle income countries, as private providers are an important source of treatment for many illnesses. In the case of malaria, a large proportion of patients purchase antimalarial treatments from for-profit providers. For example, data from 2009-2010 show that in Nigeria, Kenya and Uganda, 97%, 67% and 40% of total antimalarial volumes were distributed by for-profit providers, respectively. However, medicines obtained are often inappropriate, because non-recommended treatments are widely and cheaply available. Subsidies for artemisinin combination therapies (ACTs), the first-line treatment in most malaria-endemic countries, have been implemented in a number of settings in order to improve coverage of ACTs and discourage use of other treatments. The largest initiative of this type was the Affordable Medicines Facility - malaria (AMFm), which was implemented at a national scale in eight pilots from 2010-2013. By the end of 2013, over 310 million treatments were ordered for the private for-profit sector through the initiative, and an Independent Evaluation reported large improvements in ACT availability, price and market share in six of the eight pilots. However, little is known about the causes of inter- and intra- country variations in performance. The success of private sector ACT subsidy programmes is determined by providers' decisions on whether to stock subsidized medicines and decisions on the pricing of subsidized medicines. This study used nationally-representative outlet-survey data from Kenya, Nigeria and Uganda to model provider decisions to stock ACTs subsidized through AMFm, and set markups for the drugs. These three countries were selected, because they have diverse contexts and AMFm had differing effects. For each country, multiple regression analysis was used to examine the determinants of markups and stocking of subsidized ACTs. The determinants investigated were a set of product, provider and market characteristics, including measures of competition. The analysis addressed the endogenous and hierarchical nature of the data. The evidence presented will help identify settings suitable for ACT subsidies and the types of supporting interventions and their targeting that are most appropriate.

1584

OPTIMAL PRICE SUBSIDIES FOR APPROPRIATE MALARIA TESTING AND TREATMENT BEHAVIOR

Kristian S. Hansen[1], Tine H. Lesner[2], Lars P. Østerdal[2]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]University of Southern Denmark, Odense, Denmark

Malaria continues to be a serious public health problem particularly in Africa. Limited access to antimalarials and over-treatment with antimalarials are two seemingly contradicting phenomena that co-exist. A global subsidy on selected antimalarial drugs has been suggested to increase access to the most effective treatment both in the public and private sectors. In order to also reduce over-treatment we propose a combined price subsidy on malaria rapid diagnostic tests and antimalarial drugs. Focusing on the private sector, we analyse the optimal subsidy combination that incentivises individuals suspecting themselves to have malaria to purchase a parasitological test before buying the recommended treatment using an expected utility model describing the health-seeking behaviour of a representative individual. Solving our model numerically for individuals with a range of different health-seeking behaviours shows that the optimal policy of the health planner is to redirect some of the subsidy money from antimalarial drugs to parasitological tests.

1585

IDENTIFYING MINIMAL EPITOPES ON THE SURFACE OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN REACTIVE WITH NEUTRALIZING MONOCLONAL ANTIBODIES

Miriam T. George[1], Jesse L. Schloegel[2], Francis B. Ntumngia[1], Samantha J. Barnes[1], Edwin Chen[3], Christopher L. King[4], Niraj H. Tolia[3], Joanne L. Casey[2], Michael Foley[2], John H. Adams[1]

[1]University of South Florida, Tampa, FL, United States, [2]La Trobe University, Victoria, Australia, [3]Washington University School of Medicine, St. Louis, MO, United States, [4]Case Western Reserve University, Cleveland, OH, United States

Plasmodium vivax Duffy Binding protein region II (PvDBPII) is an essential ligand for reticulocyte invasion, thereby making this molecule an attractive vaccine candidate to protect against asexual blood-stage *P. vivax*. Similar to other blood-stage vaccine candidates, DBP allelic variation elicits a strain-specific immunity that may be a major challenge for development of a broadly effective vaccine against vivax malaria. This study aims to identify conserved epitopes of neutralizing anti-DBP monoclonal antibodies (mAbs) using immunochemical and structural approaches to help design a strain-transcending vaccine. The crystal structure of PvDBPII consists of 2 α-helical bundles with an antiparallel β-hairpin near the N-terminus and may be assigned into three subdomains delineated by six disulphide bonds. The various subdomains and combination of subdomains were expressed in their correctly refolded and disulphide bonded conformation on the surface of the M13 filamentous phage. Additionally, a PvDBPII gene fragment library was used for biopanning to screen phage clones reactive with anti-DBP mAbs. Comparative analysis of specific targets of non-inhibitory anti-DBP mAbs with neutralizing anti-DBP mAbs will help determine essential regions of PvDBP for a subunit vaccine designed to protect against blood-stage *Plasmodium vivax* malaria.

1586

EVALUATION OF FUNCTIONAL IMMUNOGENICITY OF *PLASMODIUM FALCIPARUM* TRANSMISSION-BLOCKING ANTIGEN PFS25 PRODUCED IN *E. COLI* ADJUVANTED IN VARIOUS NANOPARTICLES AND ADJUVANTS

Rajesh Kumar[1], Geetha Bansal[1], Grace Ledet[2], Richard Graves[2], Tarun Mandal[2], Dibyadyuti Datta[1], Evelina Angov[1], Nirbhay Kumar[1]

[1]Tulane University School of Public Health & Tropical Medicine, New Orleans, LA, United States, [2]Center for Nanomedicine and Drug Delivery, Xavier University College of Pharmacy, New Orleans, LA, United States

Pfs25, expressed on the surface of gametes, zygotes and ookinetes, is an established target antigen for malaria transmission blocking vaccines. Previously, we have reported that codon harmonized recombinant Pfs25 (CH-rPfs25) produced in E. coli elicited highly potent transmission blocking antibodies using Montanide ISA51, Alum and CFA as adjuvants. In the current study, we have undertaken extensive evaluation of various nanoparticles/adjuvants via different routes of immunization to identify safer and effective adjuvants. Mice were immunized with CH-rPfs25 via IM route (10 µg, three doses at 4 week intervals). Mice immunized with CHrPfs25 in Alum via IP and IM routes induced comparable antibody titers (640,000). Since the protein was equally immunogenic by IP and IM routes, other adjuvant formulations were tested by IM route only. CH-rPfs25 was adsorbed to nano-emulsions (4% & 8%NE) and PLGA particles (2 different amounts). CH-rPfs25 formulated in 4% NE gave highest antibody response (ELISA titer 1,280,000) as compared to 320,000 in 8% NE. Antibody titers with PLGA (10 and 20 mg PLGA) were only 160,000. We also evaluated NE formulations combined with MPLA and chitosan, and both demonstrated 640,000 antibody titers. Functional activity of antibodies was evaluated by standard membrane feeding assay using purified IgG (50- 400 µg/ml) from immunized animals. 100 % transmission blocking activity (no oocysts detected) was observed at 400 ug/ml of IgG from Alum group (both routes IP and IM), NE (4%), and NE-MPLA. Purified IgG from various adjuvant groups at lower doses (100 µg/ml) still exhibited >90% transmission blocking activity, while 52-81% blocking was seen at 50 ug/ml. Our results suggest that CH-rPfs25 is strongly immunogenic by different routes and as formulations with Alum and NE. We are continuing these studies to develop effective vaccine formulations for further evaluation and investigations into immune correlates of relative immunogenicity of CH-rPfs25 in various adjuvants.

1587

ENHANCING PRE-ERYTHROCYTIC STAGE VACCINE EFFICACY WITH THE DEVELOPMENT OF A HIGHLY IMMUNOGENIC VIRUS-LIKE PARTICLE VACCINE AND A MULTI-COMPONENT VACCINE STRATEGY

Katharine A. Collins, Matthew Cottingham, Rebecca Hillson, Sarah Gilbert, Adrian V. Hill

University of Oxford, Oxford, United Kingdom

CSP-based subunit vaccines have been shown to protect against malaria in a range of models, but to date, none have been able to elicit high levels of durable sterilising efficacy in human field trials. Our aim is to enhance pre-erythrocytic stage vaccine efficacy by two methods. Firstly by increasing the immunogenicity of a CSP-based virus-like particle (VLP) vaccine, and secondly by combining the VLP vaccine with a liver-stage viral vector vaccine regimen. To enhance the immunogenicity of the CSP-based particle we have developed an improved RTS,S like vaccine called R21. RTS,S is the leading CSP-based vaccine and consists of particles formed from a mixture of two proteins, with only ~20% of the total protein content being a CSP-HBsAg fusion protein. In the R21 particles, 100% of the total protein content is a CSP-HBsAg fusion protein and hence R21 will contain a much higher percentage of CSP than RTS,S. This could result in enhanced immunogenicity and efficacy and is currently under evaluation. The immunogenicity and efficacy of R21 + adjuvant was compared to non-particulate recombinant CSP + adjuvant in BALB/c mice. R21 was found to be more immunogenic and induced 10 fold greater levels of anti-CS antibodies as well as higher frequencies of CS specific T cells than CSP. These serum antibody titres were also durable and were maintained at high titres when measured 3 months after vaccination. R21 was also significantly more protective than non-particulate CSP in a BALB/c model against *P. berghei* transgenic for *P. falciparum* CSP. Vaccination with R21 + Matrix M sterilely protected 82.5% of mice compared to only 42.5% with CSP + Matrix M ($p = 0.014$). R21 was also assessed in combination with the ChAd63 ME.TRAP - MVA ME.TRAP vaccine regimen and there was no interference with the induction of vaccine specific immune responses when the vaccines were mixed and administered together. In addition, sterile efficacy against sporozoite challenge was significantly enhanced in the mixed vaccine group. R21 is now being taken forward for evaluation in Phase I/IIa clinical trials.

1588

PROJECTED COST-EFFECTIVENESS OF RTS,S VACCINATION IN 43 SUB-SAHARAN AFRICAN COUNTRIES

Katya Galactionova, Fabrizio Tediosi, Thomas A. Smith, Melissa A. Penny

Swiss Tropical and Public Health Institute, Basel, Switzerland

Phase III trials of the RTS,S malaria vaccine are generating new estimates of its efficacy and decay rate. To assess the appropriateness of introducing RTS,S into the EPI in endemic African countries cost-effectiveness estimates need to be updated with the most recent clinical efficacy data. We used methods for rapidly updating projections of country specific public health impact of the vaccine, and combined these with costing models for malaria case management and immunization to assess the cost-effectiveness of RTS,S introduction. We consider deployment with a 3 dose schedule targeting infants 6, 10 and 14 weeks of age, an older cohort vaccinated at 6, 7.5 and 9 months, and a 4 dose schedule as above, which includes a booster at 18 months after the third dose. Allowing for differences in epidemiological context and health systems we generate

predictions tailored to countries examined and apt to inform malaria control policy. An ensemble of individual-based stochastic simulation models of *Plasmodium falciparum* dynamics, with varied assumptions about immune decay, transmission heterogeneity, and access to treatment fit to an extensive library of field data were used to predict the impact of RTS,S. For each country average and incremental cost-effectiveness ratios were calculated relative to the routine case management and alternate vaccine deployment strategies. We show that RTS,S is likely to be a highly cost-effective intervention with a significant impact on malaria burden in endemic settings and cost per DALY averted generally comparable to routine malaria control interventions. Depending on vaccine properties, coverage, and age-related disease burden vaccinating younger cohorts may avert more disease and at a lower cost compared to older age groups, even if initial efficacy is lower. As the vaccine targets only a small fraction of the population susceptible to malaria and provides limited protection it does not eliminate the need for other control programs. Access to effective treatment is particularly important to sustain health gains achieved with the RTS,S.

1589

METHODOLOGY TO ESTIMATE THE COST OF INTRODUCING RTS,S VACCINE INTO A NATIONAL IMMUNIZATION PROGRAM IN SUB-SAHARAN AFRICAN COUNTRIES

Katya Galactionova, Fabrizio Tediosi

Swiss Tropical and Public Health Institute, Basel, Switzerland

Having demonstrated moderate levels of efficacy in Phase II and III trials in Africa, the RTS,S vaccine against *P. falciparum* malaria is currently considered for use within the Expanded Programme on Immunization in a large number of endemic settings. Introduction of a new vaccines demands extensive resources from the health system. Aside from the vaccine and related immunization supplies, resources are required for storage and supply chain, training, development of educational materials, social mobilization, supervision, monitoring, vaccine delivery, and waste management. We propose a generalizable methodology to estimate these costs related to vaccine introduction in African countries. Costs are evaluated from a broad provider perspective using the ingredients approach; these reflect the economic value of resources, and take into account overheads and cost of inputs shared with other health interventions. To address the uncertainty about the level of existing capacity in the health system we consider several states of the EPI: no spare capacity, estimated current capacity, and sufficient spare capacity to accommodate the new vaccine. At each capacity level we develop a series of normative scenarios for service delivery and capacity scale-up in accordance with the current operational guidelines. Scenarios are adapted to a given country setting to take into account among other the structure of the EPI program, distribution model, geography, and population dynamics. Resource lists and quantity assumptions defined for each immunization scenario are matched with price and unit cost data via cost functions to assess the overall cost of the program. The methodology takes advantage of country data on prices of key inputs using routinely collected data from the cMYP, UNICEF, and WHO-CHOICE. The methodology is applied to assess cost of RTS,S introduction in 6 endemic countries. We test the robustness of estimates generated by varying core assumptions and prices of key inputs and validate against the literature on cost of EPI program and introduction of other new vaccines.

1590

EFFICACY, SAFETY AND IMMUNOGENICITY OF HETEROLOGOUS PRIME-BOOST IMMUNIZATION WITH THE CANDIDATE MALARIA VACCINES CHAD63 ME-TRAP AND MVA ME-TRAP IN 5-17 MONTHS OLD BURKINABE INFANTS AND CHILDREN

Alfred B. Tiono[1], Issa N. Ouedraogo[1], Jean-Baptist Yaro[1], Katie Ewer[2], Edith C. Bougouma[1], Issiaka Soulama[1], Rachel Roberts[2], Amidou Diarra[1], Alphonse Ouedraogo[1], Alison Lawrie[2], Sam Coulibaly[1], Thomas Rampling[2], Carly Bliss[2], Aïssata Barry[1], Ines Petersen[3], Georgina Bowyer[2], Nick Anagnostou[2], Nicola Viebig[3], Egeruan Babatunde Imoukhuede[2], Philip Bejon[4], Adrian Hill[2], Sodiomon B. Sirima[1]

[1]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [2]University of Oxford, Oxford, United Kingdom, [3]European Vaccine Initiative, Heidelberg, Germany, [4]Kenya Medical Research Institute, Kilifi, Kenya

The development of an effective vaccine against malaria is a high priority and of great importance in the context of coordinated efforts to reduce the burden of malaria. The protective efficacy of the candidate malaria vaccines ChAd63 ME-TRAP and MVA ME-TRAP is being evaluated in an ongoing phase I/IIb double-blind randomized trial. This immunization regime has shown significant partial efficacy in controlled human malaria infection trials and an initial time-to-infection trial in adults in Kenya. Initial efficacy results from this first efficacy trial in African infants will be available in May 2014. The primary objective is to evaluate the protective efficacy against clinical malaria, for a period of 6 months after the last vaccination. Clinical malaria is defined as fever (axillary temperature ≥37.5°C) together with *Plasmodium falciparum* count > 5,000 p/µl. Seven hundred infants and children aged 5-17 months were randomized in 1:1 ratio to receive either ChAd63 ME-TRAP / MVA ME-TRAP in prime-boost immunization or rabies vaccine. Immunization schedule was 0, 8 weeks. Clinical malaria episodes were captured through a health-facility based passive case surveillance method. Venous blood samples for cellular and humoral immunogenicity were collected at various timepoints. Vaccine efficacy will be assessed using Cox regression models. For analysis of first or only episodes of *P. falciparum* malaria, the incidence of episodes for each group will be presented. Secondary analysis will examine multiple episodes, using the robust clustering method by individual. Analysis of vaccination immunogenicity will describe the arithmetic and geometric means and median spots per million PBMC by vaccine group and timepoints. The safety analysis will include all solicited and unsolicited local and systemic adverse events including clinically significant laboratory abnormalities, and serious adverse events. These results will help define the potential role of these recombinant viral vectors, either used alone or as part of a multi-component vaccine, in malaria control in Africa.

1591

ANTIBODIES AGAINST A *PLASMODIUM FALCIPARUM* RHOPTRY NECK PROTEIN PFRON12 INHIBIT MEROZOITE INVASION INTO ERYTHROCYTES

Daisuke Ito[1], Eizo Takashima[1], Shinya Hatano[1], Tsutomu Yamasaki[2], Tomoyuki Hasegawa[1], Kazutoyo Miura[3], Amporn Thongkukiatkul[4], Carole A. Long[3], Jetsumon Sattabongkot[5], Motomi Torii[6], **Takafumi Tsuboi**[1]

[1]Ehime University, Matsuyama, Ehime, Japan, [2]Okayama University of Science, Okayama, Japan, [3]National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [4]Burapha University, Chonburi, Thailand, [5]Mahidol University, Bangkok, Thailand, [6]Ehime University, Toon, Ehime, Japan

Proteins coating *Plasmodium* merozoite surface and secreted from its apical organelles are considered as promising vaccine candidates for the blood-stage malaria. The rhoptry neck protein 12 of *Plasmodium falciparum* (PfRON12) was recently reported as a protein specifically

expressed in schizont and localized to the rhoptry neck of merozoite by immunoelectron microscopy (IEM). The characteristics of the PfRON12 suggest that it is a potential blood-stage vaccine candidate, and here we assessed its potential in this regard. We expressed a recombinant PfRON12 protein by the wheat germ cell-free system to obtain anti-PfRON12 antibody. Immunoblot analysis of schizont lysate detected a single band at approximately 40 kDa under reducing condition, consistent with the predicted molecular weight. In contrast, the anti-PfRON12 antibody recognized a single band at approximately 80 kDa under non-reducing condition, consistent with two-fold molecular weight of its reduced form, suggesting the native PfRON12 forms a disulfide-bond-mediated homodimer. Immunofluorescence assay and IEM revealed that PfRON12 localized to the rhoptry neck of merozoite in schizonts and to the surface of free merozoites. The biological activity of anti-PfRON12 antibody was tested by an *in vitro* growth inhibition assay, and the antibody significantly inhibits the merozoite invasion of erythrocytes. Since anti-PfRON12 antibody inhibited the merozoite invasion *in vitro*, we decided to investigate whether PfRON12 is exposed to the human immune system in *P. falciparum*-infected individuals. The sera from *P. falciparum* infected individuals in Thailand and Mali reacted with the recombinant PfRON12, indicating PfRON12 is immunogenic in humans. Our findings suggest that PfRON12 plays an important role in the merozoite invasion process, and that it merits an additional evaluation as a *P. falciparum* blood-stage vaccine candidate.

1592

ADMINISTRATION OF PFSPZ VACCINE BY DIRECT VENOUS INOCULATION IN AFRICA

Freda Omaswa[1], Sara Healy[2], Mahamadou S. Sissoko[3], Abdoulaye Katile[3], Amagana Dolo[3], Karamoko Niare[3], Amadou Niangaly[3], Peter Billingsley[4], Anita Manoj[4], Eric James[4], B. Kim Lee Sim[4], Michael Walther[2], Ogobara Doumb Doumbo[3], Patrick Duffy[2], Stephen L. Hoffman[4]

[1]University of British Columbia, Vancouver, BC, Canada, [2]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [3]Malaria Research and Training Center, Mali-NIAID ICER, University of Science, Techniques and Technologies of Bamako, Bamako, Mali, [4]Sanaria Inc., Rockville, MD, United States

Malaria is a major cause of morbidity and mortality despite considerable investment in existing anti-malarial measures, highlighting the need for a vaccine. The vaccine candidate, Sanaria® PfSPZ Vaccine, contains radiation-attenuated, aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ). Healthy 18-35 year Malians were enrolled in a randomized, double-blind, placebo-controlled trial to assess safety, tolerability, immunogenicity, ease of administration, and protective efficacy against naturally occurring malaria of PfSPZ Vaccine administered by direct venous inoculation (DVI). PfSPZ Vaccine was thawed, diluted to a 0.5 mL volume in a 1 mL syringe with a 25-gauge needle, and then passed to local physicians for administration. Local and systemic reactogenicity were solicited through 7 days after each vaccination. A survey on subject perception of vaccination procedure will be administered at the end of the vaccination phase in July 2014. In total, 105 volunteers have been vaccinated, 12 were enrolled in a pilot safety group and 93 were randomized to receive 5 doses of $2.7x10^5$ PfSPZ or normal saline placebo. At this time, 207 DVIs with PfSPZ Vaccine or placebo have been administered. The time from vaccine request by the clinical team to thaw and formulation in a syringe to completion of injection was on average 6 minutes and injections on average took < 10 sec. Of the 207 vaccinations, only one has required a second injection attempt. This was for inability to locate a vein for the 1st dose in the pilot group. Vaccinations have been well tolerated with no local reactogenicity. Seven episodes of solicited systemic reactogenicity, all grade 1, have been reported. Complete data on safety, tolerability, and administration after 5 doses of PfSPZ Vaccine will be presented. DVI administration of PfSPZ has been rapid, efficient and extremely well-tolerated. This is a 1st step toward establishing the conditions for operational implementation and logistics for mass administration of PfSPZ Vaccine in Africa.

1593

CHLOROQUINE NEITHER ELIMINATES NOR DELAYS LIVER STAGE DEVELOPMENT OF *PLASMODIUM* DURING CHEMOPROPHYLAXIS VACCINATION

Tejram Sahu[1], Lynn E. Lmbert[2], Jessica Hinderer[2], Solomon Conteh[1], Sachy Orr Gonzalez[2], Patrick E. Duffy[1]

[1]National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [2]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States

Chloroquine (CQ) has been used in Chemoprophylaxis Vaccination (CVac) against malaria, whereby sporozoite inoculations under the umbrella of CQ prophylaxis induce liver stage-specific immunity and long-lasting sterile protection against homologous parasite strain. CQ is known to kill blood stage parasites, but its effect on liver stages is poorly studied, and the mechanism by which CVac-CQ induces strong protective immunity is not understood. We used a luciferase expressing rodent parasite, *Plasmodium yoelii*-Luc (Py-Luc), to monitor the effect of CQ on *Plasmodium* liver stage development. Balb/c mice with or without CQ prophylaxis were infected with Py-Luc sporozoites. Primaquine (PQ), a liver stage-specific anti-parasitic drug, was included as a positive control. We followed parasite development by intra-vital imaging at 44h, 54h and 60h post-infection. Parasite burden in liver was measured by quantifying bioluminescence of whole body and isolated livers, as well as by quantifying liver stage parasite transcripts by qRT-PCR. Delay in appearance of parasites in the blood was monitored by microscopic observation of Giemsa-stained thin blood smears. The parasite load in livers of CQ treated and untreated mice did not differ ($p=0.714$), and this was consistent at all three timepoints. PQ treated mice had a significant reduction in parasite burden as compared to both CQ treated and untreated groups ($p=0.008$), and were similar to the non-infected control mice. Parasites appeared in the blood stream of both CQ treated and untreated mice at the 54h time point. Taken together, our findings indicate that CQ neither eliminates liver stage parasites nor delays their development. Further investigations into the mechanisms by which CVac-CQ induces protective immunity are required, and may give insights relevant to drug and vaccine development.

1594

DEVELOPMENT OF A METABOLICALLY ACTIVE, NON-REPLICATING, ASEPTIC, PURIFIED, CRYOPRESERVED, GENETICALLY ATTENUATED *PLASMODIUM FALCIPARUM* SPOROZOITE VACCINE-PFSPZ (Δ*SLARP*Δ*B9*) VACCINE

Adam M. Richman[1], B. Kim Lee Sim[1], Ben C. van Schaijk[2], Takeshi Annoura[3], Ivo H. Ploemen[2], Tao Li[1], Abraham Eappen[1], Patricia de la Vega[1], Sumana Chakravarty[1], Adam J. Ruben[1], Shahid M. Khan[3], Chris J. Janse[3], Robert W. Sauerwein[2], Stephen L. Hoffman[1]

[1]Sanaria Inc., Rockville, MD, United States, [2]Department of Medical Microbiology, Nijmegen, Netherlands, [3]The Leiden Malaria Research Group, Parasitology, Leiden, Netherlands

Sanaria® PfSPZ Vaccine, composed of radiation attenuated, aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ) protected 6/6 (100%) of volunteers, who received the highest dose. By mid 2014 PfSPZ Vaccine will be in clinical trials in the U.S. and 5 countries in Africa and Europe. It is intended for elimination campaigns and prevention of malaria in travelers. There would be potential manufacturing, potency and regulatory advantages if radiation-attenuated parasites were replaced with genetically attenuated parasites (GAPs). We recently reported on a new target gene, *b9* and its deletion in combination with the *slarp* gene. In rodent malarias Δ*slarp*Δ*b9* SPZs elicit excellent protective immunity and do not lead to blood stage infection.

Elimination of Pf *slarp* and *b9* genes leads to attenuation similar to radiation. All prior Pf GAPs showed leaky attenuation and breakthrough liver stage development *in vivo* or *in vitro*. After characterization in Nijmegen and Leiden, the parasites were transferred to Sanaria where master and working cell banks (MCB and WCB) were made and an engineering production run performed to demonstrate that Pf*Δslarp*Δ*b9* GAP was suitable for producing aseptic, purified, cryopreserved PfSPZ. The parasites demonstrated all growth characteristics necessary for cGMP production. The Pf*Δslarp*Δ*b9* SPZ were assayed in Sanaria's 6-day hepatocyte attenuation, 3-day hepatocyte potency, and sporozoite membrane integrity (viability) assays. For the 6-day assay we used Pf wild type and Pf*Δp52*Δ*p36* SPZ as positive controls; the wild type Pf, Pf*Δp52*Δ*p36*, and Pf*Δslarp*Δ*b9* SPZ produced 21±1, 1.5±1.25 and 0, 6-day liver stage schizonts respectively. Pf*Δslarp*Δ*b9* SPZ were potent and viable. We will next use this genetically attenuated double-mutant parasite (Pf*Δslarp*Δ*b9)* to manufacture, characterize and release a corresponding PfSPZ Vaccine, PfSPZ (*Δslarp*Δ*b9*) Vaccine (also known as PfSPZ-GA1 Vaccine) in compliance with cGMPs, conduct pre-clinical studies, submit to the appropriate U.S. and Dutch regulatory agencies, and conduct a clinical trial.

1595

FINE MAPPING OF ANTIBODY ISOTYPES AND IMMUNODOMINANT B CELL EPITOPES INDUCED BY MALARIA VACCINE, PFCELTOS/GLA-SE

Casey K. Storme[1], Sidhartha Chaudhury[2], Sathit Pichyangkul[3], Susan L. Baldwin[4], Elizabeth Duncan[1], Heather Hosie[1], Darrick Carter[4], Steven G. Reed[4], Anders Wallqvist[2], Christian F. Ockenhouse[1], Jessica Cowden[1], Elke Bergmann-Leitner[1], Randall F. Howard[4], Evelina Angov[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Biotechnology HPC Software Applications Institute, Telemedicine and Advanced Technology Research Center, Fort Detrick, MD, United States, [3]Armed Forces Research Institute of Medical Sciences, U.S. Army Medical Component, Bangkok, Thailand, [4]Infectious Disease Research Institute, Seattle, WA, United States

The secreted malarial protein Cell-Traversal protein for Ookinetes and Sporozoites (CelTOS) is highly conserved among *Plasmodium* species, essential to host hepatocyte invasion, and critical to malaria pathogenesis. We previously reported that immunization of mice with full-length recombinant CelTOS from *P. falciparum* (*Pf*CelTOS) adjuvanted in Montanide ISA-720 achieved 60% heterologous protection against a *P. berghei* sporozoite challenge. The immune mechanisms leading to this cross-species protection are based on both cellular and humoral effector mechanisms. Formulating *Pf*CelTOS with the clinically relevant adjuvant GLA-SE resulted in similar levels of protection in mice against challenge as seen with ISA-720, and these studies provided evidence to support its evaluation in a Phase 1 safety, immunogenicity with Controlled Human Malaria Infection (CHMI) clinical trial. Since protection is in part mediated by antibodies, we sought to identify protective B-cell epitopes and, thereby, we determined the antibody fine specificity of preclinical and clinical samples for *Pf*CelTOS. To this end, various protein fragments were generated in *E. coli* or as synthesized peptides, and their reactivity with sera from *Pf*CelTOS/GLA-SE immune mice, rats, non-human primates and human subjects was determined. We are currently implementing *in vitro* functional assays such as inhibition of sporozoite gliding motility and the inhibition of sporozoite invasion and development within hepatocytes (ILSDA) to identify whether this vaccine formulation induces responses to functional B-cell epitopes within the identified immunodominant regions of CelTOS. Such characterizations will determine the role of these epitopes in mediating antibody-mediated protection from preclinical and clinical studies of the CelTOS antigen.

1596

GENETIC DIVERSITY OF THE PLACENTAL MALARIA VACCINE CANDIDATE VAR2CSA IN TWO MALARIA ENDEMIC SETTINGS IN AFRICA USING PACBIO SEQUENCING

Antoine Dara[1], Mark A. Travassos[1], Matthew Adams[1], Sarah Schaffer DeRoo[1], Joana C. Silva[2], Christopher V. Plowe[1], Miriam K. Laufer[1]

[1]Howard Hughes Medical Institute/Center for Vaccine Development, University of Maryland, Baltimore, MD, United States, [2]Institute of Genome Sciences and Department of Microbiology and immunology, University of Maryland, Baltimore, MD, United States

Pregnancy-associated malaria, a leading cause of maternal anemia and low birth weight, is characterized by the sequestration of *Plasmodium falciparum*-infected erythrocytes in the placental microvasculature due to expression of VAR2CSA. Epidemiological and serological studies indicate that VAR2CSA is a target of naturally acquired immunity, suggesting that VAR2CSA could be a potential target for a pregnancy-associated malaria vaccine. However, based on limited data, there appears to be extensive genetic diversity within the var2csa gene that must be taken into account in the design of an effective vaccine. Genetic diversity has prevented the successful sequencing of var2csa from field isolates using standard sequencing platforms. Due to the low sequence complexity and high variant diversity of var2csa, applying a traditional Sanger sequencing strategy on field samples is inefficient and costly. To overcome this obstacle, we are characterizing var2csa genetic diversity in malaria parasite isolates from two different endemic regions in Africa using a combination of long range PCR amplification and the Pacific Bioscience next generation sequencing platform. We performed a multiple alignment of the publicly available 20 coding sequences and 12 upstream promoter region sequences of var2csa, and designed primers based on three of the 40 identified conserved regions with a minimum length of 25 nucleotides. A first set of primers targets a 5 kilobase (kb) region spanning the upstream promoter region to the DBLepam4 domain, and a second primer set targets a 5 kb region spanning the DBLpam3 domain to the intracellular acidic terminal segment region. We have successfully amplified the two fragments for the reference strains 3D7 and NF54 and the first fragment from the Dd2 and HB3 strains. We are employing this approach to determine the var2csa sequence of clinical isolates and characterize the extent of natural diversity in var2csa in Mali and Malawi.

1597

SIMULATION OF B CELL AFFINITY MATURATION EXPLAINS ENHANCED ANTIBODY CROSS-REACTIVITY INDUCED BY THE POLYVALENT MALARIAL VACCINE AMA1

Sidhartha Chaudhury, Jaques Reifman, Anders Wallqvist

BHSAI, U.S. Army Medical Research and Materiel Command, Fort Detrick, MD, United States

Polyvalent vaccines use a mixture of antigens representing distinct pathogen strains to induce an immune response that is cross-reactive and protective. However, such approaches often have mixed results, and it is unclear how polyvalency alters the fine specificity of the antibody response and what those consequences might be for protection. Here, we present a coarse-grain theoretical model of B cell affinity maturation during monovalent and polyvalent vaccinations that predicts the fine specificity and cross-reactivity of the antibody response. We stochastically simulate affinity maturation using a population dynamics approach where the host B cell repertoire is represented explicitly, and individual B cell sub-populations undergo rounds of stimulation, mutation, and differentiation. Antigens contain multiple epitopes and are present in subpopulations of distinct pathogen strains, each with varying degrees of cross-reactivity at the epitope level. This epitope and strain-specific model of affinity maturation enables us to study the composition of the polyclonal response in granular detail and identify the mechanisms driving serum specificity and cross-reactivity. We applied this approach to predict the antibody

response to a polyvalent vaccine based on the highly polymorphic malarial antigen AMA1. Our simulations show that polyvalent AMA1 vaccination induces an enhanced cross-reactive antibody response primarily through a shift in affinity maturation that favors B cells specific to shared and cross-reactive epitopes, and demonstrates how a polyvalent vaccine with a small number of strains and only moderate allelic coverage may be broadly neutralizing. These results present broad implications for general polyvalent vaccine design.

1598

HEPATITIS B, HEPATITIS C AND HIV INFECTION FREQUENCIES AMONG VOLUNTEERS SCREENED FOR MALARIA VACCINE CLINICAL TRIALS IN MALI

Merepen A. Guindo[1], Moussa L. Diakite[1], Boucary Ouologuem[1], Souleymane Traore[1], Amadou Konate[1], Abdoulaye Katile[1], Yacouba Samake[1], Bourama Kamate[1], Sarro Yeya[2], Mahamadoun H. Assadou[1], Mahamadou S. Sissoko[1], Issaka Sagara[1], Boubacar Traore[1], Joseph P. Shott[3], Sara A. Healy[4], Stephen L. Hoffman[5], Patrick E. Duffy[4], Ogobara Doumbo[1]

[1]Malaria Research and Training Center, Mali-NIAID ICER, University of Science, Techniques and Technology, Bamako, Mali, [2]SEREFO, University of Science, Techniques and Technology, Bamako, Mali, [3]Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [4]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [5]Sanaria Inc., Rockville, MD, United States

Two clinical trials of malaria vaccine candidates (transmission blocking vaccine Pfs25-EPA/Alhydrogel® in collaboration with LMIV-NIAID) and whole organism vaccine PfSPZ (in collaboration with LMIV-NIAID and Sanaria) have begun in Bancoumana and Doneguebougou, Mali. Both villages are located within 70km of Bamako, but are different in terms of ethnicity, surrounding terrain, and primary employment. We have screened 509 men and women volunteers aged 18-45 years in Bancoumana and 18-35 years in Doneguebougou. Screening has been conducted to exclude those with human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV; by hepatitis B surface antigen (HBsAg)) and hepatitis C (HCV; by anti-HCV core antibodies)). For HCV and HBV testing, AccuDiag ELISA was used in Doneguebougou and standard rapid diagnostic tests (RDT) in Bancoumana. Determine HIV1/2 Alere® RDT with positivity confirmation by ELISA GENSCREEN ULTRA Ag-Ab were used at both sites for HIV 1/2. Ten of 204 (4.9%; 95%CI [2.37-8.82]), and ten of 218 (4.6%; 95% CI [2.22-8.27]) volunteers were positive by HIV RDT respectively in Doneguebougou and Bancoumana. Four (2.0%; 95% CI [0.54-4.94])and five (2.3%; 95% CI [0.75-5.27]) were confirmed positive by HIV ELISA GENSCREEN ULTRA Ag-Ab. HIV prevalence rates were comparable *(p> 0.05)* at the two sites. Forty-six volunteers (22.5%; 95% CI [17.01-28.90]) and 21 volunteers (9.6%; 95% CI [6.06-14.35]) were HBsAg positive respectively in Doneguebougou and Bancoumana, while six (2.9%; 95% CI [1.08-6.29]) and four (1.8%; 95%CI [0.5-4.63]) were anti-HCV positive at the sites respectively. The HBsAg positive frequency in Doneguebougou is higher than in Bancoumana, and higher than in previous studies, indicating that the prevalence of HBV infections may be increasing in this area. Together these viral diseases are an important consideration in the screening process for malaria vaccine trials.

1599

PHASE 1 MALARIA VACCINE STUDIES IN AFRICA: WHAT DEFINES A NORMAL, HEALTHY VOLUNTEER?

Sara A. Healy[1], Issaka Sagara[2], Mahamadou S. Sissoko[2], Merepen A. Guindo[2], Freda Omaswa[3], Joseph P. Shott[4], Stephen L. Hoffman[3], Ogobara Doumbo[2], Patrick E. Duffy[1]

[1]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]Malaria Research and Training Center, Mali-NIAID ICER, University of Science, Techniques and Technologies of Bamako, Bamako, Mali, [3]Sanaria, Inc., Rockville, MD, United States, [4]Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

As the malaria vaccine landscape continues to expand with new candidates entering phase 1 trials in Africa, the concept of a normal, healthy volunteer becomes difficult to consistently and accurately define in these settings. Inclusion/exclusion criteria and toxicity grading for these studies can vary significantly between sites, impacting adverse event reporting, study stopping criteria, immunogenicity responses, known susceptibility to malaria infection, and severity of disease. From May 2013 to February 2014, 509 volunteers were screened for either a phase 1 malaria transmission blocking vaccine trial or a phase 1 whole sporozoite malaria vaccine trial in Mali. All volunteers were screened by history, physical examination, and standard laboratory testing (hematological and biochemical parameters, urinalysis, Hepatitis B/C virus and HIV testing) with consistent screening ratios of 2 to 2.5 volunteers screened to 1 volunteer enrolled. However, other exclusion criteria, such as sickle cell disease/trait, electrocardiogram abnormalities, helminthiases, schistosomiasis, and syphilis were not consistently evaluated nor universally managed prior to enrollment, potentially creating variability in adverse event reporting. The impact on immunogenicity responses, malaria infection, and disease severity given the inconsistency in the definition of a healthy volunteer in this population prior to enrollment, is to be determined in these trials. Defining standard inclusion and exclusion criteria increases the likelihood of producing reliable and reproducible results, but must be closely balanced with the research being representative of the general healthy population under study. The creation of extremely narrow inclusion exclusion criteria can have significant implications on the ability of the study to be representative of the population under study and generalizability of the research results.

1600

A GENOMIC EPIDEMIOLOGY APPROACH TO ASSESSING AND IMPROVING STRAIN-SPECIFIC WHOLE ORGANISM VACCINE EFFICACY

Kara Moser[1], Mark Travassos[2], Amed Ouattara[2], Elliot Drábek[1], Cesar Arze[1], Claire M. Fraser[1], Kim Lee Sim[3], Stephen L. Hoffman[3], Ogobara K. Doumbo[4], Mahamadou A. Thera[4], Shannon Takala-Harrison[2], Joana C. Silva[1], Christopher V. Plowe[2]

[1]Institute for Genome Sciences, University of Maryland, Baltimore, MD, United States, [2]Howard Hughes Medical Institute/Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, [3]Sanaria Inc., Rockville, MD, United States, [4]Malaria Research and Training Center, University of Sciences, Techniques and Technology of Bamako, Bamako, Mali

Genetic diversity in *Plasmodium falciparum* is an obstacle for broadly efficacious malaria vaccines. A subunit blood stage malaria vaccine provides allele-specific efficacy, preventing only clinical malaria caused by parasites identical to the vaccine antigen at key polymorphic loci. A similar phenomenon may apply to whole-organism vaccines, albeit potentially at many, currently unknown, loci throughout the parasite genome. The attenuated whole-organism malaria vaccine PfSPZ Vaccine, which protects against homologous challenge, is based on the African strain NF54, the parent stock of the reference *P. falciparum* strain, 3D7. Controlled human

malaria infection trials to assess heterologous protection will initially use the South American strain 7G8. To determine how NF54 and 7G8 relate to genetic variation in natural populations, whole genome sequence data from NF54, 7G8 and from twelve mono- or polyclonal clinical samples of *P. falciparum* from a Malian village were analyzed. SNPs for all 12 Malian strains, NF54 and 7G8 were called against the 3D7 genome using GATK. Using the most reliable of the SNP filters tested, an average of 37,000 SNPs were called for Malian strains. In contrast, 383 and 17,667 SNPs were called for NF54 and 7G8, respectively. As expected, NF54 was nearly identical to 3D7, and the Malian strains were on average considerably more dissimilar genetically from 3D7, and by proxy from NF54, than is 7G8. Principal coordinate analysis was used to compare genetic diversity between strains, both at the genome-wide level and in a subset of 26 single-copy antigenic genes. This analysis placed NF54 centrally among the 12 Malian samples, suggesting a fairly representative genetic composition. NF54 and 7G8 clustered tightly with one another and with a subset of the monoclonal strains, possibly representing an artifact of polyclonality. Principal coordinate analysis of single-copy antigens showed some of the field isolates to be more distant from NF54 than 7G8 is, suggesting that additional suitable challenge strains can be easily identified.

1601

HUMANIZED DRAG MICE SUSTAIN THE VERTEBRATE LIFE CYCLE OF *PLASMODIUM FALCIPARUM* AND ELICIT PARASITE-SPECIFIC IMMUNE RESPONSES

Wathsala Wijayalath[1], Sai Majji[1], Eileen F. Villasante[1], Teodor D. Brumeanu[2], Thomas L. Richie[1], Sofia Casares[1]

[1]Naval Medical Research Center/Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Uniformed Services University of Health Sciences, Bethesda, MD, United States

Plasmodium falciparum is one of the deadliest protozoan parasites among the five species of human malaria, which accounts for the highest morbidity and mortality in tropical and sub-tropical countries. In addition of having multiple life cycle stages in the human host, *P. falciparum* parasites shows high antigenic diversity and cytoadherence properties, which increases the severity and complexity of the disease. Numerous efforts have been conducted over decades to address disease pathogenesis, immunity, and vaccine development, mostly in in-vitro or in rodent or non-human primate models. However, none of these models completely represent the disease, as it is in the natural human host, thus demanding the necessity of developing an accurate animal model. We generated a HLA- class II expressing humanized DRAG mice, which develop a functional human immune system, following human hematopoietic stem cell infusion. DRAG mice develop human hepatocytes, kupffer cells, liver endothelial cells and erythrocytes and sustain the complete life cycle of *P. falciparum* malaria parasite. Our data also demonstrate that the infected DRAG mice self-cure blood-stage infection following intravenous inoculation of live *P. falciparum* sporozoites and elicit humoral responses characterized by IgM and IgG antibodies against ring, trophozoite and schizont stage parasites. The infected DRAG mice also elicit cellular responses mediated by TNF-alpha against *P. falciparum* blood-stage parasites. Thus the DRAG mice represent the first small animal model, which has the ability to sustain the complete *P. falciparum* life cycle and to elicit parasite-specific immune responses.

1602

INJECTION OF PURIFIED, ASEPTIC CRYOPRESERVED *PLASMODIUM FALCIPARUM* SPOROZOITES (PFSPZ CHALLENGE) IS AN ALTERNATIVE TO MOSQUITO BITE ADMINISTRATION FOR CONTROLLED HUMAN MALARIA INFECTION (CHMI)

Thomas L. Richie[1], Peter F. Billingsley[1], B. Kim Lee Sim[1], Eric R. James[1], Meta Roestenberg[2], Else M. Bijker[3], Kirsten E. Lyke[4], Matthew B. Laurens[4], Susanne H. Hodgson[5], Adrian V. Hill[5], Seif Shekalaghe[6], Salim Abdulla[6], Benjamin Mordmüller[7], Peter G. Kremsner[7], Gloria P. Gómez-Pérez[8], Almudena Legarda[8], Pedro L. Alonso[8], Bernhards Ogutu[9], Kevin Marsh[10], Robert W. Sauerwein[3], Stephen L. Hoffman[1]

[1]Sanaria, Inc., Rockville, MD, United States, [2]Leiden University Medical Center, Leiden, Netherlands, [3]Radboud University Medical Center, Nijmegen, Netherlands, [4]Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, [5]The Jenner Institute, University of Oxford, Oxford, United Kingdom, [6]Ifakara Health Institute, Bagamoyo, United Republic of Tanzania, [7]Tropical Medicine Institute, University of Tübingen, Tübingen, Germany, [8]Barcelona Centre for International Health Research (CRESIB), Hospital Clínic - University of Barcelona, Barcelona, Spain, [9]Centre for Clinical Research, Kenya Medical Research Institute, Nairobi, Kenya, [10]KEMRI Wellcome Trust Research Programme, Kilifi, Kenya

Plasmodium falciparum (Pf) sporozoite (SPZ)-infected mosquitoes for controlled human malaria infections (CHMI) are produced routinely only in the USA and the Netherlands, by a small number of restricted-access insectaries. In the 28-day CHMI procedure, healthy adults are infected with malaria by the bites of 5 PfSPZ-infected mosquitoes and followed to assess efficacy of antimalarial drugs or vaccines. Although CHMI permits critical decisions regarding product advancement without the need for expensive and lengthy field evaluations, its use is limited by the requirement for a secure insectary capable of producing infected mosquitoes. This restricts performance of CHMI to local sites or requires transport of PfSPZ-infected mosquitoes to sites with a mosquito-secure facility. Costs may exceed $100K per CHMI. These limitations have been largely bypassed by Sanaria® PfSPZ Challenge, a cGMP product consisting of highly purified, aseptic, cryopreserved PfSPZ for parenteral use that is easily stored and transported to distant sites. Seven trials enrolling 178 volunteers have been conducted to test the safety, tolerability and infectivity of PfSPZ Challenge given by intradermal (ID), intramuscular (IM), intravenous (IV), or direct venous inoculation (DVI) routes, first in the Netherlands and subsequently in the UK, Tanzania, USA, Germany, Spain and Kenya. PfSPZ Challenge has been uniformly safe, and has infected 100% of volunteers by each route in 5 of the 7 trials using well-tolerated doses. IV, DVI and IM injection have achieved pre-patent periods of 11.0-11.5 days, matching those following mosquito bites, and IV and IM demonstrated a dose response. PfSPZ Challenge has enabled CHMI in Africa, emphasizing the potential for this "challenge in a bottle" to accelerate development of novel antimalarial drugs and vaccines and to promote understanding of innate and acquired immunity to malaria.

1603

CD8 T CELLS MEDIATE STERILE PROTECTION OF OUTBRED MICE FROM *PLASMODIUM YOELII* CHALLENGE FOLLOWING RECOMBINANT DNA-PRIME/AD5-BOOST IMMUNIZATION EXPRESSING TWO CANDIDATE VACCINE ANTIGENS

Emily C. Smith[1], Rebecca Danner[1], Jessica S. Bolton[1], Andrea K. Strein[1], Nonenipha Rangel[1], Esteban Abot[1], Arnel Belmonte[1], Noelle Patterson[1], Keith Limbach[1], Martha Sedegah[2], Eileen F. Villasante[2], Thomas L. Richie[2], Joao C. Aguiar[3]

[1]Henry M. Jackson Foundation/Naval Medical Research Center, Silver Spring, MD, United States, [2]Naval Medical Research Center, Silver Spring, MD, United States, [3]CAMRIS/Naval Medical Research Center, Silver Spring, MD, United States

Previously, we identified two antigens, UIS3 and Falstatin, which conferred sterile protection of outbred mice from *P. yoelii* sporozoite challenge following recombinant DNA-prime/Adenovirus serotype 5 (Ad5)-boost immunization. In the present study, we aimed to identify the immune component(s) mediating this protection. CD1 mice were primed with Py UIS3 and Py Falstatin DNA vectors followed by a boost with recombinant Ad5 vectors. Protection ranged from 7% to 57% in multiple experiments, with the highest level of protection observed for Ad5 boost of 10^{10} pu. Sporozoite IFA titers ranged from 1:40 to 1:640, while blood-stage titers ranged from 1:2560 to 1:20480. A wide range of ELISA titers was observed to UIS3 protein (range 1:25 to 1:78125) while those to Falstatin were more uniformly high (range 1:34576 to 1:144317). High frequencies of CD8 T cells producing IFN-g following Falstatin stimulation were observed in the spleens of immunized mice (range 9.6% to 18.9% of CD8 T cells). A fraction of the responding T cells also produced TNF and/or IL-2 in addition to IFN-g. Interestingly, the frequencies of Falstatin-specific CD8 T cells producing IFN-g were significantly increased among mice immunized with both UIS3 and Falstatin (above) compared to those immunized with Falstatin alone (range 4.7% to 7.4% of CD8 T cells, $p<0.01$). Similarly, endpoint serum ELISA titers targeting Falstatin were also increased among mice immunized against both antigens (above) compared to those immunized against Falstatin alone (range 1:15663.4 to 1:108539, $p<0.05$). *In vivo* depletion of CD8 T cells prior to challenge resulted in complete loss of the protection. These data indicate that UIS3 and Falstatin are promising candidate malaria vaccine antigens. Further study is required to fully understand the individual antigen contribution to protection and its duration, as well as to identify an optimal platform eliciting high level durable T cell immunity.

1604

D/HUAD5-PFCSLAM, A *PLASMODIUM FALCIPARUM* MULTI-ANTIGEN MULTI-STAGE ADENOVIRUS VECTORED VACCINE CANDIDATE, IS IMMUNOGENIC IN BALB/C MICE

Noelle B. Patterson[1], Maureen E. Stefaniak[1], Joseph J. Campo[1], Kalpana Gowda[1], Keith Limbach[1], Joseph T. Bruder[2], C. Richter King[2], Pamela Resch[3], Marilyn Ferrari[3], Larry R. Smith[3], Thomas L. Richie[1], Denise L. Doolan[1], Eileen Villasante[1]

[1]Naval Medical Research Center, Silver Spring, MD, United States, [2]GenVec, Inc., Gaithersburg, MD, United States, [3]Vical, Inc., San Diego, CA, United States

We have demonstrated that a malaria DNA/human adenovirus serotype 5-vectored vaccine platform (Ad) prime/boost vaccine expressing *Pf*CSP and *Pf*AMA1 antigens (*Pf*CA) is well tolerated, immunogenic, and efficacious in a Phase 1 clinical study (27%, 4/15 volunteers sterilely protected against controlled human malaria infection administered by mosquito bite) (Chuang et al., 2013). We are now evaluating strategies to increase efficacy. Here, we report the addition of antigens to broaden vaccine immunogenicity, with a focus on inducing a multi-valent, multi-stage, and multi-immune (cellular and humoral) response against both pre-erythrocytic and erythrocytic parasite life cycle stages. *Pf*CSLAM is a cocktail of *Pf*CSP, *Pf*SSP2/TRAP, *Pf*LSA1, *Pf*AMA1, and *Pf*MSP1 DNA and Ad vectors; the first four antigens are designed to induce T cell responses targeting sporozoite and liver stages, while those encoding AMA1 and MSP1 are designed to induce antibody responses targeting asexual blood stages. We have previously demonstrated that Ad and DNA/Ad prime/boost vaccines are immunogenic in murine, swine, and nonhuman primate models, and that a *Pf*CA DNA/Ad vaccine is protective in humans. Here, BALB/c mice were immunized i.m. with either the individual components, the 5 antigen *Pf*CSLAM mixture, or a 4 antigen *Pf*CLAM mixture, administered as a single Ad dose ($1x10^8$ pu) on study day (SD) 28, two Ad doses on SD1 and SD28, or pDNA (50 µg) on SD1 and Ad ($1x10^8$ pu) on SD28. Animals were bled pre-, 2 and 6 weeks post-boost for antibody assays, and spleens were harvested 2 and 6 weeks post-boost for T cell assays. Results established that the 5- and 4-antigen mixtures, CSLAM mixture ± SSP2, induced antigen-specific T cell and antibody responses to each antigen comparable to those induced by the individual components, as assessed by IFN-γ ELISpot or ELISA. These data support that the addition of antigens to the *Pf*CA mixture which is protective in humans can broaden the vaccine specificity. Future plans include GMP manufacture and clinical testing of the CSLAM or CLAM DNA prime /Ad boost vaccine.

1605

PLASMODIUM FALCIPARUM AMA1-BASED SUBUNIT VACCINE FMP2.1/AS02A ELICITS A DIVERSE AND STRONG YET UNPROTECTIVE IMMUNE RESPONSE IN A PEDIATRIC COHORT IN BANDIAGARA, MALI

Jason A. Bailey[1], Mark A. Travassos[1], Christopher G. Jacob[1], Amed Oattara[1], Drissa Coulibaly[2], Matthew B. Laurens[1], Shannon Takala-Harrison[1], Carter L. Diggs[3], Lorraine Soisson[3], Tyler S. Brown[1], Kirsten E. Lyke[1], David E. Lanar[4], Sheetj Dutta[4], D. Gray Heppner[4], Jozelyn Pablo[5], Rie Nakajima[5], Algis Jasinskas[5], Amadou Niangaly[2], Andrea A. Berry[1], Bourema Kouriba[2], Mahamadou A. Thera[2], Ogobara K. Doumbo[2], Philip L. Felgner[5], Christopher V. Plowe[1]

[1]Howard Hughes Medical Institutes, Center for Vaccine Development, Baltimore, MD, United States, [2]Malaria Research and Training Center, Bamako, Mali, [3]United States Agency for International Development, Washington, DC, United States, [4]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [5]Department of Medicine, University of California Irvine, Irvine, CA, United States

FMP2.1/$AS02_A$ is a blood stage malaria subunit vaccine candidate based on the ectodomain of apical membrane antigen 1 (AMA1) of the 3D7 strain of *Plasmodium falciparum*. In a Phase 2 vaccine trial in 400 children aged 1-6 years in Bandiagara, Mali, West Africa, the vaccine had only a statistically insignificant 17% efficacy against all clinical malaria episodes when compared to the rabies control vaccine. However, it had 64% efficacy against clinical malaria caused by homologous strains with respect to eight pre-specified polymorphic amino acid positions. To assess anti-AMA1 antibody specificity in AMA1 vaccine trials, we developed a protein microarray for measuring seroreactivity to 263 unique AMA1 ectodomain variants detected by sequencing the *ama1* gene in field samples. We evaluated AMA1 seroreactivity in a random sample of 40 children (aged 1-6 years) and 20 adults (aged 18-55 years) pre- and post-vaccination with FMP2.1/$AS02_A$ or rabies control vaccine. Both children and adults immunized with the AMA1 vaccine had broad and strong immune responses to diverse AMA1 variants compared to controls 90 days after the first immunization. Due to the broad cross-reactivity of antibodies generated by the vaccine, we were unable to pinpoint specific AMA1 variants or polymorphic epitopes associated with protection from clinical malaria. Further analysis using multivariable logistic regression, as well as principle components analysis, Random Forest, and receiver-operating characteristic curves, suggest that antibodies stimulated by the FMP2.1/$AS02_A$ vaccine may be biased, binding preferentially to immunodominant but unprotective AMA1 epitopes. This form of deceptive imprinting has been described in HIV and influenza as a tool for immune escape and also has been characterized in malaria as the 'smokescreen effect'. These

results suggest that *a priori* knowledge of a functional epitope map could inform the selection of malaria subunit vaccine antigens that would generate protective antibody populations.

1606

A FULL-LENGTH *PLASMODIUM FALCIPARUM* RECOMBINANT CIRCUMSPOROZOITE PROTEIN EXPRESSED BY PSEUDOMONAS FLUORESCENS PLATFORM AS A MALARIA VACCINE CANDIDATE

Gabriel Gutierrez[1], Amy R. Noe[1], Steve Giardina[1], Diego Espinosa[2], Ryan Haverstock[3], Jeff Allen[3], Hongfan Jin[3], Xiangming Li[4], Jordana Coelho-dos-Reis[4], Ryota Funakoshi[4], John Sacci[5], Diane Retallack[3], Tom Vedvick Vedvick[6], Chris Fox[6], Ramses Ayala[1], Steven G. Reed[6], Fidel Zavala[1], Moriya Tsuji[4]

[1]Leidos Life Sciences, Frederick, MD, United States, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]Pfenex Inc., San Diego, CA, United States, [4]Aaron Diamond AIDS Research Center, New York, NY, United States, [5]University of Maryland School of Medicine, Baltimore, MD, United States, [6]Infectious Disease Research Institute, Seattle, WA, United States

The circumsporozoite protein (CSP) of *Plasmodium falciparum* is a major surface protein, which forms a dense coat on the sporozoite's surface. Preclinical research on CSP and clinical evaluation of a CSP fragment-based RTS,S/AS01 vaccine have demonstrated a modest degree of protection against *P. falciparum*, mediated in part by humoral immunity and in part by cell-mediated immunity. Given the partial protective efficacy of the RTS,S/AS01 vaccine in a recent Phase 3 trial, further improvement of CSP-based vaccines is crucial. Here we describe the preclinical evaluation of a full-length, recombinant CSP (rCSP)-based vaccine candidate against *P. falciparum* malaria suitable for current Good Manufacturing Practice (cGMP) production. Utilizing a novel high-throughput Pseudomonas fluorescens expression platform, we demonstrated greater efficacy of full-length rCSP as compared to N-terminally truncated versions, rapidly down-selected a promising lead vaccine candidate, and developed a high-yield purification process to express immunologically active, intact antigen for clinical trial material production. The rCSP, when formulated with various adjuvants, induced antigen-specific antibody responses as measured by ELISA and immunofluorescence assay (IFA), as well as CD4+ T-cell responses as determined by ELISpot. The adjuvanted rCSP vaccine conferred protection in mice when challenged with transgenic *P. berghei* sporozoites containing the *P. falciparum* repeat region of CSP. Furthermore, heterologous prime/boost regimens with adjuvanted rCSP and an adenovirus type 35-vectored CSP (Ad35CS) showed modest improvements in eliciting CSP-specific T-cell responses and anti-malarial protection, depending on the order of vaccine delivery. Collectively, these data support the importance of a further clinical development of adjuvanted rCSP, either as a stand-alone product or as one of the components in a heterologous prime/boost strategy, ultimately acting as an effective vaccine candidate for the mitigation of *P. falciparum*-induced malaria.

1607

A MONOCLONAL ANTIBODY AGAINST THE N-TERMINAL REGION OF THE *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN STRONGLY INHIBITS SPOROZOITE INVASION OF HEPATOCYTES

Diego Espinosa[1], **Amy Noe**[2], Photini Sinnis[1], Gabriel Gutierrez[2], Fidel Zavala[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Leidos Life Sciences, Frederick, MD, United States

Studies in animals and human volunteers have demonstrated that antibodies against the Circumsporozoite Protein (CSP) can protect against infection by *Plasmodium* sporozoites. Due to its repetitive nature and tandem disposition, the epitopes at CSP's repeat region are the most prominent target of protective antibody responses. However, it has been long hypothesized that antibodies raised against epitopes outside the repeat domain can also confer significant protection against sporozoite invasion. Using a newly developed chimeric *Plasmodium berghei* strain bearing the N-terminal region of the *P. falciparum* CSP, we report the characterization of a monoclonal antibody (MAb) recognizing the *P. falciparum* CSP. We mapped the fine epitope specificity of this MAb (5D5) and established that it recognizes an amino acid sequence immediately adjacent to Region I of the *P. falciparum* CSP. Using both the novel *P. berghei- P. falciparum* chimera and *P. falciparum* parasites, we further characterized the 5D5 MAb epitope specificity and show that it can bind both live and air-dried fixed sporozoites. Most importantly, we demonstrate that 5D5 can strongly inhibit parasite infection *in vivo* and can inhibit cleavage of CSP, and so provide additional evidence that antibodies targeting epitopes other than those at CSP's repeat region can be highly protective. Furthermore, we propose that this MAb could be utilized as an antibody-based, therapeutic prophylaxis, which may be a critical tool in the face of growing malaria drug resistance.

1608

SAFETY AND PROTECTIVE EFFICACY OF INTRAVENOUS IMMUNIZATION WITH CRYOPRESERVED *PLASMODIUM FALCIPARUM* SPOROZOITES UNDER CHEMOPROPHYLAXIS - TUECHMI-002

Benjamin Mordmueller[1], B. Kim Lee Sim[2], Peter F. Billingsley[2], Eric R. James[2], Meral Esen[1], Markus Gmeiner[1], Guezin Surat[1], Heimo Lagler[1], Thomas L. Richie[2], Stephen L. Hoffman[2], Peter G. Kremsner[1]

[1]University of Tuebingen, Tuebingen, Germany, [2]Sanaria Inc., Rockville, MD, United States

Exposure to 12-15 *Plasmodium falciparum* (Pf)-infected mosquitoes, administered three times at monthly intervals under continuous chemoprophylaxis with chloroquine is highly efficacious in preventing asexual blood stage infection following subsequent controlled human malaria infection (CHMI) in healthy, adult, malaria-naïve individuals. To translate and expand this immunization strategy, mosquito bites need to be replaced by a pharmaceutical product that can be easily administered and exactly dosed. In addition, the chemoprophylactic regimen needs to be adjusted to the specific requirements of this approach. Our previous study (TUECHMI-001) showed that one mosquito bite corresponds to ~620 intravenously (IV) injected, cryopreserved Pf sporozoites (PfSPZ) produced by Sanaria Inc. Here, we report first results on the safety, tolerability, immunogenicity and preliminary protective efficacy of escalating doses of Sanaria's PfSPZ (PfSPZ Challenge) under chemoprophylaxis (PfSPZ-CVac approach) with chloroquine. During immunization, PfSPZ were injected by direct venous inoculation (DVI) three times at 4-week intervals. Volunteers receive staggered doses of 3,200 (Group A), 12,800 (Group B) or 51,200 (Group C) PfSPZ per injection, corresponding to approximately 5, 20 or 80 mosquito bites. In every group, 9 volunteers received PfSPZ and 5 placebo, while all received 10 mg/kg chloroquine 2 days before the first injection, followed by 5 mg/kg every week for a total of 10 doses. To assess efficacy of the immunization regimen, CHMI will be done 8 weeks after completion of chemoprophylaxis by DVI of 3,200 PfSPZ Challenge. Subsequently, a PfSPZ-CVac dose that shows at least 75% efficacy, good safety and tolerability will be tested using an experimental ultra-short chemoprophylaxis with azithromycin and chloroquine. Here, 2 g extended-release azithromycin will be given on the day of each PfSPZ injection followed by 10 mg/kg chloroquine 5 days later. Complete results for the first two dose groups will be presented together with safety, tolerability and preliminary immunogenicity data of the highest dose (51,200 PfSPZ).

1609

LONGITUDINAL ANALYSIS OF HUMORAL AND CELLULAR IMMUNITY FOLLOWING VACCINATION WITH AN ATTENUATED *PLASMODIUM FALCIPARUM* SPOROZOITE VACCINE

Andrew Ishizuka[1], Kailan-Sierra Davidson[1], Kim Lee Sim[2], Stephen Hoffman[2], **Robert A. Seder**[1]

[1]Vaccine Research Center, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [2]Sanaria Inc., Rockville, MD, United States

A highly effective and durable vaccine for preventing *Plasmodium falciparum* (Pf) malaria infection is a critical need for preventing the substantial morbidity and mortality incurred by this infection. Pf sporozoites (PfSPZ) administered by mosquito bites are the only immunogens shown to induce high-level sterilizing protection (>80%) in humans. We previously reported that attenuated, aseptic, purified, cryopreserved PfSPZ (PfSPZ Vaccine) administered 4 or 5 times intravenously (IV) conferred high-level protection in humans in a dose-dependent manner. Moreover, initial analysis of humoral and cellular immunity showed that there was a dose-dependent increase in CSP antibody titer, functional inhibition of sporozoite invasion *in vitro* and the frequency of sporozoite specific IFN-g producing CD4 and CD8+ T cell responses. Here, we substantially expanded this analysis and performed a longitudinal assessment of antibody and cellular responses through the course of vaccination and after controlled human malaria ~ 3 weeks after the final immunization and ~ 5 months later. For T cell assessment, multi-parameter flow cytometry with two recently developed 16-color panels as used to assess the magnitude, phenotype and quality of sporozoite specific T cell responses. Together, these data provide insights into the durability of immunity and protection after vaccination with the PfSPZ Vaccine and will guide ongoing and future studies to define the mechanistic correlates of protection.

1610

CLINICAL DEVELOPMENT OF THE PFSPZ VACCINE TO PROTECT THE WARFIGHTER FROM MALARIA

Judith E. Epstein[1], Kristopher Paolino[2], Sharina Reyes[1], Robin Nielsen[2], Alexandra Singer[1], Martha Sedegah[1], Richard Ruck[2], Carlos Vasquez[1], Elizabeth Saverino[3], Silas Davidson[2], Peter F. Billingsley[3], Anita Manoj[3], Sumana Chakravarty[3], Kavita Tewari[3], Eric James[3], Anusha Gunasekera[3], Adam Richman[3], Matthew Stewart[3], Deborah Padilla[3], Tooba Murshedkar[3], Thomas L. Richie[3], B. Kim Lee Sim[3], Eileen Villasante[1], Stephen L. Hoffman[3]

[1]Naval Medical Research Center, Silver Spring, MD, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Sanaria Inc., Rockville, MD, United States

A vaccine to prevent malaria for use by the Department of Defense (DoD) must provide sustained sterile protection against infection. The Sanaria® PfSPZ Vaccine has been developed to address this need. It is composed of aseptic, purified, cryopreserved, vialed PfSPZ manufactured in compliance with all regulatory standards. In a recent clinical trial, PfSPZ Vaccine protected 6/6 (100%) subjects against controlled human malaria infection (CHMI) at the highest dosage regimen administered (5 intravenous doses of 1.35x10^5 PfSPZ), and there was a dose response in regard to antibody and T cell responses. The protective regimen was safe and well tolerated, indicating that further dose escalation could be undertaken. Since completion of that study, 6 additional PfSPZ Vaccine trials have been or will be initiated, designed to optimize dose size, dose interval, number of doses and duration of protection. This study, conducted by the DoD, addresses the following questions: (1) Is PfSPZ Vaccine safe and tolerable administered by direct venous inoculation? (2) Can 5 doses of 2.7x10^5 PfSPZ provide protection 3 weeks (short term) after immunization against CHMI carrying a heterologous Pf strain? (3) Can 5 doses provide protection when subjects undergo a second CHMI at 24 weeks (long term) with homologous and heterologous Pf parasites? 4) Can the number of doses required to provide short term and long term homologous protection be reduced (to 3 doses of 4.5x10^5 PfSPZ)? We will present safety and immunogenicity results for both the 3 and 5 dose regimens. Additionally, we will present our plans to conduct late Phase 2 and Phase 3 trials supporting a BLA for licensure in adults. The potential for worldwide benefit results from the fact that DoD requirements - excellent safety and tolerability, efficient administration, and sterile protection lasting for at least six months - are characteristics that equally support deployment to malaria endemic areas to prevent disease and death and to promote campaigns aiming to halt malaria transmission and eliminate the disease from defined geographical areas.

1611

MAN VERSUS MOSQUITO: HOW VECTOR-BORNE PATHOGENS MOVE AROUND THE WORLD

Michael Johansson[1], Andrew Tatum[2]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]University of Southampton, Highfield, United Kingdom

Vector-borne pathogens, such as malaria and dengue, are major causes of morbidity and mortality, worldwide. These pathogens often move between endemic areas and from endemic to non-endemic areas via airplanes, which can rapidly transport infected humans and vectors over long distances. Upon introduction, either the human or vector may initiate new transmission cycles in other locations under the right ecological conditions. While numerous countries have established regulations for disinsection of airplanes arriving from certain locales, it has not been clear whether this significantly reduces transmission risk. We developed branching process models to assess the probability of traveling humans and vectors initiating transmission resulting in at least one local human infection in a new location. For humans, this depends on the probability of an infected person traveling, infecting a mosquito, and that mosquito infecting a human, as well as several constituent processes. For mosquitos, it is the probability of an infected mosquito traveling and infecting a human, again with constituent processes. We assessed these models for *Plasmodium falciparum*, a causative agent of malaria. For a plane moving from a highly endemic area to another area highly suitable for *P. falciparum* transmission, the probability of introduction of *P. falciparum* by a human is approximately 100%. However, for a mosquito it is less than 0.1%. Analysis of the sequence of events leading to introduction makes it clear that mosquitoes have lower probabilities of travel, infection, and further transmission compared to humans for whom the risk of pathogen introduction is many times larger. While controlling the transportation of mosquitoes may be critical for avoiding the introduction of vector or pest species, our model indicates that it has little benefit for vector-borne pathogens. Given the ever-increasing volume of travel, it is critical to develop new ways to reduce the risk of pathogen spread by infected humans.

1612

DURABILITY OF POLYESTER-BASED LONG-LASTING INSECTICIDAL NETS IN THREE GEOGRAPHICAL ZONES OF NIGERIA - A THREE YEAR FOLLOW-UP OF NETS DISTRIBUTED THROUGH CAMPAIGNS

Albert Kilian[1], Emmanuel Obi[2], Richmond A. Selby[3], Hannah Koenker[4], Matt Lynch[4]

[1]Tropical Health LLP, Montagut, Spain, [2]Malaria Consortium, Abuja, Nigeria, [3]Malaria Consortium, Kampala, Uganda, [4]Centers for Communication Programmes, Baltimore, MD, United States

The average survival of Long-lasting Insecticidal Nets (LLIN) has increasingly become of interest to malaria program managers and the international community as this measure of durability will determine the frequency of LLIN replacements at household level and the cost of sustaining universal coverage. With the recent publication of a WHO-recommended method

to estimate net survival, comparative analyses from different areas or for different brands have now become possible. Following the mass distribution campaigns of 2010/11 in Nigeria annual follow-up surveys to measure attrition and physical integrity of campaign LLIN (polyester, 100 Denier) were undertaken in three locations of Nigeria representing different eco-geographic and climatic zones: Shinkafi district in Zamfara State in the northern dry-savannah, Toto district in Nasarawa State in the central guinea-savannah and Abi district in the rain-forest area of Cross River State. In each district a population representative sample was drawn using a 20 cluster sampling design and in each selected community 15 households that had received nets from the campaign were included in the interview and net assessment. In the questionnaire reasons for any loss of campaign nets were explored as well as attitudes and practices towards net care and repair. The assessment of physical integrity of the nets was done according to WHO recommendations and the proportionate Hole Index used to evaluate the outcome for each net. In the first round of surveys a total of 900 households were sampled and 1,571 campaign nets assessed while for the second round the figures were 896 and 1,367 respectively. Two years after the campaign the survival of LLIN varied considerably between locations with 69.6% (95% CI 62.7-75.7) still in serviceable condition in Nasarawa State, 81.4% (75.0-86.5) in Zamfara and 89.4% (84.2-93.5) in Cross River State. The final round of data collections is currently ongoing and will be presented together with estimates of median LLIN survival. Reasons for differences by location will be explored and discussed.

1613

MODELING THE EFFECTS OF TRANSNATIONAL MIGRATION ON PUBLIC HEALTH POLICIES IN SOUTHEAST ASIA: AN INVESTIGATION INTO THE IMPACT ON ELIMINATION STRATEGIES

Amruta Dixit[1], Ming-Chieh Lee[1], Edward Wenger[2], Guiyun Yan[1]

[1]University of California Irvine, Irvine, CA, United States, [2]Institute for Disease Modeling, Seattle, WA, United States

Human migration plays an important role in the spread of infectious diseases. In order to determine the impact of human movement between areas of unequal malaria endemicities on malaria transmission and the resulting control and elimination strategies, we need to improve our understanding of: the migration patterns present in the study areas of interest, the malaria acquisition risk borne by the migrants, and the impact of migration on malaria transmission. We hypothesized that persistent and unmonitored flow of people between porous borders between areas of unequal transmission, presents a significant challenge to the elimination of malaria in the area of low disease transmission. The present study aims to provide a quantitative assessment of malaria risks due to transnational migration and to evaluate subsequent intervention strategies for malaria control and elimination. Within the EMOD framework developed by the Institute for Disease Modeling, we have constructed simulations with two geographically-connected populations in order to model the impact of varying degrees of human migration rates and intervention methods on malaria transmission. Our preliminary simulation results suggest that the proportion of humans infected was markedly different between the various rates of human migration. We will continue to develop increasingly complex simulations that explore both human and vector-based interventions such as the administration of primaquine and the application of long-lasting microbial larvicides. The simulations results can be used to consider various feasible pathways to sustained local elimination as a function of cross-border migration rates. This approach has the ability to make a timely and significant contribution to public health by filling a critical gap in our knowledge of human migration patterns, especially in Southeast Asia. The utility of these data and this novel modeling tool is multidisciplinary and has the potential to inform sophisticated models in other research fields.

1614

ANALYSIS OF DETERMINANTS OF INSECTICIDE TREATED NETS (ITNS) USE AMONG CHILDREN UNDER FIVE YEARS USING LOGISTIC REGRESSION

Wahjib Mohammed[1], Anthony J. Turkson[2], David Y. Mensah[1]

[1]National Malaria Control Programme, Accra, Ghana, [2]Takoradi Polytechnic, Accra, Ghana

Malaria causes between three hundred and fifty (350) and five hundred (500) million clinical episodes and over one million deaths annually. Children and pregnant women are the most vulnerable group and most endangered by the disease. Insecticide-Treated bednets (ITNs) range among the most effective measures of malaria prophylaxis, yet its implementation level in sub-Saharan Africa is still low. The goal of this study was to assess factors influencing the use of ITNs by children under five years in Ghana. A cross-sectional study was conducted using pre-tested, interviewer-administered questionnaires. Possible factors were measured using five hundred (500) mothers or guardians of children under five years, in twenty three (23) communities in the Asamankese sub-municipality in the West Akim District in the Eastern Region of Ghana. Logistic regression was used to assess the influence of five possible factors on ITNs use. In order of importance in determining one's use of LLIN, 'Sleeping area allowing for the use of Long Lasting Insecticides Net (LLIN)' was the most important factor influencing the use of ITNs by children under five years in Ghana, followed by 'Household's expected monthly income', then 'Mother's SHS level of education' compared to 'none', while the least was 'Number of children under five years'. The study also revealed that 86.2% of the participants owned ITNs out of which 92.81% used it the night before the study. The study revealed that there is a relationship between influencing factors and use of LLINs. To improve ITNs usage, there should be continuous distribution of LLINs and Insecticide Treated Materials (ITMs), such as insecticide treated curtains for doors and windows. This should be heavily supported by education and Behavior Change Communication (BCC) via radio, TV, and other media especially on the hanging techniques and need to provide adequate space for sleeping area.

1615

EVALUATING DIFFERENCES IN HOUSEHOLD INSECTICIDE-TREATED BEDNETS (ITN) OWNERSHIP BETWEEN UGANDA AND ZIMBABWE DURING 2005-2011: AN ECONOMETRIC APPROACH

Yesim Tozan[1], Selver Derya Uysal[2]

[1]New York University, New York, NY, United States, [2]Institut für Höhere Studien, Vienna, Austria

The rapid scale-up of insecticide-treated bednets (ITNs) in African countries have received heightened attention with the availability of significantly greater resources for national malaria control efforts, particularly after 2005. In this study, we investigate the mean differences in household ITN ownership in Uganda and Zimbabwe for the years 2005 and 2011, using DHS and GPS data and MARA malaria endemicity maps and econometric methods. The probability of owning an ITN in Uganda was 12.8% higher than in Zimbabwe in 2005. Although ITN ownership increased steadily in both countries, the difference in the probability of owning an ITN widened significantly and became 33.5% over this period. The Blinder-Oaxaca technique can be used to study the mean outcome differences between two groups (in our case two countries). Using this technique, we divide the ITN ownership differential between two countries into a part that is "explained part" by group differences in ITN ownership determinants, such as household characteristics and malaria risk, and a residual part that cannot be accounted for by such differences in ITN ownership determinants. This "unexplained" part captures the effects of group differences in unobserved predictors of ITN ownership. Our preliminary results showed that the larger fraction of the increase in the ITN ownership differential in this period was due to the unexplained part. To understand

this result we investigated what happened in these countries in terms of malaria control efforts between 2005 and 2011. Published literature points to a significant difference in malaria financing in these countries, yet the earmarked proportion of funding for increasing the ownership and use of ITNs is usually high in all countries. Next we will study to what extent malaria control efforts in these two countries affected ITN usage and all-cause child mortality rates and potentially extend our analysis to include other African countries.

1616

UNDERSTANDING INTRA-HOUSEHOLD DECISION MAKING FOR INSECTICIDE-TREATED MOSQUITO NET ALLOCATION IN UGANDA

Emily E. Ricotta, Steve A. Harvey, Hannah Koenker, Matthew Lynch

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Intra-household allocation of insecticide-treated mosquito nets (ITNs) is an important concern for malaria prevention programs. Behavior change communication campaigns stress the need to prioritize children under five and pregnant women for net distribution and use, as these two groups are the most vulnerable to malaria infection. This study utilized a pile sort activity to help understand net allocation decision making in Luwero and Nebbi, two Ugandan districts. Sixty-four respondents, half men and half women, were asked to assign 10 individuals ranging in age from infant to older adult and including adults and children of both sexes as well as one visibly pregnant woman to one of four beds. One bed had a new net, one a slightly used net with a few holes, one an old net with large holes, and the last with no net. After sorting, respondents were asked why they allocated each individual as they had. The number of times a household member was placed under each net was evaluated, and a hierarchical cluster analysis was completed to determine which household members were typically grouped together. Responses were compered between male and female, urban and rural, and district of residence. Results demonstrate that net allocation differs by gender, rural/urban, and district, however the pregnant woman and baby were always clustered together and were given the best net greater than 50 and 60 percent of the time, respectively. Children ages 5-14 were also typically clustered together and given the best or second-best net. Young adults (around age 25), older adults, and elders were given the worst net or no net a majority of the time. Reasons given for these placements varied, but common themes include the baby and pregnant women being the most vulnerable to malaria, the older children being able to help repair nets, and the heads of household and young adults being robust enough to not succumb to malaria infection and having sufficient resources to purchase additional nets. The general consensus was that this household did not have enough nets, and that the family should strive to provide nets for all of their household members. This exercise supports current behavior change campaign messaging, demonstrating that, at least in their reported behavior, people are likely to prioritize vulnerable populations when it comes to net use.

1617

REDUCTION IN DISPARITY OF INSECTICIDE-TREATED NETS OWNERSHIP AND USE AMONG SOCIOECONOMIC GROUPS AFTER SCALE UP IN UGANDA

Cameron Taylor, Ana Claudia Franca-Koh, Samantha Herrera, Jui Shah, Lia Florey, Yazoume Ye

ICF International, Rockville, MD, United States

The increase in funding for malaria control in the past decade resulted in an increase in Insecticide-Treated Nets (ITNs) ownership and use in many countries in sub-Saharan Africa, particularly in Uganda. However, with the shift in programmatic focus from target groups to universal coverage there is a need to ensure equal access and use of ITNs for all sub-populations regardless of their socioeconomic status. This study assessed change in disparity in ITN ownership and use among different socioeconomic groups in Uganda between 2006 and 2011. The authors used Lorenz Concentration Curve and Index (C-Index) to assess equity in household ITN ownership and use among children under five between wealth quintiles separately in 2006 (Demographic and Health Survey data) and 2011(Malaria Indicator Survey data). C-Index values range between -1 to 1, a value of 0 suggests no difference in ownership and use among different socioeconomic groups. Household ownership of at least one ITN rose significantly from 16% (2006) to 60% (2011). Similarly, ITN use among children under five was very low (10%) in 2006 and increased substantially to 47% in 2011. The increase in ITN ownership was associated with significant reduction in inequity among wealth quintiles (C-Index 0.11, 95% CI: 0.08;0.34) in 2006 versus 0.02, 95% CI: 0.01;0.04 in 2011). Similarly the disparity in use of ITN use among children under five from different wealth quintiles greatly reduced from 2006 (C-Index: 0.04, 95% CI:-0.10;0.19) to 2011 (C-Index: 0.01, 95% CI:-0.04;0.06). This achievement is probably due to the shift to universal coverage in 2009 which led to free mass distribution campaigns of Long-lasting Insecticidal Nets (LLINs), with 7.2 million LLINs distributed by 2010. This achievement in parity between wealth quintiles should be sustained; however, efforts are needed to further increase overall ITN ownership coverage and use in Uganda. This is achievable through additional free mass campaign distribution combined with traditional distribution channels.

1618

ASSESSING THE CONTRIBUTION OF MALARIA CONTROL INTERVENTIONS ON REDUCTIONS IN ALL-CAUSE UNDER-5 MORTALITY IN ZAMBIA

Katherine E. Colson[1], Laura Dwyer-Lindgren[1], Nancy Fullman[1], Tom Achoki[2], Matthew Schneider[3], Peter Hangoma[4], Marie Ng[1], Felix Masiye[5], Emmanuela Gakidou[1]

[1]Institute for Health Metrics and Evaluation, Seattle, WA, United States, [2]Ministry of Health of Botswana, Gaborone, Botswana, [3]United States Agency for International Development, Washington, DC, United States, [4]Department of Economics, University of Bergen, Bergen, Norway, [5]Department of Economics, University of Zambia, Lusaka, Zambia

Between 2000 and 2010, Zambia rapidly scaled-up malaria control interventions such as insecticide-treated nets and indoor residual spraying. At the same time, Zambia recorded substantial declines in under-five mortality. Zambia's experience is heralded as a malaria-control success story, but it is unclear whether the expansion of malaria control contributed to improved childhood survival beyond the impacts of other child health interventions. To quantify the impact of malaria control efforts in Zambia, we estimated trends for all-cause under-five mortality and a range of child and maternal health interventions at the subnational level. We quantified the reduction in child mortality associated with malaria control while taking into account trends in other key interventions as well as socio-demographic, health system, and environmental factors across districts. Our estimation methods included generalized linear models and functional data analysis and were validated with cross-validation and simulation techniques. We found that the bivariate relationship between malaria control and child mortality was strong and significant, but this relationship was attenuated when other factors were considered. Several other child health interventions also scaled up dramatically during the same time period, including pentavalent immunization, prevention of mother-to-child-transmission of HIV/AIDS, exclusive breastfeeding, and nutrition programs. Because of this simultaneous expansion, it was statistically infeasible to isolate the effects of malaria control efforts. In the absence of the combined scale-up of these interventions between 2000 and 2010, we estimated that child mortality would have been 11% higher in 2010. The scale up of these interventions accelerated declines in mortality by 1% each year. Our findings emphasize the importance of constructing a comprehensive landscape of the drivers of progress in child mortality. A greater quantity of high-quality and localized data is critical for evaluating the independent impact of each intervention on childhood survival.

1619

INCREASING ROLE OF *ANOPHELES FUNESTUS* AND *AN. ARABIENSIS* IN MALARIA TRANSMISSION IN THE KILOMBERO VALLEY, TANZANIA

Dickson W. Lwetoijera[1], Caroline Harris[2], Samson Kiware[1], Stefan Dongus[3], Gregor Devine[4], Philip McCall[5], Silas Majambere[1]

[1]Ifakara Health Institute, Ifakara, Morogoro, United Republic of Tanzania, [2]Lancaster University, Lancaster, United Kingdom, [3]Swiss Tropical and Public Health Institute, Basel, Switzerland, [4]QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia, [5]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

This longitudinal study demonstrates the trends in malaria vector dynamics and their relative contribution to malaria transmission in hyper-endemic transmission settings in Tanzania. The study was conducted in two villages within the Kilombero valley, in rural Tanzania for five consecutive years (2008 - 2012). 72 houses were selected per village and each house was sampled for mosquitoes monthly using a CDC light trap. Collected mosquitoes were assessed for species identity and sporozoite infection status using PCR and ELISA respectively. *Anopheles funestus* susceptibility to insecticides was assessed using WHO guidelines. A total of 100,810 malaria vectors were collected, of which 76% were *An. gambiae* s. l. and 24% were *An. funestus*. Of all *An. funestus* samples that amplified with PCR (n = 2,737), 97% were *An. funestus* s.s., 2% were An. rivorulum and 1% An. leesoni. Whereas for *An. gambiae* s.l. (n = 8,117), 93% were *An. arabiensis* and 7% were *An. gambiae* s.s. The proportion of *An. gambiae* s.s. identified by PCR (2,924) declined from 0.2% in the year 2008 to undetectable levels in 2012. *An. arabiensis* dominated the wet season whereas *An. funestus* dominated the dry season. Malaria transmission intensity significantly decreased from an EIR of 78.14 infectious bites/person/year in 2008 to 35ib/p/yr in 2011 but rebounded to 226 ib/p/yr in 2012 coinciding with an increased role of *An. funestus* in malaria transmission. Insecticide susceptibility tests indicated full susceptibility of *An. funestus* to deltamethrin (100% mortality), reduced susceptibility to dieldrin (95%), permethrin (93%), and confirmed resistance to DDT (86%). Similar findings were also recorded for An. arabiensis, in separate study in same area. The results indicate the continuing role of *An. arabiensis* and the increasing importance of *An. funestus* in malaria transmission. These findings call for complementary vector control and surveillance tools that target these specific vector species, their behaviour and their ecology and an insecticide resistance management plan to preserve the efficacy of LLINs.

1620

DEVELOPMENT OF AN *IN VITRO* TRANSMISSION BLOCKING (ITB) ASSAY AGAINST *PLASMODIUM FALCIPARUM* WITHOUT THE USE OF MOSQUITOES

Abraham G. Eappen, Tao Li, Meghan Marquette, Peter F. Billingsley, Adam Richman, B. K. Sim, Stephen L. Hoffman

Sanaria Inc., Rockville, MD, United States

Major efforts are underway aimed at developing malaria vaccines that can be used for elimination of *Plasmodium falciparum* by preventing the development of the parasite within the mosquito and thereby preventing transmission of the parasite to humans. To assess candidate vaccines, antibodies are induced by immunization of animals and humans, and assessed for their transmission blocking activity against sexual erythrocytic and mosquito stage parasites in a standard membrane-feeding assay (SMFA). These SMFAs are biologically relevant, but highly consumptive of personnel time and resources, require a mosquito colony and are not easily adaptable to high through-put. Thus, it is difficult, time consuming and expensive to assess large numbers of candidate anti-sera in SMFAs. Sanaria's technology platform generates live, aseptic, purified, cryopreserved *P. falciparum* sporozoites (PfSPZ) that can be administered as a highly protective malaria vaccine. PfSPZ are produced using *Anopheles stephensi* mosquitoes as bioreactors. Sanaria, in its quest for developing PfSPZ-based products without the use of mosquitoes, developed technology for the *in vitro* production of Pf oocysts. We have optimized gametocyte culture conditions that are optimal for the *in vitro* production of ookinetes and oocysts, established culture conditions to reproducibly produce and quantify 3 and 7 to 8 day oocysts, and demonstrated that *in vitro* produced oocysts are similar in size and morphology to mosquito produced oocysts. These developments have made it possible to assess the transmission blocking activities of candidate vaccines in an *in vitro* transmission blocking (iTB) assay without the need for mosquitoes. Preliminary data indicate correspondence between the results of SMFA and iTB assays. The transmission blocking activity of antibodies against Pfs25 and Pfs48/45 in SMFA and iTB assay will be presented.

1621

IDENTIFICATION OF A NOVEL *PLASMODIUM FALCIPARUM* HEAT SHOCK PROTEIN 70 (HSP70Z), ALSO IDENTIFIED AS CG4, AS A TRANSMISSION BLOCKING VACCINE CANDIDATE

Mark DeCotiis, Joan Aebig, Buddhadeb Mallik, Nikiah Dawson, Olga Muratova, Karine Reiter, Martin Burkhardt, Vu Nguyen, Patrick E. Duffy, Nicholas J. MacDonald, **David L. Narum**

National Institutes of Health, Rockville, MD, United States

A malaria transmission blocking vaccine (TBV) is critical to achieve the goal of malaria elimination in some areas. To date, only one sexual stage protein, Pfs25, has been evaluated in humans and a second sexual stage protein, Pfs230, will begin human testing soon. Other sexual stage proteins have failed to reach the clinic due to an inability to produce them at pilot-scale following cGMP. In order to expand the number of TBV candidates, a panel of monoclonal antibodies (mAbs) was produced against *Plasmodium falciparum* macrogametes to identify novel surface proteins that, when targeted by antibodies could interfere with parasite development in the mosquito midgut. Here we report on the evaluation of one mAb, identified as 1C7, which recognized a heat shock protein (HSP), identified as HSP70z or Cg4, by Western blot of a sexual stage parasite lysate and by pull-down studies using LC/MS/MS techniques. HSP70z is expressed in asexual and sexual stage parasites with a molecular mass of approximately 100 kDa. In macrogametes, HSP70z may be localized on the macrogamete cell surface by a live immunofluorescence assay. Most importantly, 1C7 blocked *P. falciparum* transmission in mosquitoes, with similar activity to that of a Pfs230 domain 1 specific mAb using an *ex vivo* membrane feeding assay. A recombinant form of HSP70z (named rHSP70z) was produced in *Pichia pastoris* that was comprised of approximately 20% of the native protein which was recognized by 1C7 in Western blots. rHSP70z specific IgG purified from sera of immunized rabbits failed to block parasite transmission. In a competition ELISA, rHSP70z specific rabbit antibodies failed to compete for 1C7 binding to rHSP70z, likely explaining the lack of transmission blocking activity. Currently, the 1C7 epitope is being mapped to evaluate whether a synthetic peptide mimicking the 1C7 epitope will induce transmission blocking antibodies.

1622

IMPACT OF INDOOR RESIDUAL SPRAYING ON ENTOMOLOGICAL INDICES OF MALARIA TRANSMISSION IN THE BUNKPURUGU-YUNYOO DISTRICT IN THE NORTHERN SAVANNAH ZONE OF GHANA

Samuel Dadzie[1], **Sylvester Coleman**[2], Peter Mumba[2], Aklilu Seyoum[2], Philip Ricks[3], Daniel Szumlas[4], Paul Psychas[5], Jacob Williams[6], Maxwell Appawu[1], Daniel Boakye[1]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Ghana Africa Indoor Residual Spraying Project, Abt Associates, Accra, Ghana, [3]Malaria Branch, Division of Parasitic Diseases and Malaria, Atlanta, GA, United States, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]University of Florida, Gainesville, FL, United States, [6]Research Triangle International, Washington, DC, United States

Malaria remains a major public health problem in Ghana, especially in the northern savannah zone. This study was conducted to evaluate the impact of U.S President's Malaria Initiative (PMI) and the Ghana Health Services Indoor Residual Spraying (IRS) program on malaria transmission in the Bunkpurugu-Yunyoo District in northern Ghana. In 2011 and 2012, the district was sprayed with a pyrethroid, alphacypermethrin, at an application rate of 25mg/m^2. In 2013, an organophosphate, pirimiphos-methyl at a rate of 1g/m^2), was used based on declining susceptibility of local vectors to pyrethroids. Indoor resting densities (IRD), parity, sporozoite rate and entomological inoculation rates (EIR) of the local vector species were monitored through pre- and post-IRS monthly human landing and pyrethrum spray collections. The IRD of *Anopheles gambiae* s.l. (the predominant vector species, 99.2% of all *Anopheles* collected) was reduced from a mean of 2.91 mosquitoes/room recorded from the baseline surveys to 2.10 mosquitoes/room (27.7% reduction) in 2012 after spraying with alphacypermethrin. In 2013, the mean IRD of *An. gambiae* s.l. was further reduced to 0.22 mosquitoes/room, representing 89.2% decline compared to 2012. In 2012 there was a non-significant reduction (p=0.289) in the mean parity rate for *An. gambiae* s.l. from 75% to 43% (57% reduction). Spraying with Actellic in 2013, resulted in 67% reduction in parity rate from 43% to 32% (p = 0.130). A comparison of the pre and post-IRS EIRs also revealed a significant (p<0.05) reduction, from 0.35 infective bites/man/night (ib/m/n) in 2011 to 0.021 ib/m/n in 2012. In 2013, there was slight decrease in EIR to 0.018 ib/m/n after spraying with Actellic 300CS that year. The results show that the IRS operations resulted in reduction in key entomologic indicators. IRS with pirimiphos-methyl had the greatest impact on indoor resting densities and parity rates of *An. gambiae* s.l. but not EIRs. The PMI funded IRS program after three years contributed to 94.8% reduction in malaria transmission in the study area.

1623

SCALING UP OF INSECTICIDE-TREATED NETS (ITN) OWNERSHIP AND USE IN MOZAMBIQUE: HAS THE SCALE-UP BEEN EQUITABLE?

Samantha Herrera, Ana Claudia Franca-Koh, Jui Shah, Cameron Taylor, Lia Florey, Yazoume Ye

ICF International, Rockville, MD, United States

Insecticide-treated nets (ITNs) are effective tools for malaria prevention and can significantly reduce morbidity and mortality due to malaria, especially among children under five in endemic areas. ITN ownership and use have rapidly increased in Mozambique, due to scaling up of ITN distribution efforts within the country over the past seven years, which have aimed to achieve universal coverage. The percent of households that owned at least one ITN rose by more than three-fold from 15.7% in 2007 to 51.4% in 2011. ITN access, defined as one ITN for every two people in the household also rose significantly, from 9.4% in 2007 to 37.0% in 2011. Similarly during this time, ITN use among children under five and pregnant women increased significantly, from 6.7% to 35.7% and from 7.3% to 34.3%, respectively. With the aim of achieving universal coverage, it is important to also assess distribution across subgroups, particularly groups from different socio-economic status. This analysis used Lorenz concentration curves and indices to assess the equity of ITN household ownership, access and use in Mozambique, using data from the 2007 Malaria Indicator Survey and 2011 Demographic Health Survey. Concentration Index (C-Index) values range between -1 and 1, with a value of 0 representing perfect equality. From 2007 to 2011, equity in ITN household ownership (C-Index: 0.06 in 2007 and 0.04 in 2011), ITN access (C-Index: 0.12 in 2007 and 0.09 in 2011), and ITN use among children under five (C-Index: -0.04 in 2007 and 0.03 in 2011) showed slight improvements, while equity in ITN use among pregnant women remained the same (C-Index: .06 for both years). It is important to highlight that while improvements where shown, equity in ITN household ownership, access and ITN use among children under five, all were fairly equitable in 2007. The results demonstrate that ITN scale-up efforts have been successful as well as equitable across the population, however further improvements in access and ITN coverage are still needed to reach targets.

1624

DIFFERENCES IN DEET AND PICARIDIN SENSITIVITY BETWEEN SOUTHEAST ASIAN VECTORS OF MALARIA AND ARBOVIRUSES, RESULTS OF A FIELD EVALUATION OF TOPICAL REPELLENTS

Lies Durnez[1], Mao Sokny[2], Karel Van Roey[1], Vincent Sluydts[1], Siv Sovannaroath[2], Tho Sochantha[2], Marc Coosemans[1]

[1]Institute of Tropical Medicine, Antwerp, Belgium, [2]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia

Scaling up of insecticide treated nets has contributed significantly to a substantial malaria decline. However, some malaria vectors, and most vectors of arboviruses, bite outdoors and in the early evening. Therefore, topically applied insect repellents may provide a crucial additional protection against mosquito-borne pathogens. Among topical repellents, DEET is the most commonly used, followed by others such as PMD or picaridin. A study was carried out in Cambodia to determine the entomological efficacy of DEET and picaridin repellents on wild populations of several mosquito genera, including vectors of arboviruses (*Aedes aegypti* and *Ae. albopictus*) and malaria (*Anopheles dirus*, *An. minimus*, *An. maculatus* and *An. barbirostris*). During 230 survey days in two consecutive years, the lower limbs of 5 persons were treated with repellents ('DEET 20%', 'picaridin 20%', or 'picaridin 10%') or ethanol (2 negative controls), followed by mosquito collections on the treated limbs during 5 consecutive hours. The treatments were grouped following a 5x5x5 Graeco-latin square to equalize the effects of treatment days, collection sites, and test persons. Protection rates were high (91-99.2%), with significant differences between treatments, genera, and species. For malaria vectors, 'DEET 20%' performed better than 'picaridin 20%' or 'picaridin 10%'. The protection rate against *An. barbirostris* was significantly lower as compared to the other vectors, especially for the picaridin repellents. As malaria endemic areas often differ in their vector species composition, this heterogeneity in repellent sensitivity between vector species might result in a geographically heterogeneous epidemiological impact of repellent use for malaria control.

1625

MOLECULAR AND ENVIRONMENTAL INTERACTIONS IN *ANOPHELES GAMBIAE* INFLUENCE BOTH REPRODUCTIVE CAPACITY AND *PLASMODIUM* DEVELOPMENT

Perrine Marcenac[1], Francesco Baldini[2], Nicola Segata[3], Abdoulaye Diabate[4], Flaminia Catteruccia[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Università di Perugia, Terni, Italy, [3]University of Trento, Trento, Italy, [4]Institut de Recherche en Sciences de la Sante/Centre Muraz, Bobo-Dioulasso, Burkina Faso

Reproduction in the major malaria vector *Anopheles gambiae* is influenced by a number of molecular and environmental factors. Male-female

molecular interactions following mating are important determinants of fertility and fecundity. At the same time, an increasing amount of evidence points to reproductive processes playing an important role in *Plasmodium* parasite development. Male transfer of the steroid hormone 20-hydroxy-ecdysone (20E) during mating activates the transcription of a *Mating-Induced Stimulator of Oogenesis* (*MISO*) gene that transduces the mating signal into an increase in egg development. Silencing *MISO* by RNA interference in laboratory colonies reduces egg development to levels observed in virgin females. This phenotype is caused by reduced expression of yolk protein precursors (YPPs) after *MISO* silencing, impairing lipid accumulation in the oocyte. Previous research shows that the same YPPs essential for lipid accumulation in the developing mosquito egg help parasites escape the immune system. We have found evidence that MISO depletion decreases *Plasmodium* infection in *A. gambiae*, further reconstructing the molecular pathways linking egg development and *Plasmodium* infection. Functional studies performed in field *A. gambiae* show that in natural populations *MISO* is essential for egg development, as its silencing abolishes oogenesis after blood feeding. These results suggest that in natural mosquito populations MISO is a key switch that directs resources derived from the blood meal towards oogenesis only in mated females. Besides molecular factors, environmental factors also affect *A. gambiae* reproductive biology in natural populations. Sequencing analysis of the microbiota from reproductive tissues of wild *A. gambiae* demonstrates enrichment of bacteria in specific villages and mating swarms, which may impact reproductive success and isolation. In addition we have identified bacteria in reproductive tissues that have been previously shown to impact *Plasmodium* survival. We are currently determining the effects of these bacteria on reproductive biology and mosquito fitness, two factors relevant for malaria transmission. By elucidating the different molecular and environmental interactions regulating reproduction and parasite development in *A. gambiae*, we hope to develop novel targets for vector control.

1626

HETEROGENEITY OF HUMAN AND MOSQUITO BEHAVIOR IN RELATION TO OUTDOOR AND EARLY MALARIA TRANSMISSION IN SOUTHEAST ASIA

Charlotte Gryseels[1], Lies Durnez[1], Sambunny Uk[2], Vincent Sluydts[1], Tho Sochantha[2], Somony Heng[2], Karel Van Roey[1], Lydie Canier[3], Didier Menard[3], Sokny Mao[2], Saorin Kim[3], Marc Coosemans[1], Koen Peeters Grietens[1]

[1]Institute of Tropical Medicine Antwerp, Antwerp, Belgium, [2]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [3]Institut Pasteur du Cambodge, Phnom Penh, Cambodia

Outdoor transmission is one of the key factors for malaria control and elimination in the Greater Mekong Region, including Cambodia, where malaria is now mainly reduced to forested regions inhabited by ethnic minorities and mobile migrants. Tackling outdoor transmission requires a better understanding of the heterogeneity in human and vector behavior during times when people are still active outdoors. Results from a mixed-methods social science study in Ratanakiri, Cambodia, indicate that local ethnic minorities have different socio-cultural characteristics than the majority society targeted by malaria control programs. (i) Mobility caused by a multiple residence system increases exposure to the sylvatic vector *An. dirus*, as during the malaria peak season people usually reside on their farms in the forest. (ii) Open housing blurs the boundary between indoor and outdoor biting. (iii) Differences in sleeping times between villages, farms and during forest activities creates diverse evening biting opportunities, if assumed that people sleep under insecticide-treated nets at night. However, (iv) evening resting is frequently done without nets, and (vi) even night sleeping often occurs under non-impregnated bought nets, which are often torn, making it hard to establish to what extent transmission actually occurs early or outdoors. Results seem to suggest that the evolving interplay of vector and human behavioral heterogeneity maintains malaria hotspots/pops of mainly asymptomatic carriers. Targeting malaria transmission in low transmission settings requires a better understanding of this heterogeneity.

1627

NO PRODUCT? NO PROGRAM: TREATMENT UPTAKE AND AVAILABILITY OF ANTIMALARIAL DRUGS FOR INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN MALAWI

Katherine Wolf[1], Loren Bausell[1], Evance Moyo[2]

[1]United States Agency for International Development | DELIVER PROJECT, Arlington, VA, United States, [2]United States Agency for International Development | DELIVER PROJECT, Lilongwe, Malawi

Throughout Africa, 30 million pregnant women are exposed to malaria each year. Several interventions are central to malaria control efforts, including dispensing sulfadoxine-pyrimethamine (SP) for intermittent preventive treatment in pregnancy (IPTp). While preventing malaria in pregnancy is a key focus of global interventions, trends in using SP to prevent malaria during pregnancy have largely stagnated. Twenty years after adopting IPTp as an official policy, the rates of pregnant women in Malawi who receive at least two doses of SP is only 55 percent. Research has identified factors impeding adherence to IPTp policies: antenatal clinic (ANC) client behavior/attitudes, provider actions/attitudes, and SP availability at facilities. Qualitative data indicate that stockouts significantly impact IPTp coverage. However, only a quantitative analysis will show if there is a relationship between IPTp use and stockouts. For four years, the USAID | DELIVER PROJECT examined trends for three complementary data sources: SP availability at heath facilities using the country's logistics management information system; SP uptake at health facilities using national ANC service statistics; and IPTp coverage in households reported in the Malawi Demographic Health Surveys. Preliminary results show a general decline in SP stockouts between 2010 and 2013. SP stockouts peaked in late 2011 and the first quarter of 2012 (80 percent); low stockout rates were reported during the last half of 2012 and all of 2013 (6 percent). High stockout rates in early 2012 correlate with a sharp drop in SP uptake during the same time (62 to 31 percent). As SP availability improved, beginning in May 2012, the IPT coverage rates also improved. Women attending their first ANC visit after 12 weeks, who received any SP, increased to almost 100 percent by mid-2013. These results show a close relationship between SP availability and the uptake of preventive treatment during pregnancy. Using a mixed-method case study approach, this analysis will explore the impact of SP stockouts on MIP programming efforts.

1628

AN EXTENDED MOLECULAR BARCODE FOR TRACKING *PLASMODIUM FALCIPARUM* PARASITE POPULATIONS

Nicholas K. Baro[1], Rachel Daniels[1], Eli Moss[2], Wesley Wong[1], Daniel L. Hartl[3], Daniel E. Neafsey[2], Sarah K. Volkman[1], Dyann F. Wirth[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Broad Institute, Cambridge, MA, United States, [3]Harvard University, Cambridge, MA, United States

The lack of a generalizable approach to malaria control requires a real-time assessment of both epidemiological and parasite population genetics at the local level. The major epidemiological shifts associated with the transition into a malaria-eliminating country are mirrored by changes in the genetic diversity profile of malaria parasite populations. These genetic signatures can be monitored using population genetic - based tools to determine efficacy of malaria intervention efforts prior to measurable changes in disease prevalence. For these reasons, and to further understand the basic biological processes of parasite transmission, we have improved upon an interim panel of neutral, unlinked, single nucleotide polymorphisms (SNPs) that is specifically designed for resolving

individual parasites in highly related parasite populations and allows for quantification of allele balance at polymorphic loci to infer changes in transmission dynamics. The extended molecular barcode includes SNPs exhibiting high minor allele frequency and low divergence (e.g FST) filtered from screening over 500 whole genome sequenced samples representing populations from Africa, South East Asia and South America. The accuracy and sensitivity of Sequenom's Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry genotyping technologies allow for quantitative output that reflects proportions of each allele in multiplexed reactions. The increased number of loci and low dynamic range of the minor allele improve our ability to predict the number of distinct parasite genomes in samples with higher complexity of infection (COI). In silico testing of the extended molecular barcode demonstrates its utility for detecting recent common ancestry among parasites in a sample on a much more cost-effective basis than using variants called with whole genome sequence data. We are exploring the use of these approaches to identify shared regions of the genome that are identical by descent among highly related Senegalese populations and assess COI in high transmission settings.

1629

RECEPTIVITY OF ANOPHELINE MOSQUITOES IN SOUTHERN ZAMBIA: TWO YEARS OF BIONOMIC DATA FROM AN AREA TARGETED FOR MALARIA ELIMINATION

Jennifer C. Stevenson[1], Limonty Simubali[2], Harry Hamapumbu[2], Sungano Mharakurwa[1], Douglas E. Norris[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Macha Research Trust, Choma, Zambia

Malaria prevalence in Macha, southern Zambia has reduced significantly over the past decade, with community parasite prevalence currently below 1%. The province is now targeted for elimination strategies. It is important, however, to establish the potential of the local mosquito population to transmit malaria to estimate the risk of resurgence if the parasite were re-introduced. To address this, light trap collections of mosquitoes were carried out monthly in randomly selected geo-referenced households in the catchment area of Macha Hospital from February 2012 to February 2014. Peak times of host-seeking were studied using collection bottle rotators. Approximately 1200 female anophelines were collected from 856 traps across 336 different households. 60% were identified as the vector *An. arabiensis* to give a mean catch of 0.88 *An. arabiensis* per trap-night over the study period. Catches ranged from 0 to 148 *An. arabiensis* in a single trap, with highest catch recorded in February 2013 (monthly mean 7.2 per trap-night). Households with anophelines appeared to be clustered. Spatial analyses are ongoing to overlay mosquito distribution on malaria risk maps. 10.8% of *An. arabiensis* were blood fed and the human blood index was calculated as 0.93. At least 9 other anopheline species were identified, some of which were highly anthropophagic. One specimen was found to be positive for *Plasmodium falciparum* sporozoites by ELISA. Preliminary analysis of host-seeking times indicated peaks between 22:30 and 02:30, but vector activity was recorded as early as 20:30. Despite substantial reduction in malaria cases in Macha, large numbers of the vector *An. arabiensis* exist at certain times of the year and in certain localities. Here *An. arabiensis* demonstrates high endophily and anthropophagy. There is the potential for human exposure outside the times of net use. Whilst drug-based elimination strategies are encouraged, vector control methods should be maintained and entomological surveillance continued to monitor any increase in dominant or secondary vectors.

1630

OPTIMAL COVERAGE AT MINIMAL COST: A DYNAMIC MODELING APPROACH TO SIMULTANEOUS ALLOCATION OF MULTIPLE ANTI-MALARIA INTERVENTIONS

Beatrix Balogh[1], David W. Hutton[2], Ravi M. Anupindi[3], Leslie Arney[1], Meiting Liu[4], Haipeng Wu[4], Peter S. Larson[1], Prashant Yadav[1], Mark L. Wilson[4]

[1]William Davidson Institute, University of Michigan, Ann Arbor, MI, United States, [2]Department of Health Management and Policy, School of Public Health, University of Michigan, Ann Arbor, MI, United States, [3]Stephen M. Ross School of Business, University of Michigan, Ann Arbor, MI, United States, [4]Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI, United States

Malaria control efforts often involve a multipronged approach, including insecticide-treated nets (ITNs), indoor residual spraying (IRS), and pharmaceutical-based treatment and prevention. Public health agencies (government, private, NGOs) are constrained by conflicting demands on limited budgets. Determining the appropriate combination and magnitude of interventions is both complicated and context specific. Using a dynamic modeling approach, we analyzed the system dynamics of multiple malaria interventions functioning in concert, evaluated the cost-effectiveness of treatment and prevention strategies, and developed a resource allocation model to apportion resources across intervention types to maximize overall cost effectiveness. This compartmental mathematical model (using MATLAB) includes age, human immunity, and seasonality in the *Plasmodium* transmission cycle, with interventions of IRS, ITNs, intermittent preventive treatment for pregnant women (IPTp), and mass screening and treatment (MSAT). We assessed individual and combined effects on various transmission parameters such as biting rate, force of infection, and mosquito death. For a set of parameters considered typical of high transmission malaria endemic settings, results show that after five years into the model run and compared to a baseline with no interventions, with a combination of 65% coverage of IRS and ITNs, infection prevalence in children under five years of age and pregnant women would decrease by about 18%. Those above five years of age exhibited decrease in prevalence by about 16% with 70% combined IRS and ITNs. The addition of IPTp and MSAT decreased long-term community prevalence in all age groups by an overall additional 12%. This modeling approach allows a careful assessment and optimization of costs and benefits to particular combinations of malaria interventions and should assist public health groups in maximizing benefit under constrained budgets.

1631

HOUSEHOLD EXPENDITURES FOR MALARIA TREATMENT FROM A POPULATION-BASED SURVEY

Donald S. Shepard[1], Peter Mangesho[2], Robert Mpagala Kihomo[1], Aggrey Kihombo[1], Joseph Mugasa[2], Amos Malongo[2], Ruth Mnzava[2], Filemoni Tenu[2], Angelique K. Rwiyereka[1], Yara A. Halasa[1], Aaron M. Samuels[3], Mark Rowland[4], Frank Mosha[5], William Kisinza[2]

[1]Brandeis University, Waltham, MA, United States, [2]National Institute for Medical Research, Tanga, United Republic of Tanzania, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom, [5]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania

Effective malaria control technologies not only improve health, but also reduce health expenditures by government and households. In Muheza, Tanzania, a randomized trial is assessing the effectiveness and cost-effectiveness of a non-pyrethroid insecticide-treated wall liner (ITWL) and indoor residual spraying (IRS). To project savings, the investigators are determining current expenditures for malaria treatment in conjunction with a cross-sectional epidemiological household survey. Following mapping and household enumeration, from December 2013 through February 2014 epidemiological teams visited 4200 randomly chosen

residents aged>6 months across 60 village clusters. Respondents who reported a malarial episode within the past 30 days were selected for a follow-up economic interview. Its questions included household income, treatment received, time required for travel and treatment, and expenses incurred. Overall, 18% of sampled residents reported a malaria illness episode within the last 30 days. Preliminary data are currently available for 467 representative malarial cases. Of these, 6% received inpatient hospital treatment, 91% received ambulatory treatment outside the home, and 3% received only in-home or no treatment. Overall combined travel and treatment time averaged 5 hours. Household expenditures per case averaged US$5.24 (TZS 8,545), but the median value of US$2.33 (TZS 3,800) was below the mean. This arose because expenditures on malaria treatment were highly skewed with a standard deviation equal to 213% of the mean. Analysis of household expenditures found 54% was for direct medical expense (consultations, beds, tests, and medications), 27% for transportation, and 19% for other expenses. Average expenditures of hospitalized patients US$32.82 (TZS53,491) were an order of magnitude above those of non-hospitalized patients of US$2.15 (TZS5,787). By comparison, household monthly revenue was under US$46.01 (TZS75,000) for 71% of respondents. Average inpatient expenses represent about a month's median income. Given low rural incomes, even routine medical expenses can strain household budgets. Adjustment of censored expenditures for episodes in progress and valuing time lost would increase estimated costs. If ITWL and IRS prove efficacious in this district, households will save on treatment expenses.

1632

IMPACT OF MALARIA INTERVENTIONS ON REDUCTIONS IN NEONATAL MORTALITY IN MALAWI, RWANDA AND MAINLAND TANZANIA

Rebecca Winter[1], Lia Florey[1], Anne Langston[2], Tom Pullum[1]

[1]The Demographic and Health Surveys Program, ICF International, Rockville, MD, United States, [2]ICF International, Rockville, MD, United States

Between 2000 and 2010 sub-Saharan Africa's impressive gains in under-five mortality have been accompanied by a more modest reduction in neonatal mortality. As a result, neonatal mortality now accounts for about a third of all under-five deaths in the region. This study selected three countries in SSA with significant reductions in NMR during this period_ Malawi, Rwanda, and mainland Tanzania_ in order to identify factors that contributed to the observed reductions, with special interest in the relative importance of scale-up of interventions to protect women against malaria during pregnancy. In Malawi, the neonatal mortality rate among women's most recent children born fell from 26 deaths per 1,000 live births in the five years preceding the 2000 DHS to 20 deaths per 1,000 live births in the five years preceding the 2010 DHS. In Rwanda, the NMR declined from 29 to 14 deaths per 1,000 live births, and in mainland Tanzania from 32 to 18 deaths per 1,000 live births between the 1999 and 2010 surveys. Multivariate decomposition procedures were used to examine the extent to which the scale-up of malaria interventions contributed to these observed reductions. Results show that in all three countries the rapid increase in mosquito net ownership was associated with the observed reductions in neonatal mortality, even after adjusting for changes in the distribution and effects of sociodemographic characteristics and key maternal and delivery services. In Malawi - where information on mothers' use of IPTp was available - the study did not find evidence that the scale-up of IPTp was associated with the reduction in NMR. In conclusion, the findings reinforce the importance of consistent and universal mosquito net use in areas with high prevalence of malaria. While malaria interventions are most often geared towards saving the lives of children at older ages (6 months to 5 years), the study findings contribute to a growing body of evidence pointing to the importance of malaria interventions to neonatal survival.

1633

FIGHT AGAINST MALARIA BY STUDENTS AND SCHOOLCHILDREN IN KINSHASA, DR CONGO

Sophia M. Mangala, Junior Musenga, Ornella L. Mampuya, Viviane Mitshiabu, Steve Tulantched, Thierry L. Bobanga, Thierry L. Bobanga

Protestant University of Congo, Kinshasa, Democratic Republic of the Congo

Malaria is a real public health problem in Africa. Despite the efforts made by countries, 655,000 deaths were recorded worldwide in 2010. And the Democratic Republic of the Congo accounts for nearly 10 % of the mortality And the fight against this disease should be of interest all groups in the community. And new and innovative approaches should be considered to reduce disease Thus, students of the Protestant University of Congo have been trained in the fight against malaria with the aim of training schoolchildren Students' knowledge was initially evaluated. And training was organized to provide knowledge about the transmission, prevention, particularly on ITN and finally what to do in case of fever. Method: 823 students in 9 schools in the city of Kinshasa sensitized by 20 university students who were trained by the managers of the National Program of Academic Medicine and University of Kinshasa Parasitology Department. The age of children was between 9 to 11 years old This awareness was marked by a strong interaction between children and sensitizers. At the end of awareness, the children were evalued to assess their understanding of the subject. And the best schoolchildren were awarded. It was noted that over 70 % of children could answer basic questions on malaria. In conclusion, at the end of this work, over 800 schoolchildren in the city of Kinshasa and 20 students have contributed to the fight against malaria, which is a tiny proportion of the population of said city. It is therefore important to continue these actions as awareness through children, future actor and relay in the transmission of knowledge from their family. This, could be, an innovative approach to malaria control.

1634

INSECTICIDE-TREATED NET (ITN) UTILIZATION AND MAINTENANCE IN KINSHASA, DR CONGO

Nono B. Mangaza[1], **Fabrice B. Ekomisa**[2], Solange E. Umesumbu[3], Billy S. Kunyu[4], Thierry L. Bobanga[2]

[1]University of Technology Belcampus, Kinshasa, Democratic Republic of the Congo, [2]Protestant University of Congo, Kinshasa, Democratic Republic of the Congo, [3]National Malaria Control Program, Kinshasa, Democratic Republic of the Congo, [4]National Institute of Biomedical Research, Kinshasa, Democratic Republic of the Congo

Malaria remains at the beginning of the first century killer in DRC with more than 60,000 deaths, especially among children under 5 and pregnant women Among the strategies, the LLIN is a tool of choice but its availability and hanging are operational problems. Furthermore its efficiency and sustainability are elements to evaluate. The present study was conducted with the aim to determine the proportion of households with at least one LLIN ; the proportion screens installed (used); the proportion of pregnant women who slept under LLINs ; the proportion of children who slept under LLINs ; determine the average age of the net and the number of washing on average per month. A cross-sectional study on the use and sustainability of ITN Long (LLINs) in the city of Kinshasa. We formed a cluster sampling and multistage. The sample size was 104 households per municipality. And 24 selected communes we got 2,512 households. The use of the net in Kinshasa was 59.4 %. Pregnant women and children under 5 were using respectively 70% and 60%. Through the city's most important use is in the center where the mosquito nuisance with the *Culex* is the greatest with 72.7 %. Whereas the periphery of the city use is low (44 to 55%) where anopheles populations are most abundant. The proportion of pregnant women and children under 5 years under nets was 70 % and 60 % respectively. In 2,512 households visited

nearly 4,812 LLINs were counted. The average duration of the net in the household was in the range 19 to 24 months with 1.5 washes by month. More than 50% of household use detergent for washing ITN. The majority of nets found in the households have probably lost their effectiveness before 18 months of utilization. In conclusion, the use of LLINs was still low in Kinshasa. The inhabitants do not respect manufacturer's recommendations in term of washing. Study on bio efficacy and durability in field use condition must be conducted to make evidence of efficacy

1635

EFFECT OF MASS DRUG ADMINISTRATION OF IVERMECTIN TO HUMANS ON MALARIA TRANSMISSION AND EPIDEMIOLOGY IN WEST AFRICA

Haoues Alout

Colorado State University, Fort Collins, CO, United States

Mass drug administration (MDA) of ivermectin to control filariasis and onchocerciasis has been shown to reduce malaria vector survivorship and the proportion of mosquitoes that are able to transmit *Plasmodium falciparum* in the same communities. Therefore, we have proposed that ivermectin MDA be considered in malaria control and elimination strategies. In our ongoing efforts to fully characterize the effect of ivermectin MDA on malaria transmission, we compared its effect on *Anopheles gambiae* and *Plasmodium falciparum* natural populations in three different West African countries (Senegal, Liberia and Burkina Faso) across different seasons. Blood fed mosquitoes were collected indoors before and after MDA in treated villages by health authorities for either lymphatic filariasis (ivermectin+albendazole) or for onchocerciasis control (ivermectin alone), and concomitant mosquito sampling was performed in untreated control villages. We compared the blood fed mosquito survivorship, sporozoite rate and parasite genetic diversity in mosquitoes, taking account for temperature, humidity, mosquito species and type of treatment. The mosquitocidal effect was consistent in all field sites and seasons, and did not vary with the addition of albendazole. The reduction in sporozoite rates were significant when compared to control villages but the observed reductions vary across field sites. In Burkina Faso in the treated village, sporozoite rate was significantly reduced by 79% following MDA (from 8% to 1.7%) and increased to pre-treatment levels after two weeks. Additionally, MDA completely eliminated sporozoite transmission from outdoor host-seeking mosquitoes for a period of two weeks. The potential impact of ivermectin on *Plasmodium* genetic diversity in mosquitoes and its consequence on malaria epidemiology and transmission will be discussed.

1636

IMPROVED ENVIRONMENTAL COMPLIANCE AND OPERATIONAL EFFICIENCY USING MOBILE SOAK PITS

Peter J. Chandonait

Abt Associates, Inc., Cambridge, MA, United States

The President's Malaria Initiative (PMI) employs thousands of spray operators and annually sprays millions of homes in Africa with a residual pesticide to kill malaria-bearing mosquitos. At the end of the work day, the spray operators must clean their spray tank and their personal protective equipment (helmets, face shields, gloves, and boots), resulting in wash water that is contaminated with pesticide residue. This contaminated water is treated before release in soak pits, which are pits 1 meter wide x 2 meters length x 1 meter deep, filled with a bed of commonly available cooking charcoal and other materials to filter out and break down the pesticide. These soak pits are centrally located within targeted spray areas so that many operators can travel to and use these facilities for clean-up at the end of the day. PMI has built and/or refurbished hundreds of soak pits over the past several years of IRS project implementation. Ensuring that these soak pits are built properly so as to minimize negative environmental impacts from IRS is a major environmental compliance responsibility of the implementing partner. However, in sparsely populated spray areas, teams may travel hours to reach targeted communities, and they may not be able to return to a centralized location for clean-up at the end of the day. AIRS has developed a mobile soak pit (MSP) that can be transported from site to site with the spray team, can be installed in less than one half hour, and can remove pesticide contamination from wash waters. Advantages of this mobile soak pit include a marked reduction in the time that spray operators spend traveling from site to wash area, better adsorption of the pesticide due to the characteristics of the filter material, better control of soak pit materials, and better protection of the community because the pesticide contamination is taken away in the filter.

1637

BASELINE STUDIES ON ANGLOGOLD ASHANTIS' INDOOR RESIDUAL SPRAYING PROGRAM (IRS) FOR MALARIA CONTROL IN GHANA

Kwaku P. Asante[1], Dominic B. Dery[1], David K. Dosoo[1], Kinsley Kayan[1], Sylvester Segbaya[2], Victor Asoala[3], Kofi Tchum[1], Frank Amoyaw[2], Nicholas Amoako[1], Abraham Oduro[4], Seth Owusu-Agyei[1]

[1]Kintampo Health Research Centre, Brong Ahafo Region, Ghana, [2]AngloGold Ashanti (Ghana) Malaria Control Limited, Ashanti Region, Ghana, [3]Navrong Health Research Centre, Upper East Region, Ghana, [4]Navrongo Health Research Centre, Upper East Region, Ghana

Malaria is still high in many parts of Africa including Ghana. Private -public partnership are essential in significantly reducing the burden of malaria in high risk countries. In Ghana, there is rapid scale up of indoor residual spraying in various parts of the country with funding from the Global Fund and the United States President's Malaria Initiative (PMI). AngloGold Ashanti (a mining company) is implementing an IRS programme in 25 districts in Ghana. The results of baseline studies (sensitivity of insecticides, prevalence of childhood malaria parasitemia and anemia) conducted prior to the IRS programme are presented here. Malathion was most effective with 100% *An. gambiae* mortalities in seven districts. Fenitrothion was effective in three districts whiles Propoxur worked in one district. Few (14) kdr susceptible strains were detected in samples analyzed with majority being homozygous kdrRR(120) resistant species compared to 32 Heterozygous kdrRS. Preliminary data shows high prevalence of malaria parasitemia (range: 30 - 50%) and anemia (range 40% - 60%). An organophosphate class of insecticide is considered most appropriate for IRS in eleven districts currently earmarked in Ghana. Rotation of different classes of insecticides over time is however recommended as it offers a practical solution for resistance management in light of rapid resistance development. Monitoring of malaria parasitemia and anemia during the IRS programme is required.

1638

TWO VARIANTS OF MULTIDRUG RESISTANT *VIBRIO CHOLERAE* O1 BIOTYPE EL TOR INVOLVED IN TWO CONSECUTIVES OUTBREAKS OF CHOLERA IN CAMEROON (2004 - 2005 AND 2010 - 2012)

Ariane A. Nzouankeu[1], Antoinette Ngandjio[1], Vanessa Nkamga[1], Pierrette Simo[1], Marie-Laure Quillici[2], Marie-Christine Fonkoua[1]

[1]Centre pasteur du Cameroun, Yaounde, Cameroon, [2]Institut Pasteur de Paris, Paris, France

Outbreaks of cholera due to toxigenic *Vibrio cholerae* are always dangerous and need a quick taking care of patient. From may 2010 to February 2012, an outbreak of cholera raged in Cameroon. The epidemic started in the North and reached the south of Cameroon 4 months later, in September 2010. The isolated strains, *V. cholerae* O1 serotype Ogawa, were multidrug resistant with additional resistance to nalidixic acid. isolated from 2 subsequent outbreaks of cholera in Cameroon, 2004 - 2005 and 2010 - 2011. Geographically, the 2004-2005 outbreak was localized in the south of Cameroon, while the current outbreak (2010-2011) covered the whole area of Cameroon. A total of 200 V. Cholerae O1

isolated during the last outbreak of cholera in Cameroon were used in this study; the strains belonged to biotype El Tor, serotype Ogawa. The strains were resistant to multiple antimicrobials especially nalidixic acid, which was the newest character. Molecular detection of their virulence factors revealed that tcpa gene which encodes the toxin coregulated pilus was characteristic to El Tor biotype, while nuleotide sequence of ctxB which encode the sub-unit B of the cholera toxin, was closer to the classical biotype. A total of 3 mutations was observed on the ctxB nucleotide sequence of which 2 were PFGE fingerprinting types showed different patterns, This study reveals that *V. cholerae* strains isolated in 2010-2011 were different from strains isolated in 2004-2005, using antimicrobial susceptibility phenotypes, characterization of antimicrobial resistance, cholera toxin genotyping, PFGE. PFGE analysis revealed two different unrelated profiles All the strains harboured tcpa El Tor allele, which was a supplemental argument ranging these strains in El Tor biotype.

1639

PREVALENCE OF ENTEROAGGREGATIVE *ESCHERICHIA COLI* VIRULENCE GENES IN YOUNG CHILDREN FROM RURAL SOUTH AFRICA AND PATHOGENESIS OF POTENTIAL VIRULENCE MARKERS IN A MOUSE MODEL: THE MAL-ED COHORT

Nicoline Tanih[1], David T. Bolick[2], Amidou Samie[1], Emanuel Nyathi[1], Leah Barrett[2], Relana Pinkerton[2], James P. Nataro[2], Araceli Santiago[2], Richard L. Guerrant[2], **Pascal O. Bessong**[1]

[1]University of Venda, Thohoyandou, South Africa, [2]University of Virginia, Charlottesville, VA, United States

Enteroaggregative *Escherichia coli* (EAEC) is recognized as a cause of growth shortfalls with or without diarrhea worldwide. A number of EAEC virulence-related genes (VRGs) have been described but their role in the clinical outcome of infection is not completely defined and may vary from one geographical location to another. Recently, we have characterized Aar (AggR activated regulator), a new negative regulator in EAEC. Deletion of Aar upregulated the expression of the EAEC master regulator AggR. Accordingly, we have found that an EAEC strain lacking Aar causes growth failure without diarrhea in our zinc deficient mouse model. We extracted DNA from 207 strains of EAEC isolated from the stool of 109 children followed from birth to 12 months of age from the Dzimuali community in the Limpopo Province of South Africa. We investigated the prevalence of EAEC VRGs using multiplex polymerase chain reaction. Samples were analyzed for identification of 18 VRGs. Plasmid encoded haemolysin (aar) was the most frequently detected (86.5%), followed by aggregative adherence regulator (*aggR*, 53.6%) and EAEC HilA homologue (*eilA*, 47.3%). Secreted autotransporter toxin gene (*sat*) was observed at lowest frequency (0%). Although only (5%) of participants had diarrhea in their first 12 months, children with EAEC had greater growth shortfalls (P= 0.03) and one child had an aar(-) EAEC with striking growth failure. These data confirm a high prevalence, endemicity and heterogeneity of EAEC strains in the Limpopo Province of South Africa and their association with growth failure. However, investigations are on-going to determine the impact of potential virulence determinants of the EAEC strains (aar (-) and *aggR* +) in a murine model and their impact on the host on diarrhea and growth impairment.

1640

SENDING PEACEKEEPING TROOPS INTO AN ONGOING CHOLERA OUTBREAK - THE CHILEAN EXPERIENCE DURING MINUSTAH (MISSION DES NATIONS UNIES POUR LA STABILISATION EN HAÏTI)

Reinaldo Rosas[1], Alberto Fica[1], Dannette Guiñez[1], Myriam Polanco[2], Alejandra Saldivia[1], Wilson Wigant[1], Vjera Triantafilo[1], Thomas Weitzel[3]

[1]Hospital Militar de Santiago, Santiago, Chile, [2]Comando de Salud del Ejército, Santiago, Chile, [3]Universidad del Desarrollo Clinica Alemana, Santiago, Chile

Since 2004, Chile participates in MINUSTAH with a permanent contingent of 500 soldiers, who change every 6 months. The Preventive Medicine Service prepares troops and medical units for potential threats. After the 2010 earthquake, a cholera outbreak rapidly spread through Haiti, which was probably related to soldiers participating in the same UN mission. This work describes the experience with pre- and post-deployment measures of the Chilean Forces to reduce the cholera risk in Haiti as well as importation into Chile by asymptomatic carriers. Those actions included sanitary and hygiene education of troops, training for medical officers, pre-deployment immunizations with whole-cell oral cholera vaccine (WC/rBS), and post-deployment microbiological surveillance. In 2011, vaccine side effects were monitored using a standardized questionnaire. After deployment, all troops were screened for *Vibrio cholerae* carriership. Data on WC/rBS vaccination were available for 569 soldiers of whom 9.7% reported systemic and 10.5% gastrointestinal (GI) side effects. A shorter interdose interval (7 vs 30 days) was associated with more GI disturbances (17.4% vs 7.7%, $p<0.001$). In the subgroup receiving WC/rBS 30 days apart, systemic side effects were more common if peacekeepers simultaneously received other pre-deployment vaccines (11.9% vs 4.3%, $p<0.05$). All surveillance stool cultures were negative, except for 2 (in 2012) which both grew strains of non-pathogenic *V. cholerae.* Cholera is still a threat for underdeveloped countries and military operations within these countries need to take measures to prevent infection and further spread the disease by deployed troops. Our experience showed an acceptable safety profile of WC/rBS, especially if doses are separated by 30 days. This vaccine provides a fairly high protection rate for the first 6 months, but its influence on the rate of asymptomatic carriers is uncertain. Therefore, additional post-deployment stool cultures seem an appropriate surveillance tool for returnees to non-industrialized countries such as Chile.

1641

NOVEL IMMUNOLOGICAL SIGNALS FOR DIAGNOSING ACUTE TYPHOID FEVER

Nga Tran Vu Thieu[1], Elizabeth Klemm[2], Gordon Dougan[2], Sabina Dongol[3], Buddha Basnyat[3], Stephen Baker[1]

[1]Oxford University Clinical Research Unit, Vietnam, Ho Chi Minh, Vietnam, [2]Sanger Institute, Cambridge, United Kingdom, [3]Oxford University Clinical Research Unit, Nepal, Kathmandu, Nepal

Salmonella Typhi is the causative agent of typhoid. There are a variety of methods for diagnosing typhoid; all lack sensitivity and specificity. Serological assays targeting LPS and/or flagella are commonly used settings to diagnose typhoid. However, cross-reactivity makes these antigens unsuitable for assessing disease burden and diagnosing acute illness. There have been few studies focusing on studying the immunological response to specific *Salmonella* antigens. Using an *S.*Typhi antigen array we identified a number of antigens that elicited a significant IgM or IgG response greater than that of uninfected controls. We selected the best twelve (6 IgM and 6 IgG) antigens that gave a differential response between the groups for further investigation. Firstly, we expressed and purified these antigens and immunized mice to study the ability of the mouse serum to stimulate bacterial killing. All of the serum samples from immunized mice were able to stimulate a bactericidal response against *S.* Typhi, inhibiting >80% of the bacterial growth over three hours.

Additionally, eight of the serum samples had a bactericidal effect on *S.* Paratyphi A. None of the serum from immunized mice demonstrated any bactericidal activity against *S.* Typhimurium. We further investigated this bactericidal response by repeating the experiments with gene knockout strains. There was a marked reduction in bactericidal activity with the immunized mouse serum on the strains of *S.*Typhi strains harboring the respective specific antigen encoding gene knockout. Specifically, serum from mice immunized with CdtB (subunit of typhoid toxin) demonstrated a significant reduction bactericidal activity against a *cdtB S.*Typhi mutant in comparison to wild-type *S.*Typhi. These preliminary data show that *S.*Typhi 12 antigens appear to stimulate specific and strong immunological responses in patients with acute typhoid. Furthermore, we suggest these antigens may be candidate diagnostics or subunit vaccines, and provide a novel insight for further understanding of host immune responses induced during acute typhoid.

1642

THE SPATIOTEMPORAL DYNAMICS AND PHYLOGENETICS OF *SALMONELLA PARATYPHI* A IN KATHMANDU, NEPAL

Duy Pham, Stephen Baker

Oxford University Clinical Research Unit, Ho Chi Minh, Vietnam

Enteric fever is a life threatening systemic disease caused by the bacteria *Salmonella* Typhi and *Salmonella* Paratyphi A, B, and C. Typhi is the major agent of enteric fever, yet Paratyphi A is emerging at an unprecedented rate. In our location in Patan Hospital, Kathmandu, standardised blood culture surveillance over the last ten years has shown annual increases in the proportions of individuals with Paratyphi A. More than two thirds of the culture confirmed enteric fever cases are now caused by this serovar. Both Typhi and Paratyphi A are systemic pathogens that induce indistinguishable syndromes. However, they exhibit contrary epidemiologies, different geographical distributions, and different propensities to develop resistance to antimicrobials. Additionally, they are genetically and phenotypically distinct, having gone through a lengthy process of convergent evolution to cause an identical disease. To understand the emergence and the molecular epidemiology of Paratyphi A in our setting we genome sequenced 182 organisms isolated from patients with acute or relapsed enteric fever, and a number isolated from the gallbladder of asymptomatic carriers. Performing phylogenetic analysis and evolutionary reconstruction we find that Paratyphi A is isolated and genetically distinct from Typhi. Our data show that Paratyphi A has been through a major clonal expansion in Kathmandu, apparently driven by resistance to fluoroquinlones and increased virulence through multi-copy effector proteins. Contemporary isolates of Paratyphi A have been introduced from other parts of Asia and induced a clonal replacement of the native strain(s). We surmise that Typhi and Paratyphi A have a dissimilar epidemiology in Nepal with Paratyphi A associated with spatiotemporal outbreaks and person-to-person transmission. Our study is the first to tackle the local phylogenetics and spatiotempral dynamics of Paratyphi A. Our work outlines new perspectives on enteric fever and will pave the way for future genomic epidemiology investigations of this important emergent pathogen.

1643

INCIDENCE AND ETIOLOGY OF INFECTIOUS DIARRHEA FROM A MULTIYEAR FACILITY-BASED SURVEILLANCE SYSTEM IN GUATEMALA, 2008-2012

Wences Arvelo[1], Aron Hall[1], Olga Henao[1], Beatriz Lopez[2], Chris Bernart[2], Juan C. Moir[3], Lissette Reyes[3], Susan P. Montgomery[1], Oliver Morgan[1], Alejandra Estevez[2], Maria R. Lopez[2], Michele B. Parsons[1], Jan Vinje[1], Nicole Gregoricus[1], Jon Gentsch[1], Umesh Parashar[1], Eric Mintz[1], Joe Bryan[1], Kim A. Lindblade[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Universidad del Valle de Guatemala, Guatemala City, Guatemala, [3]Ministry of Health and Welfare, Guatemala City, Guatemala

Diarrheal diseases are a major cause of morbidity and mortality worldwide, yet data on etiology and population-level incidence in developing countries are limited. Diarrhea surveillance was conducted at two hospitals and 10 ambulatory clinics in the departments of Santa Rosa and Quetzaltenango in Guatemala. A case was defined as a person of any age having ≥3 loose stools in a 24-hour period and was admitted or presented to the surveillance facilities. Epidemiologic and clinical data were collected. Stool specimens were tested for bacterial, parasitic, and viral enteric pathogens. Estimated incidence rates were calculated by adjusting for healthcare seeking behaviors, based on results from a survey in the surveillance area assessing the proportion of those with reported diarrhea who visited a surveillance facility during their illness. From November 2008 to December 2012 there were 5,331 diarrhea cases. The weighted estimated community incidence averaged 659 diarrhea cases per 10,000 persons per year during the four year period. The estimated incidence was highest among children aged <5 years, averaging 1,584 cases per 10,000 children per year, while among those aged ≥5 years the estimated incidence averaged 311 cases per 10,000 persons. From 2008-2009 samples from 1,401 (26%) cases were tested for all the pathogens of interest. Among these, 846 (60%) specimens were from children aged <5 years in whom a virus was identified in 211 (25%) patients; of which, 178 (84%) tested positive for norovirus and 101 (48%) for rotavirus, including co-infections. Among the 555 patients aged ≥5 years the most frequently identified etiology was bacterial with 134 (24%) cases. Diarrheagenic *Escherichia coli* was detected in 94 (70%) cases, *Shigella* spp in 31 (23%), *Campylobacter* spp in 5 (4%), and *Salmonella* spp in 4 (3%) cases. Identification of parasites was low (24 cases, 9%), and most cases were among those aged 5-19 years. These data demonstrate a substantial burden of viral and bacterial diarrheal diseases in Guatemala, which may help guide public health policies aimed at reducing the burden of illness and death due to diarrhea.

1644

SALMONELLA ENTERICA SEROVAR ENTERITIDIS OUTBREAK AT A LODGE IN MOKOPANE, LIMPOPO PROVINCE, JANUARY 2014

Akhona N. Tshangela

National Institute for Communicable Diseases, Johannesburg, South Africa

Salmonella enterica serovar Enteritidis is a leading cause of foodborne disease worldwide, but there is little data available in South Africa. Asymptomatic food-handlers have also been associated as a source of infection. We investigated the aetiology of an acute gastroenteritis outbreak among persons staying at a lodge in Limpopo Province, South Africa in January 2014. A retrospective cohort analysis was used to determine the risks of illness associated with consuming foods and/or beverages at the lodge. The at-risk population were contacted to complete a standard questionnaire related to food and beverages consumed at the lodge, symptoms of illness, visits to healthcare facilities and specimen submission for pathogen testing. Food and water samples were tested, as well as completion of an environmental assessment questionnaire by staff and external caterers. The data was categorised and STATA version 12 was used for multivariate analyses. A total of 73 ill persons, including

3 laboratory-confirmed infections, were identified: 69/109 (63%) of the selected cohort were seen at health facilities. Of the at-risk population 87% (109/124) completed the standard questionnaire: 66 cases of gastrointestinal illness and 43 healthy individuals were identified, with a corresponding attack rate of 61%. Most of the cases were females (86%, n=57) with a mean age of 33 years (S.D=7.1), and 36% (n=24) of the cases were hospitalised. Epidemiological data suggested a point source outbreak with no further transmission. Statistical analysis of survey data indicated consumption of diluted fruit juice (from concentrate) adjusted by other food and beverage items, presented a risk ratio of 1.5 (95% CI, 1.1-1.8, p=0.032). Environmental analysis indicated increased risks for cross-contamination. The outbreak was possibly due to cross-contamination of food/ beverages prepared in the lodge kitchen, and fruit juice consumption was the main exposure associated with ill cases. Feedback on food safety and hygiene practices to prevent cross-contamination at the kitchen lodge were provided.

1645

FECAL SHEDDING OF ROTAVIRUS FOLLOWING ROTARIX VACCINATION IN A COHORT OF BOLIVIAN INFANTS

Rachel M. Burke[1], Paulina A. Rebolledo[2], Anna Fabiszewski de Aceituno[1], Lucia Inchauste[3], Volga Iñiguez[3], Rita Revollo[4], Parminder Suchdev[1], Juan Leon[1]

[1]Rollins School of Public Health, Emory University, Atlanta, GA, United States, [2]Emory University School of Medicine, Atlanta, GA, United States, [3]Universidad Mayor de San Andrés, La Paz, Plurinational State of Bolivia, [4]Hospital Municipal Los Andes, El Alto, Plurinational State of Bolivia

Worldwide children under the age of five suffer from an estimated 138 million cases of rotavirus (RV) diarrhea per year. Currently available oral RV vaccines, such as the human attenuated monovalent vaccine Rotarix, increase RV-specific immunity and prevent severe diarrhea. Following vaccination, RV vaccine strains are expected to replicate in the intestinal tract and be excreted in feces. Fecal shedding of vaccine strains could lead to horizontal transmission of such strains and theoretically afford protection to unvaccinated contacts, which would be of substantial benefit in impoverished countries such as Bolivia where rates of immunization are suboptimal or RV associated morbidity is high. With the ongoing rollout of RV vaccines in low-middle income countries, it is imperative to evaluate and describe RV vaccine-virus shedding in vaccine recipients. Between June 2013 and April 2014 a birth cohort of 462 Bolivian infants were enrolled and followed through receipt of Rotarix to quantify the prevalence of fecal shedding within 7 days of the initial vaccine dose (at approximately 2 months of age). Shedding was assessed using enzyme immunoassay (EIA) and in a subset of infants real-time reverse transcription-polymerase chain reaction (RT-PCR) was used for confirmation. Bivariate logistic regression was used to identify potential predictors of shedding. The mean age at the initial (pre-vaccine) visit was 35 days (SD 8 days), and 55% of the infants were male. Baseline prevalence of stunting was 20%, prevalence of preterm birth was 19%, and prevalence of low birth weight was 7%. The mean maternal age was 26 years (SD 6 years), and 61% of mothers had completed secondary school. Shedding was identified in 6 infants out of 305 tested (2%). All before-mentioned predictors were tested, but no significant associations with shedding were detected (likely due to lack of power). Initial hypotheses for the low shedding rate include the high prevalence of exclusive breastfeeding, role of maternal antibodies, and circulating RV in the community; these and other potential explanations will be addressed in future investigation.

1646

INTESTINAL INFLAMMATION AND ALTERED BONE METABOLISM IN PERUVIAN INFANTS

Gwenyth Lee, Pablo Peñataro Yori, Margaret Kosek

Johns Hopkins School of Public Health, Baltimore, MD, United States

Low-grade inflammation resulting from frequent enteric episodes and resulting enteropathy is believed to be a causes of growth faltering among children in the developing world. In order to clarify these associations, markers of bone metabolism may be beneficial, as they are more dynamic over short intervals than anthropometry. In order to test this hypothesis, urine samples from 139 6-month olds and 64 15-month old Peruvian Amazonian infants, and serum samples from the same children at 7 and 15 months, were tested for markers of bone collagen formation (plasma osteocalcin (OC)) and resorption (urinary deoxypyridinoline (DPD)/ creatinine (Cr)), as well as the acute phase protein plasma alpha-1-acid glycoprotein (AGP) and the plasma cytokines TNF-α, interleukin-6 (IL-6), and IL-1β , and fecal alpha-1-antitrypsin (AAT), a marker of intestinal inflammation. The mean plasma osteocalcin at seven months was 41.2µg/L in boys and 36.4µg/L in girls, by 15 month this had fallen to and 34.1 µg/L and 25.2 µg/L, respectively. The mean DPD/Cr at 6 months was 58.9 nmol/mmol Cr and 65.1 nmol/mmol Cr for boys and girls, respectively, and at 15 months, 68.3 nmol/mmol Cr and 52.1 nmol/mmol Cr for boys and girls, respectively. The mean length-for-age Z score (LAZ) at 6 months was -1.3 and 19.4% were stunted (LAZ< -2). By15 months of age the mean LAZ was -2.0 and 53.1% were stunted. The mean weight-for-length Z-score (WLZ) was 0.9 at 6 months , 0.8 at 7 months, and 0.4 at 15 months. 76.4% of children at 6mo, and 69.1% at 15 month were classified as having subclinical inflammation, defined by AGP> 1g/L. Bone collagen metabolism was altered by nutritional status, as OC was inversely associated with both length-for-age and weight-for-length, and DPD/Cr was positively associated with length-for-age. Correspondingly, the ratio of OC/DPD was highest among shorter and leaner children. After adjusting for anthropometric status, age, and gender, OC and the ratio of OC/DPD were both inversely associated with fecal AAT, but not with plasma AGP, tnf-alpha, IL-6, or IL-1beta. Our findings suggest that bone metabolism is suppressed among children with chronic intestinal inflammation.

1647

EPIDEMIOLOGY OF SELF-REPORTED HEALTH EVENTS AMONG DEPLOYED U.S. MILITARY PERSONNEL

Ruvani M. Chandrasekera[1], Mark S. Riddle[2], Chad K. Porter[2]

[1]Cherokee Nation Technology Solutions, Silver Spring, MD, United States, [2]Naval Medical Research Center, Silver Spring, MD, United States

Deployed military personnel are at risk of experiencing numerousadverse health events to include diarrhea, vomiting, fever and muscle aches while on humanitarian and war-fighting operations. These symptoms can negatively impact servicemember effectiveness and epidemiologic studies are needed to describe their frequency and associated risk factors; however, extensive prospective epidemiological studies in operational environments are challenging. We utilized self-reported data collected through the Post-Deployment Health Assessment from individuals following operational deployments and compared prevalence estimates across region/country of deployment, multiple demographic characteristics as well as pre-existing medical conditions. Univariate and multivariate logistic regression methods were also used to identify unique risk factors while controlling for important covariates. Of 21,982 subjects, the top five self-reported symptoms included back pain (15.9%), feeling tired/problems sleeping (15.7%), swollen, stiff or painful joints (13.6%), diarrhea (12.7%) and muscle aches (11.1%). Among those reporting diarrhea/vomiting, a high proportion were assigned to limited duty/bed rest (36% and 54%, respectively). Further data will be presented on the estimated level of care required for reported adverse health events, and potential risk factors for increased self-report of diarrhea and vomiting. While these results are limited to self-report, the data support prior studies highlighting diarrhea

and vomiting as significant causes of morbidity and troop down-time during operational deployment. Furthermore, recent studies highlighting the link between acute gastroenteritis and long-term adverse health outcomes raise the importance of these common, deployment-related health events. Continued evaluation of primary prevention strategies is needed.

1648

REGULATION OF SMALL RHO GTPASES IN REDUCING INTESTINAL CELLS MIGRATION INDUCED BY STRAINS OF ENTEROPATHOGENIC *ESCHERICHIA COLI*

Paloma A. Cavalcante[1], Mara M. Prata[1], Antônio V. Silva[1], Josiane S. Quetz[1], Alexandre B. Havt[1], Ila F. Lima[1], Eunice B. Carvalho[1], Pedro H. Quintela[1], Tamara S. Rodrigues[1], Rafael H. Gonzaléz[1], Rosemeyre S. Freire[1], Sean R. Moore[2], James Nataro[3], Richard L. Guerrant[3], **Aldo A. Lima**[1]

[1]Federal University of Ceara, Fortaleza, Brazil, [2]University of Cincinnati, Cincinnati, OH, United States, [3]University of Virginia, Charlottesville, VA, United States

Intestinal epithelial migration provides an important early response when intestinal pathogens damage the host intestinal barrier. Small Rho GTPases Rac1, RhoA and Cdc42 are important regulators of this migratory response. We sought to test the hypothesis that EPEC impairs intestinal epithelial migration *in vitro* via small Rho GTPase-dependent mechanisms. Methods: We investigated the effects of EPEC strain E2348/69, EPEC strain LDI001 (isolated from a malnourished child), and commensal E. coli HS on IEC-6 cell migration; as well as on the regulation of transcription and gene expression of small Rho GTPases by qPCR and confocal immunofluorescent microscopy, respectively. Results: We observed a significant reduction in IEC-6 cell migration for all E. coli strains tested. However, pathogenic EPEC strains reduced migration to a greater degree than E. coli HS. Only EPEC E2348/69 induced significant cellular necrosis. Gene analyses of small Rho GTPases revealed an increase in rac1 transcription in EPEC LDI001 infected cells and upregulation of rhoA transcription following infection with all strains. Confocal imaging showed an increase in Rac1 expression and decrease in RhoA in response EPEC LDI001 infection. We further observed increased expression of Cdc42 in all infected groups. Conclusions: The results suggest differential suppression of migration and co-regulation of small Rho GTPases in response to infection with enteropathogenic vs. commensal E. coli strains. These *in vitro* data corroborate an emerging *in vivo* and clinical understanding of the pathobiology of this infection and its associations with malnutrition and intestinal barrier injury.

1649

INCORPORATING HERD PROTECTION INTO A COST-BENEFIT ANALYSIS OF TYPHOID FEVER VACCINE INTERVENTIONS

Marina Antillon, Virginia E. Pitzer

Yale University, New Haven, CT, United States

Recent studies have shed light on the costs and savings of typhoid fever vaccination campaigns, but no studies been able to quantify the potential economic savings attributable to realistic estimates of herd protection, the protection conferred to individuals who did not receive the vaccine. In light of numerous budget constraints in resource-poor settings, it is necessary to more accurately estimate the indirect effects of vaccine campaigns as well as the additional potential financing mechanisms that may lower the costs of vaccination per DALY saved. Field studies are now available to assess the burden of typhoid fever and the possible impact of Vi-polysaccharide and Vi-conjugate vaccines. Using mathematical models for typhoid transmission, we can quantify the indirect protection of vaccines under different vaccine strategies. Moreover, surveys on the private demand for these vaccines in South Asian contexts, where the disease is endemic, inform calculations on the optimal vaccine subsidies necessary to achieve a desired level of vaccine coverage while recuperating some of the programmatic costs through user fees. With that in mind, we will show that past estimates of the costs of vaccination per life year saved have been overestimated when compared to an analysis that takes into account accurate estimates of indirect vaccine protection at different pricing levels.

1650

CHARACTERIZING THE REGIONAL AND GLOBAL DISTRIBUTION AND BURDEN OF CHOLERA

Sean M. Moore, Andrew A. Azman, Carla E. Zelaya, Justin Lessler

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

There are an estimated 2.8 million cholera cases per year globally, but the majority of these cases are not detected or reported. Because of inadequate surveillance and reporting the global distribution of cholera risk and its public health burden are poorly known. Previous attempts to determine the global burden of cholera have relied on a limited number of case studies that do not capture the broad range of settings and environmental conditions where cholera occurs. Here we assemble a database of cholera surveillance and incidence reports from a variety of government, scientific, and non-governmental agency sources, with a particular focus on sub-Saharan Africa where a majority of cholera cases have been reported in the past several decades but where the distribution of risk and burden is still poorly understood. We will then use a formal modelling framework to associate cholera transmission with environmental and socioeconomic variables and map the global distribution of cholera risk and incidence. As a preliminary analysis we developed cholera incidence maps for the West African country of Guinea-Bissau using a hierarchical Bayesian framework with cholera data at spatial scales ranging from neighborhood-level incidence in the capital city of Bissau to country-level reports. Cholera incidence from 1986-2009 was highest in the island and coastal districts (including Bissau city). Incidence did not change significantly between the 1990s (which included outbreaks in 1994 and 1996-1997) and the 2000s (which included outbreaks in 2005 and 2008), except for an increase of 26-280% in the islands of the Bijagos Archipelago. Improving our understanding of the spatial distribution of cholera in Guinea-Bissau and associating incidence with climate, environmental and socioeconomic factors will provide a basis for planning public health preventions to reduce cholera transmission in this region.

1651

BIOMARKERS OF ENVIRONMENTAL ENTEROPATHY FOR POSITIVELY ASSOCIATED WITH TOXIN-SPECIFIC B AND T CELL RESPONSES TO AN ORAL CHOLERA VACCINE IN BANGLADESHI CHILDREN

Daniel T. Leung[1], Taufiqur R. Rahman[2], Muhammad Ikhtear Uddin[2], Naoshin S. Nishat[2], Mohammad Rubel Hoq[2], Amit Saha[2], Jason B. Harris[1], Stephen B. Calderwood[1], Firdausi Qadri[2], Edward T. Ryan[1]

[1]Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Environmental enteropathy (EE) is a poorly understood condition that refers to chronic alterations in intestinal permeability, absorption, and inflammation that affects young children in resource limited settings. While EE has been linked to suboptimal oral vaccine performance in children, the causative immunological mechanisms are poorly defined. The objective of this study was to determine how markers of enteropathy are associated with immune responses to an oral cholera vaccine (OCV). We collected blood and stool from 40 Bangladeshi children who received two doses of an OCV given 14 days apart. We measured five EE markers, including stool myeloperoxide (MPO), a marker of intestinal inflammation, stool alpha anti-trypsin (AAT), a marker of intestinal absorption, as well as plasma endotoxin core antibody (EndoCab), plasma intestinal fatty acid binding protein (iFABP), and plasma soluble CD14 (sCD14), all markers of microbial translocation. We measured cholera toxin (CT)- and lipopolysaccharide

(LPS)-specific antibody responses by ELISA, toxin-specific memory T cell responses by flow cytometry following whole blood culture, and T cell culture cytokines by Luminex array. Using a multiple linear regression model, we assessed each vaccine-associated immune response outcome as separate dependent variables, and used log-transformed EE marker measurements, along with gender, blood group, and age, as independent variables. We found stool MPO to be a positive predictor of antibody responses to CT, plasma iFABP a positive predictor of gut-homing memory T cell responses, and stool AAT a positive predictor of interferon-gamma responses. No marker predicted antibody responses to LPS. Variance inflation factor for all independent variables were < 1.6, suggesting no multi-collinearity. In summary, we demonstrate that biomarkers of environmental enteropathy are positive predictors of toxin-specific immune responses to an OCV.

1652

LEPROSY IN NIGERIA (2008-2012): AN EVALUATION OF THE NATIONAL SURVEILLANCE SYSTEM

Aishatu Abubakar Sadiq[1], Joshua A. Obasanya[2], Patrick Nguku[1], Gabrielle Poggensse[1], Kabir Sabitu[3]

[1]Nigeria Field Epidemiology and Laboratory Training Programme, Abuja, Nigeria, [2]National Tuberculosis and Leprosy Control Programme, Abuja, Nigeria, [3]Department of Community Medicine, Ahmadu Bello University, Zaria, Nigeria

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* an acid fast, rod shaped bacillus. It was historically associated with social isolation and psychological consequences. Although eradicated in most countries, ongoing transmission in African and Asian countries requires enhanced surveillance and monitoring. In 2012 Nigeria reported 3805 cases and no deaths. We evaluated the national Leprosy surveillance system to assess its usefulness and attributes. Centers for Disease Control(CDC) guidelines for evaluating surveillance systems were used. Ten Stakeholders from partner agencies, national and state level were interviewed using structured questionnaires. Secondary data (2008-2012) was abstracted from surveillance data submitted to the National Tuberculosis and Leprosy Control Program. Laboratory diagnostic capacity was also assessed.Out of 20,623 cases reported, 3805(18.45%) were received in 2012, 3623(17.57%) in 2011,3913(18.97%) in 2010,4383(21.25%) in 2009 and 4899 (23.76%) in 2008.The prevalence reduced from 4.3x10-3 /10,000 population in 2008 to 1.2x10-4/10,000 population in 2011.Child proportion was 10.7% in 2008 and 8.0% in 2011.Grade 2 disability rate ranged between 11.7% and 13.4%.Being an adult (OR=1.97; 95% CI =1.37-2.82) and male (OR=1.25; 95% CI=1.05-1.48) was found associated with Multibacillary Leprosy. Residents of the Northwest region (OR=0.73; 95%CI=0.59-0.90) were less likely to have Multibacillary Leprosy. The system is active and rated simple by 17(85%) of respondents. Review of weekly,quarterly and monthly reporting forms and records at the national level showed timeliness. All suspected cases (100%) were laboratory tested within 24hours of presentation.There is a high laboratory turnover of staff and low numbers of personnel trained in laboratory diagnosis.The system is fully integrated with surveillance of Tuberculosis at all levels. Leprosy transmission is still ongoing and the WHO elimination target (<1 case/10,000 population) has been achieved at the national level. Pockets of leprosy exist in the northwest region.We recommend intensification of surveillance activities in all zones,improvement of Laboratory diagnostic capacity and recruitment of additional personnel. The system is acceptable,flexible, simple and timely. The system is meeting its purpose and promotes the achievement of the global elimination target for leprosy.

1653

OPTIMIZING THE CHEMOTHERAPEUTIC APPROACH FOR THE TREATMENT OF BURULI ULCER: POSSIBLE OPTIONS AND RESEARCH NEEDS

Charitha Gowda[1], Martina Casenghi[2], Julien Potet[2], Eric Comte[3], Jennifer Cohn[2]

[1]University of Pennsylvania, Philadelphia, PA, United States, [2]Medecins Sans Frontieres Access Campaign, Geneva, Switzerland, [3]Medecins Sans Frontieres, Geneva, Switzerland

Buruli ulcer (BU) is a serious necrotising skin infection caused by the environmental pathogen *Mycobacterium ulcerans*, and represents the third most common mycobacterial infection. Current WHO treatment has simplified the delivery of care for BU by recommending that early, limited lesions be treated with antibiotics alone. However, these recommendations still present significant disadvantages including the use of injectable agents. There is an urgent need to identify alternative oral regimens that are effective, short-course, have few drug-drug interactions with antiretrovirals and can be used in children. We performed a systematic literature review for publications focused on chemotherapy for *M. ulcerans* infection. We searched PubMed, EMBASE, Scopus, WHO Global Index Medicus, CAB Abstracts and Cochrane Library with standardized search terms, screened abstracts submitted to international conferences and assessed the ClinicalTrials.gov registry. While there were no restrictions by publication date or type, only articles in English, French and Italian as of December 31, 2012 were included. We included *in vitro* and clinical studies, with the primary outcomes of clinical resolution of the ulcer without surgery (clinical studies) and assessment of *in vitro* activity (pre-clinical data). We excluded all studies without microbiological confirmation of *M. ulcerans* infection. 49 clinical studies including 6 RCTs, 14 observational cohorts, 11 case series and 18 case reports were identified. Various drugs and drug combinations were identified as having clinical efficacy against BU disease in resource-poor settings. In particular, the combinations of clarithromycin+rifampin and clarithromycin+fluoroquinolones demonstrated good efficacy and safety. *In vitro* data reveal a number of promising compounds. Although recent studies indicate that a fully oral regimen for BU may be as equally effective as regimens containing aminoglycosides, further research is needed to identify and evaluate new treatments. The anti-tuberculosis research & development (R&D) pipeline represents a potentially rich source of novel compounds for BU treatment. We propose an R&D agenda aimed at delivering new, more efficacious and readily implementable treatments against Buruli ulcer in resource-limited settings.

1654

PERSISTENT GUT MICROBIOTA IMMATURITY IN MALNOURISHED BANGLADESHI CHILDREN

Sathish Subramanian[1], Sayeeda Huq[2], Tanya Yatsunenko[3], Rashidul Haque[2], Mustafa Mahfuz[2], Mohammed A. Alam[2], Amber Benezra[4], Joseph DeStefano[3], Martin F. Meier[3], Brian D. Muegge[3], Michael J. Barratt[3], Laura G. VanArendonk[3], Qunyuan Zhang[5], Michael A. Province[5], William A. Petri[6], Tahmeed Ahmed[2], Jeffrey I. Gordon[3]

[1]Center for Genome Sciences and Systems Biology, Washington University, St. Louis, MO, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]Center for Genome Sciences and Systems Biology, Washington University, St. Louis, MO, United States, [4]Department of Anthropology, New School for Social Research, New York, NY, United States, [5]Division of Statistical Genomics, Washington University, St. Louis, MO, United States, [6]Departments of Medicine, Microbiology and Pathology, University of Virginia School of Medicine, Charlottesville, VA, United States

Therapeutic food interventions have reduced mortality in children with severe acute malnutrition (SAM) but incomplete restoration of healthy growth remains a major problem. The relationships between the type of

nutritional intervention, the gut microbiota, and therapeutic responses are unclear. In the current study, bacterial species whose proportional representation define a healthy gut microbiota as it assembles during the first two postnatal years were identified by applying a machine-learning-based approach to 16S rRNA datasets generated from monthly fecal samples obtained from a birth-cohort of children, living in an urban slum of Dhaka, Bangladesh, who exhibited consistently healthy growth. These age-discriminatory bacterial species were incorporated into a model that computes a 'relative microbiota maturity index' and 'microbiota-for-age Z-score' that compare development (defined here as maturation) of a child's fecal microbiota relative to healthy children of similar chronologic age. The model was applied to twins and triplets (to test for associations of these indices with genetic and environmental factors including diarrhea), children with SAM enrolled in a randomized trial of two food interventions, and children with moderate acute malnutrition. Our results indicate that SAM is associated with significant relative microbiota immaturity that is only partially ameliorated following two widely used nutritional interventions. Immaturity is also evident in less severe forms of malnutrition and correlates with anthropometric measurements. Microbiota maturity indices provide a microbial measure of human postnatal development, a way of classifying malnourished states, and a parameter for judging therapeutic efficacy. More prolonged interventions with existing or new therapeutic foods and/or addition of gut microbes may be needed to achieve enduring repair of gut microbiota immaturity in childhood malnutrition and improve clinical outcomes.

1655

CONTRIBUTION OF THE COMMUNITY HEALTH VOLUNTEERS IN THE CONTROL OF BURULI ULCER IN BENIN

Yves T. Barogui[1], Ghislain E. Sopoh[2], Roch C. Johnson[3], Janine de Zeeuw[4], Ange D. Dossou[2], Jean G. Houezo[2], Annick Chauty[5], Julia Aguiar[6], Didier Agossadou[7], Patrick A. Edorh[3], Kingsley Asiedu[8], Tjip S. van der Werf[4], Ymkje Stienstra[4]

[1]Centre de Dépistage et de Traitement de l'Ulcère de Buruli de Lalo, Lalo, Benin, [2]Centre de Dépistage et de Traitement de l'Ulcère de Buruli d'Allada, Allada, Benin, [3]Université d'Abomey-Calavi, Abomey-Calavi, Benin, [4]University of Groningen, University Medical Center Groningen, Department of Internal Medicine/Infectious Diseases, Groningen, Netherlands, [5]Centre de Dépistage et de Traitement de l'Ulcère de Buruli de Pobè, Pobè, Benin, [6]Centre de Dépistage et de Traitement de l'Ulcère de Buruli de Zangnanado, Zangnanado, Benin, [7]Programme National de Lutte Contre la Lèpre et l'Ulcère de Buruli, Ministère de la Santé, Cotonou, Benin, [8]Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland

Buruli ulcer (BU) is a neglected tropical disease caused by *Mycobacterium ulcerans*. Usually BU begins as a painless nodule, plaque or edema, ultimately developing into an ulcer. Striking is the high number of patients presenting with ulcers in an advanced stage. Such late presentation will complicate treatment and have long term disabilities as a consequence. The disease is mainly endemic in West Africa. Strategy for control this disease is early detection using community village volunteers. This study aims to understand the contribution of different actors in the current referral pattern in Benin, the role of the different referral systems on the stage of disease at presentation in the hospital and the diagnostic precision of Buruli ulcer. Patient information of Buruli ulcer patients that reported to one of the four BU centers in Benin between January 2008 and December 2010 was collected using the WHO/BU01 forms. Information traced from these forms were general characteristics of the patient, the results of diagnostic tests, the presence of functional limitations at start of treatment, lesion size, patient delay and the referral system. The role of the different referral systems on the stage of disease at presentation in the hospital was analyzed by a logistic regression analysis. About a quarter of the patients (26.5%) were referred to the hospital by the community health volunteers. In our data, community health volunteers seemed to refer patients more frequently in an earlier stage of disease but after adjustment for the health center, this effect could not be seen anymore. The Polymerase Chain Reaction (PCR) for IS2404 positivity rate among patients referred by the community health volunteers was not systematically lower than in patients referred by other systems. This study clarifies the role played by community health volunteers. It highlights that in Bénin, the community health volunteers are an important link in the control of BU.

1656

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING FLORAL EXTRACT OF *GOMPHRENA GLOBOSA* AND ITS ANTIMICROBIAL ACTIVITY AGAINST MULTI DRUG RESISTANT BACTERIA

Kovendan Kalimuthu[1], Arokiyaraj Selvaraj[2], Murugan Kadarkarai[1], Vincent Savariar[3]

[1]Bharathiar University, Coimbatore, India, [2]National Institute of Animal Science, Suwon, Republic of Korea, [3]Loyola College, Chennai, India

Antibiotic resistance has become a major clinical and public health problem within the lifetime of most people living today. Silver nanoparticles (AgNPs) are well known biocidal substances that can be incorporated as antimicrobial agents in phar¬macology, veterinary medicine, implants, wound dressings, and topical ointments. AgNPs were also found to exhibit antimicrobial activities. The present work reports one step ecofriendly method for the synthesis of AgNPs using *Gomphrena globosa* and its antibacterial effects against drug resistant bacteria. In the present results, AgNPs was characterized by ultraviolet-visible spectroscopy, X-ray diffraction spectroscopy, Transmission electron microscopy and particle size analyzer. The synthesized particles were found to be spherical in shape and sizes ranged between 55-60 nm. Further energy-dispersive X-ray spectroscopy confirmed the presence of silver. Furthermore these green synthesized AgNPs were found to show significant antimicrobial effect against the drug resistant Methicillin resistant *Staphylococcus aureus*, cipro flaxin resistant *Escherichia coli*, and carbapenem resistant *Acetobacter baumanii.* This outcome may pave a way for using floral extract of the AgNPs a drug carrier system to cure bacterial diseases.

1657

FREQUENCY OF VIRULENCE GENOTYPES IN *ESCHERICHIA COLI* STRAINS ISOLATED FROM URINARY TRACT INFECTIONS OF MEXICAN PATIENTS

Gloria Luz Paniagua Contreras[1], Bianca Hidalgo Hernández[1], Selene Acebo Falcón[1], Eric Monroy Pérez[1], Raymundo Rodríguez Moctezuma[2], Pablo Domínguez Trejo[2], Sergio Vaca[1]

[1]Universidad Nacional Autónoma de México, Tlalnepantla, Edo. de México, Mexico, [2]Instituto Mexicano del Seguro Social, Tlalnepantla, Edo. de México, Mexico

Uropathogenic *Escherichia coli* (UPEC) is responsible for a high percentage of urinary tract infections (UTIs) worldwide, among them cystitis and pyelonephritis. The purpose of this work was to determine the frequency of the genotypes: pap (pilus associated with pyelonephritis), papGI and papGII (pilus associated with pyelonephritis GI and GII), hlyA (haemolysin), afa (afimbrial adhesin), sfa (S fimbriae), iron (iron), iuc (aerobactin), cnf (cytotoxic necrotizing factor), astA (enteroaggregative toxin), sap (she pathogenicity island marker) and set (Shigella enterotoxin 1) in a group of E. coli strains isolated from Mexican patients suffering UTIs. E. coli strains were identified by biochemical tests and by PCR amplification of 16S rRNA. Genes pap, papGI, papGII, hlyA, afa, sfa, iron, iuc, cnf, astA, sap and set were detected by multiplex PCR and by end-point PCR. Urine samples of 100 urinary tract infected patients were microbiologically analyzed. E. coli was identified in 60% of the samples (n=60). Of the E. coli strains, 48.3% (n=29) carried the set gene; 41.6% (n= 25) carried papGI; 26.6% (n=16) carried hlyA; 23.3% (n=14) carried afa; 21.6% (n=13) carried pap; 20% (n=12) carried papGII; 18.3% (n=11) carried sfa; 16.6% (n=10) carried iron; 13.3% (n=8) carried iuc; 6.6% (n=4) carried cnf1; 10% (n= 6) carried

astA and 5% (n=3) carried sap. The high frequency of the identified genes in the UPEC strains suggests that they are virulent and able to produce cystitis and/or pyelonephritis.

1658

COMMUNITY-ACQUIRED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* INTRODUCED INTO A BRAZILIAN PUBLIC PEDIATRIC CLINIC

Mariel A. Marlow[1], Claudete Araújo Cardoso[2], Marcos Gabriel Pinheiro[3], Ivete Martins Gomes[2], Cláudia Aguiar Barros[2], Lee W. Riley[1], Fábio Aguiar-Alves[3]

[1]University of California Berkeley, Berkeley, CA, United States, [2]School of Medicine, Fluminense Federal University, Niterói, Brazil, [3]Pathology Program, Laboratory of Molecular Epidemiology and Biotechnology, Rodolfo Albino University Laboratory, Fluminense Federal University, Niterói, Brazil

Low-income communities, health care professionals, and adults in frequent contact with children are all populations known to be at higher risk for methicillin-resistant *Staphylococcus aureus* (MRSA) colonization. The objective of our investigation was to determine risk factors for colonization with MRSA as well as staphylococcal cassette chromosome *mec* (SCC*mec*) genotypes among pediatric health care workers from a public hospital in Rio de Janeiro, Brazil. We collected nasal swabs and data on potential risk factors from 178 health care workers from all pediatric sectors from January to December 2012. Swab cultures were evaluated for antimicrobial resistance against Cefoxitin and Oxacillin, and resistance was confirmed by identifying the *mecA* gene by PCR. MRSA colonization was 5.1% (n=9/178). Logistic regression analysis showed being a nurse and working in an inpatient unit as potential risk factors for MRSA colonization (adjusted OR= 11.6, 95% CI 1.2 - 110.7 and 2.7, 95% CI 0.3 - 23.7, respectively). No non-work related risk factors were identified. Four of the nine isolates were found to be SCC*mec* type IV, the genotype most commonly associated with community-acquired MRSA (CA-MRSA). Five isolates were observed to be SCC*mec* type III, with four collected from nurses of various pediatric sectors in August/September alone. We then compared these isolates with nasal MRSA isolates sampled from children within 48 hours of being admitted to the pediatric ward from December 2011 to July 2012. Of 11 CA-MRSA isolates from 92 children sampled (12.0%), ten were found to be SCC*mec* IV, including two which preceded those collected from nurses in the same sector. Given SCC*mec* type III isolates appear to have circulated among various pediatric sectors, the introduction of the more virulent CA-MRSA genotype to the pediatric inpatient ward is of high concern. Prevention in the hospital setting may also depend on interventions at the community level in low-income settings.

1659

EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL)-PRODUCING *ENTEROBACTERIACEAE* ISOLATED FROM HEALTH CARE WORKERS' CELL PHONES IN FIVE PERUVIAN INTENSIVE CARE UNITS: ANTIBIOTIC RESISTANCE PATTERNS AND MOLECULAR CHARACTERIZATION

Steev Loyola[1], Luz Gutierrez[2], Gertrudis Horna[2], Jorge Osada[2], Andres G. Lescano[1], Kyle Petersen[1], Jesus Tamariz[2]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Universidad Peruana Cayetano Heredia, Lima, Peru

Technological advances allow rapid and efficient communication through cell phones; their unsupervised use in hospital environments however, is common. Outbreaks associated with ESBL-producing (Extended-spectrum beta-lactamase) *Enterobacteriaceae* have been widely described worldwide in intensive care units (ICU), we therefore hypothesized that cell phones might represent a source of these infections in Peru. We conducted a 5-month passive surveillance in 3 Pediatric ICUs and 2 Neonatology ICUs from 3 hospitals during February to June 2012. Swabs were collected from ICU health care workers' cell phones twice monthly. Microbiological identification and resistance patterns were determined by standard methods. Suspicious ESBL-producing bacteria in the antibiogram were confirmed by the phenotypic CLSI ESBL confirmatory test. We then performed PCR for detection of blaTEM, blaSHV and blaCTX-M genes to characterize the ESBL. A total of 114 employees were enrolled, 114 devices were tested, resulting in 491 samples. Twenty-two percent (25/114) of providers phones were colonized with nosocomial pathogens. Among 105 Enterobacteriaceae isolated, 33.3% (35/105) produced ESBLs, including 18.8% (9/48) of *Enterobacter spp.,* 55.9% (19/34) of *Escherichia coli*, 26.1% (6/15) of *Klebsiella pneumoniae* and 12.5% (1/8) of *Klebsiella oxytoca*. blaCTX-M was the most prevalent ESBL. ESBLs resulted in a phenotype of Multidrug resistance: Tobramycin resistance represented 74.3% of isolates, both Ciprofloxacin and Sulfamethoxazole/Trimethropim 68.6%, Gentamicin 62.9%, Amikacin 17.1%, and Cefoxitin 5.7%. No carbapenem resistance was detected and Metallo-beta-lactamases (MBLs), Carbapenemases and AmpC beta-lactamases were not identified in isolated *Enterobacteriaceae*. Our data suggest cell phones can be an important source of ESBL spread in developing world ICUs. Methods to prevent outbreaks and transmission of these bacteria from commonly used fomites, such as cell phones, are needed.

1660

THE ROLE OF *YERSINIA PESTIS* PRESENSIBILIZATION AND GENETIC BACKGROUND IN RESISTANCE OF BLACK RATS AGAINST PLAGUE

Voahangy Andrianaivoarimanana[1], Guillaume Soubigou[2], Minoarisoa Rajerison[1], Michel Ranjalahy[1], **Ronan Jambou**[2]

[1]Institut Pasteur de Madagascar, Antananarivo, Madagascar, [2]Institut Pasteur, Paris, France

In Madagascar, plague remained endemic in the rural areas of the central highlands with the black rat, *Rattus rattus*, as the main reservoir. The occurrence of plague cases from one season to another in the same villages is questioning. One hypothesis is that part of black rats can acquire resistance to plague allowing survival of rat populations and maintenance of infected fleas and thus of the disease. We previously described resistance of field black rats living in endemic area against plague whereas those from non endemic ones remained sensitive. This study investigates whether this resistance is genetically driven or if pre immunization of rats with *Yersinia pestis* could increase survival during subsequent infections. F1 generation of black rats, originating either from plague endemic or plague free zones were obtained and challenged once or twice with *Y. pestis*. Rat survival, antibody production and gene expression were compared during the acute phase of the disease. First inoculation of a low dose of *Y. pestis* greatly increases survival of rats against a lethal dose of the bacteria. This protection of primed rats can likely be related to anti-F1 IgG. Transcriptome analysis of leukocytes five days after infection revealed that genes related to inflammation but also to apoptosis were more expressed in rats from non endemic than in those from endemic ones. In the other hand, anti-apoptotic BcL2 pathway was highly expressed in resistant rats. This suggested that rat susceptibility to infection could be driven by apoptosis of activated leukocytes. Transmission of a resistance phenotype to the F1 generation for *R. rattus* from endemic plague foci is highlited. These findings highlight the role of low transmission of bacteria in a resistance phenotype of *R. rattus* to plague. A genetic component of this resistance is also supported This study provides critical insights on the role of *R. rattus* in plague persistence in Madagascar

1661

CHARACTERIZATION OF *ACINETOBACTER* ISOLATES POSITIVE FOR IMP CARBAPENAMASE FROM PERUVIAN HOSPITALS

Nathanael D. Reynolds

U.S. Naval Medical Research Unit - 6, Lima, Peru

Carbapenem antibiotics constitute the last resort in treating multi-drug resistant *Acinetobacter*infections typically acquired during hospitalization. The effectiveness of carbapenems against *Acinetobacter* has been compromised in the last decade largely due to the emergence of carbapenem-hydrolyzing metallo-beta-lactamases (MBL). Metallo-beta-lactamases include the enzymes IMP, VIM, NDM, GIM, and SIM and account for the majority of carbapenem resistance in Gram negative bacteria. While extensively characterized in East Asia and Europe, currently there exist limited reports on MBL resistance in *Acinetobacter* infections from South America. Surveillance of hospital acquired *Acinetobacter* infections in Lima and Iquitos, Peru between March 2011 and February 2013 identified 32 suspected nosocomial isolates (11 from Lima and 21 from Iquitos) and 20 clinical and environmental isolates associated within ICU outbreaks in Lima. Four of the 52 *Acinetobacter spp.* isolates were positive for *blaIMP*, consisting of one *A. baumannii*, one *A. haemolyticus*, and two *A. junii*. Phenotypic carbapenem resistance as defined by minimum inhibition concentrations to imipenem indicates resistance in one *A. junii* and *A. haemolyticus*. Whole genome sequencing of the *blaIMP*-positive isolates identified multiple resistance genes and characterized the IMP-16 variant in the *A. baumannii* and *A. junii* isolates and IMP-18 in the *A. haemolyticus* isolate. Phylogenetic analysis indicates no relativeness between the *A. junii* isolates or any IMP-positive isolates with previously identified the IMP-positive *Acinetobacter* isolates referenced by the NCBI Whole Genome Shotgun Database.

1662

CHARACTERIZATION OF CARBAPENEMASE-POSITIVE *PSEUDOMONAS AERUGINOSA* ISOLATES IN LIMA HOSPITALS

Drake H. Tilley

U.S. Naval Medical Research Unit - 6, Lima, Peru

Pseudomonas aeruginosa is an opportunistic pathogen that accounts for ten percent of all hospital-acquired infections. Therapeutic options for treatment of *P. aeruginosa* infections are increasingly limited due to inherent antimicrobial resistance an ability to acquire new mechanisms of resistance. Recently, carbapenemases have emerged as one of the major mechanisms of acquired resistance in *P. aeruginosa* and represent a significant clinical concern due to its ability to hydrolyze the majority of beta-lactam antibiotics. The most clinically relevant and widely disseminated carbapenemases include KPC, IMP, VIM and NDM. Currently, little information has been reported on the prevalence of carbapenemase genes present in *P. aeruginosa* isolates in Peru. To begin to define carbapenemase resistance in Peru, 124 carbapenem-resistant *Pseudomonas aeruginosa* isolates were collected from nosocomial and outbreak infections from three hospitals in Lima. Antibiotic resistance to beta-lactam was identified in 18 percent (23/124) of the *P. aeruginosa,* as defined by disk diffusion assay according to the CLSI guidelines. PCR was performed on all 124 isolates to detect the carbapenemase genes *blaKPC*, *blaIMP*, *blaVIM* and *blaNDM*. From the 124 isolates, 22 (18%) *P. aeruginosa* isolates were identified as the IMP-16 variant, one (1%) isolate positive for the VIM-2 varient, and none positive for KPC or NDM, being this 23 isolates extensively drug-resistant. Finally, in order to determine genomic-relatedness, the 23 isolates with carbapenemase genes were analyzed using rep-PCR on the Diversilab system. The 23 isolates clustered into four genomically distinct groups. Interestingly, several of IMP positive isolates clones were dispersed throughout different hospitals, suggesting possible clonal spread of IMP-16 positive *P. aeruginosa* between Lima hospitals. Also, the VIM-2 positive isolate demonstrate great than 95% homology with a reference to wild type strain reflecting the capacity of carbapenem-sensitive isolates to acquire to carbapenem resistant.

1663

GENETIC CHARACTERIZATION OF RECOVERED *BACILLUS* ISOLATES FROM THE ENVIRONMENTAL SURVEILLANCE SWABS BY SEQUENCING OF *GYR*B GENE: A PUBLIC HEALTH APPROACH

Irshad M. Sulaiman, Emily Jacobs, Steven Simpson, Khalil Kerdahi

Food and Drug Administration, Atlanta, GA, United States

The primary mission of FDA is to enforce the Food, Drug and Cosmetic Act and regulate food, drug and cosmetic products. To assess adulteration in these commodities, FDA uses presence of pathogenic microorganisms in manufacturing and distribution areas as one of the regulatory action criteria and to ensure that the firm is following good manufacturing practices. Further, FDA provides guidance to achieve the said goal by establishing an environmental monitoring program in these facilities. This study was conducted to verify the effectiveness of pathogen control in a pharmaceutical compounding facility located in Southeast region of United States. A total of 28 environmental swabs were collected from several locations of a compounding company premises. The swab samples were initially examined by conventional microbiologic protocols. Of these, several swabs were found positive for the presence of rod-shaped, gram-positive bacteria, *Bacillus*. It is a diverse group of bacteria, and some of its species are human-pathogenic that can cause range of infections including ear infections, meningitis, urinary tract infections and septicemia. Species-identification of recovered *Bacillus* isolates were completed by our recently developed protocol based on nucleotide sequencing of PCR amplified *gyr*B gene products. Analysis of data confirmed four species of *Bacillus* (*B. cereus*, *B. pumilus*, *B. subtilis*, and *B. thuringiensis*) in the swabs examined. This newly developed *gyr*B-based molecular diagnostic protocol can be used as a suitable genetic marker for rapid detection of *Bacillus* in the environmental monitoring program of public health importance.

1664

PRODUCTION AND EVALUATION OF A32 KDA FRAGMENT OF THE IMMUNOGLOBULIN-LIKE B PROTEIN FROM *LEPTOSPIRA*

Hua-Wei Chen, Giulia Weissenberger, Chien-Chung Chao, Wei-Mei Ching

Naval Medical Research Center, Silver Spring, MD, United States

Leptospirosis is caused by spirochaetes of the genus *Leptospira*. It is considered to be the most widespread zoonotic disease in the world. Symptoms of leptospirosis are easily confused with a variety of other febrile illnesses (e.g., dengue and malaria) that require different treatment regimens. Currently, the Microscopic Agglutination Test (MAT) is the standard method for the diagnosis of leptospirosis. It is not only technically complex but also time-consuming. With the publication of the whole genome sequences of several pathogenic species of *Leptospira*, hundreds of genes encoding surface-exposed lipoproteins and outer membrane proteins have been identified as candidates for the development of rapid diagnostics of leptospirosis. An ELISA using recombinant antigens (rLipL32, rLipL41, and rLigA-Rep) for the detection of *Leptospira*-specific antibodies has been developed in our laboratory with sensitivity close to 90%. Here, we prepared a recombinant protein containing the coding region of amino acids 630-931 of LigB (rLigB-Rep). The over-expressed rLigB-Rep, which contains a six-histidine tag at the N-terminus, was primarily found in the inclusion body. The solubilized rLigB-Rep in 8 M urea was purified with a nickel column under denatured conditions. We achieved greater than 90% purity as demonstrated by SDS-PAGE. The purified rLigB-Rep was refolded by dialysis in buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA) containing 6M, 4M, 2M, 1M, and no urea at 4°C. The refolded rLigB-Rep

has been shown that it was recognized by confirmed leptospirosis patient sera in western blot. These data suggest that rLigB-Rep antigen can be used to further improve the ELISA assay's sensitivity.

1665

EPIDEMIOLOGICAL PROFILE OF LEPTOSPIROSIS CASES, GUATEMALA: 2008-2013

Maria R. Lopez[1], John P. McCracken[1], Danilo Alvarez[1], David Moran[1], Rafael Ciraiz[2], Joe Bryan[3], Celia Cordon-Rosales[1]

[1]Universidad del Valle de Guatemala, Guatemala, Guatemala, [2]Ministry of Health Guatemala, Guatemala, Guatemala, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

Leptospirosis is a widespread zoonosis with a higher incidence in countries with humid subtropical or tropical climates. *Leptospira* are usually transmitted to humans through soil or water contaminated with infected urine from mammal hosts (mainly rodents). Outbreaks are associated with flooding, agriculture, as well as recreational, sporting and military activities. In Guatemala, there are reports of cases in humans and animals but the epidemiology of leptospirosis is unknown. Active surveillance for suspected leptospirosis cases was conducted during 2008-2013 among patients with acute febrile illness (AFI) attending the Cuilapa National Hospital (932 M) or Nueva Santa Rosa ambulatory facilities (1005 M) in the Department of Santa Rosa, in southern Guatemala. Leptospirosis is typically an undifferentiated AFI but suspected cases are seldom confirmed with laboratory diagnosis. In this study, AFI was defined as self-reported fever or measured temperature ≥38°C that began <7 days before presentation with no other diagnosis (e.g. pneumonia or diarrhea). Blood samples were taken and tested for IgM anti-*Leptospira* by enzyme-linked immunosorbent assay. Of 553 patients studied (396 hospitalized cases and 157 ambulatory cases), 25 (6%) hospitalized patients were positive while 8 (5%) ambulatory patients were positive. The median of age (IQR) of cases was older (22 years (16-34)) than the leptospirosis negative cases (16 (7-29)). Most cases (79%) were between 10-39 years and 53% were male. The majority of the patients presented with nonspecific signs, such as headache (91%), nausea (85%), myalgia (81%), vomiting (79%), arthralgia (67%), and hemorrhages (12%). During 2008-2013, 22 cases (67%) were detected in the rainy season (May-Oct). Cases were higher in 2010, the year with the most rain in a decade due to tropical storm Agatha, with 18 (55%) cases detected and 14 (78%) in the rainy season. Given endemic nature of leptosporosis in Guatemala and Central America, efforts on prevention and control should focus on these events and the greater risk among adults.

1666

EXTENDED EMPIRICAL TREATMENT CONTRIBUTES TO CHANGES IN BACTERIAL RESISTANCE PROFILE

Wellington F. Rodrigues[1], Camila B. Miguel[1], Ana Paula O. Nogueira[2], Carlo J. Oliveira[1], Marcelo C. Araújo[1], Javier E. Lazo-Chica[1]

[1]Universidade Federal do Triângulo Mineiro, Uberaba, Brazil, [2]Universidade Federal de Uberlândia, Uberlândia, Brazil

Extra intestinal infections acquired in the community with *Escherichia coli (E. coli*) showed in the United States, a rate of frequency of six million to 8 million cases of uncomplicated cystitis and 127,500 cases of sepsis per year. Empirical treatment has been associated with the emergence of bacterial resistance. although some countries are concerned to switch drugs used to treatment infectious, others keep the same treatment (for treatment empirical) over a long period This study aims to assess the sensitivity and resistance profile of E. coli across the different drugs used for treatment. A cross-sectional and retrospective study (January 2008 to December 20013) at a public hospital, university, belonging at System of Health of Minas Gerais/Brazil (empirical treatment = fluoroquinolones), was considered only in patients ambulatory that presented symptoms (dysuria). The antibiotics tested were as follows (potency in µg/disc): ampicilin + sulbactam (10/10), cephalothin (30),ciprofloxacin (5), norfloxacin (10) e nitrofurantoin (300) (standard disc diffusion method as per CLSI guidelines using discs of standard potency. Furthermore the costs of different classes of antibiotics were compared. Statistical analysis was performed using the program "Prism" from Graphpad. The results showed that all antibiotics tested here are effective ($p < 0.05$). However there was an increase in the sensitivity of nitrofurantoin in relation to other antibiotics with a decrease of the resistance of the same antibiotic in compared with others ($p < 0.05$), and a variation in the sensitivity and resistance among the fluoquinolonas with the ampicillin more inhibited beta-lactamase. There was also a significant difference in cost, and showed are more affordable the nitrofurantoin ($p < 0.05$), followed by fluoroquinolones, beta-lactamase inhibitor and cefalotinas. In conclusion, this work shows that should be considered an alternation of treatments for infection by E. coli, thus favoring the control of bacterial resistance and the cost effective to the population.

1667

ANTIBIOTIC RESISTANCE PATTERNS OF *ENTEROBACTER* SPECIES ISOLATED FROM CHILDREN WITH CYSTITIS IN IRAQ

Maitham G. Yousif

Al-Qadysia University, Dewania, Iraq

Awareness of antibiotic sensitivity patterns among gram negative bacterial pathogens is clinically important not only to empirically guide therapy but also to monitor the emergence of new drug resistant strains. We report here a survey of antibiotic resistance in Enterobacter species cultured from children with cystitis in Iraq. We collected 1,474 urine cultures from children aged 1-7 years treated at Al-Hakeem Hospital in Najaf governate, Iraq between January and September of 2010. Enterobacter species were identified in 3.9% of cultures (n=57), and were found more commonly in females (68.4%) than males (13.6%). Cultures positive for *Enterobacter* occurred most frequently in February followed by July. The following seven antibiotics were tested on isolates: cephalexin, cefotaxime, ceftriaxone, gentamicin, nalidixic acid, ciprofloxacin and amikacin. Antibiotic resistance variations were measured monthly and appeared to have a seasonal dependence. In January *Enterobacter* isolates were strongly resistant to cephalexin, in February to cefotaxime, in March to ceftriaxone, cephalexin and gentamycin, and in April to cefotaxime and nalidixic acid. In July isolates showed no resistance to amikacin and low resistance to ciprofloxacin, while in August and September strong resistance to cephalexin. Identification of factors which lead to an apparent seasonal variations in antibiotic resistance patterns among *Enterobacter* will require further study that includes careful evaluation of demographic and therapeutic histories of patients from whom *Enterobacter* strains are isolated, speciation of these isolates and collection of larger numbers of isolates over longer periods of time in order to control for sampling variability.

1668

SIGNIFICANT DIFFERENCES IN ULTRASOUND FINDINGS BETWEEN MALNOURISHED AND NON-MALNOURISHED SCHOOLCHILDREN IN MADAGASCAR

Maria Teresa Giordani[1], Daniela Sardei[2], Indi Trehan[3], Tom Heller[4], Jacopo Nicoletti[5], Enrico Brunetti[5]

[1]San Bortolo Hospital, Vicenza, Italy, [2]Pediatrician, Carrè, Vicenza, Italy, [3]Washington University, St. Louis, MO, United States, [4]Klinikum Muenchen-Perlach, Muenchen, Germany, [5]University of Pavia, IRCCS S. Matteo Hospital Foundation, Pavia, Italy

Childhood malnutrition contributes to high mortality rates and decreased educational and adult capacity among survivors. Not just closely linked to an increasing burden of infectious diseases, it is also increasingly recognized as a cause of chronic morbidity in later life. We explored the utility of bedside ultrasound in identifying specific pathologic findings in malnourished children in Madagascar, with the aim of improving the

management of co-morbidities. 83 children with stunting and/or severe acute malnutrition underwent bedside abdominal sonography and compared with 76 non-malnourished children. Liver and spleen size, liver echogenicity, intraluminal bowel evidence of massive helminthic infections, enlargement of abdominal lymph nodes, thickening of the gallbladder wall, and other pathologic findings were assessed. Malnourished children had hepatosplenomegaly (36% vs 18%) and fatty liver (41% vs 18%) more frequently than non-malnourished children. Hepatosplenomegaly was more common in the Antaimoro area, where malaria and sickle-cell anemia are more prevalent. Evidence of intestinal helminth infections were common in both groups, but in non-malnourished children were mostly associated with fever and acute diarrhea. Other pathologic findings were present In 17 malnourished children (20%) compared with 8% of non-malnourished children. Preliminary results of this study suggest that ultrasound evaluation of malnourished children is feasible and can be aid in identifying co-morbidities. The high rates of fatty liver infiltration particularly deserves more attention, as a possible marker for the development of metabolic diseases and liver fibrosis in adulthood.

1669

LEPROSY, A MIMICKER OF OTHER DISEASES IN A DEVELOPED COUNTRY

Rone Lin[1], Winnie Ooi[2], Samuel Moschella[2], Stephanie Burns[2]

[1]Tufts Medical Center, Boston, MA, United States, [2]Lahey Hospital and Medical Center, Burlington, MA, United States

Leprosy (Hansen's disease) is an uncommon disease in the U.S. and therefore unfamiliar to many health-care providers. This disease primarily occurs in immigrants from countries with higher endemicity such as India and Brazil. Initial misdiagnosis can lead to significant delay in therapy, morbidity and loss of function. Here we reviewed our database of 172 patients with leprosy, who were evaluated and treated in our Hansen's disease public health satellite clinic from 1998 to 2014. There were 18 patients (10%) with prior misdiagnoses; eleven of them initially presented with skin rash, six with neuropathy and one with rheumatologic symptoms. Skin rash from Hansen's disease can be difficult to distinguish from more common dermatologic conditions such as fungal skin infection, allergic dermatitis and cutaneous sarcoidosis. Idiopathic mono-neuropathies with wrist or foot drops, as well as mononeuritis multiplex, were among the common misdiagnoses for patients with leprous neuritis. Leprosy can also mimic rheumatoid arthritis with solely joint symptoms without initial involvement of skin or nerve. In our review, the diagnosis could be delayed for a significant amount of time, for even up to 10 years in 2 cases. This led to irreversible loss of neurologic function (foot/wrist drops), neuropathic ulcers and osteomyelitis. We will present 3 illustrative cases: one of leprous neuritis misdiagnosed as mononeuritis multiplex from sarcoidosis, a second of polyarthritis from leprosy mimicking rheumatoid arthritis, and lastly, a case of borderline tuberculoid leprosy with skin rash thought to be from cutaneous sarcoidosis based on initial evaluation of a skin biopsy. To summarize, it is of crucial importance to include leprosy in the differential diagnoses for patients with chronic rash, neuropathy or joint symptoms in the appropriate epidemiologic setting, because early recognition and treatment can prevent progression of the disease and its morbidities.

1670

TWO FATAL CASES OF MELIOIDOSIS ON THE THAI-MYANMAR BORDER

Miriam B. Torchinsky[1], Cindy S. Chu[2], Clare Ling[2], Francois Nosten[2]

[1]University of British Columbia, Vancouver, BC, Canada, [2]Shoklo Malaria Research Unit, Mae Sot, Thailand

Melioidosis, caused by the gram-negative environmental bacterium *Burkholderia pseudomallei*, is an infectious disease of clinical importance in endemic areas, and is associated with a high case-rate fatality in humans and animals. Once considered an esoteric tropical disease, research on *B. pseudomallei* has gained prominence due to its potential for epidemic spread, increasing numbers of case reports from non-endemic regions, and classification by the United States as a potential bioterrorism agent. Lack of awareness among physicians, along with a wide variability in disease manifestations, contributes to underdiagnosis and delayed treatment, and also confounds accurate assessment of global prevalence. Although melioidosis is endemic in Northern Australia and parts of Southeast Asia, there are no published reports from the Thai-Myanmar border. Here we report the first two documented cases of fatal melioidosis in this region. The discussion of cases in as-yet-unrecognized foci of disease is of great public health importance and may help to better elucidate environmental and host determinants of infection. Our study highlights the need to both increase clinical awareness of melioidosis on the Thai-Myanmar border, and to better assess the true burden of disease in the region through improved case detection and rigorous *B. pseudomallei* prevalence studies.

1671

HISTOPLASMOSIS IN OREGON EX ECUADOR

Jon E. Lutz[1], Jonathan Brewer[1], John Blizzard[2]

[1]Bend Memorial Clinic and St. Charles Medical Center, Bend, OR, United States, [2]Department of Cardiothoracic Surgery, Bend, OR, United States

A 41 year old river raft guide returned from a 2 week long rafting trip in a jungle region of Ecuador on March 30, 2012. He had received appropriate travel vaccinations and had taken mefloquine for malaria prophylaxis. Shortly after his return he developed fever to 103 F range, chills, sweats, headache, myalgias, arthralgias and fatigue. He self-medicated with amoxicillin and subsequently ciprofloxacin was prescribed by a physician. On examination on April 9th he was afebrile, had some shotty cervical and inguinal lymphadenopathy but otherwise had a normal physical examination including that of the lungs. Initial laboratory investigations including malaria smears, blood cultures and stool studies were unrevealing. A chest x-ray had evidence of a right sided infilatrate. Ceftriaxone and doxycycline were given but fevers persisted. A CT scan of the chest was carried out and revealed extensive mediastinal and hilar lymphadenopathy and too numerous to count lung nodules. Fiberoptic bronchoscopy was nondiagnostic. On April 20th therapy with ketoconazole was initiated to treat possible paracoccidioidomycosis. On April 24th, histoplasmosis serologies were reported positive. He was started on liposomal amphotericin B. On April 25th he underwent a minithoracotomy for definite diagnosis as there was concern about possible coexisting malignancy. Pathology and intraoperative cultures were consistent with histoplasmosis. He subsequently completed a 3 month course of itraconazole and did well. We herein discuss briefly travel-associated histoplasmosis and the current recommendations for drug therapy of severe primary histoplasmosis infection.

1672

DESCRIPTION OF DENGUE-RELATED HOSPITALIZATION AND DISEASE SEVERITY FROM AN ENHANCED DENGUE SURVEILLANCE SYSTEM IN PUERTO RICO

Janice Pérez-Padilla[1], Eric González[2], Aidsa Rivera-Sánchez[1], Brenda Torres-Velásquez[1], Juan P. Torres[2], Luisa Alvarado[2], Kay M. Tomashek[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]San Lucas Episcopal Hospital, Ponce, PR, United States

Dengue is an acute febrile illness (AFI) that is endemic in Puerto Rico. The clinical spectrum of dengue ranges from mild AFI to a life-threatening illness. Although timely identification of dengue patients can reduce medical complications and mortality, this complicated by clinical manifestations that overlap with other AFI. To identify early clinical features that can be used as predictors for severe dengue, we evaluated the clinical course of laboratory-positive (i.e., DENV nucleic acid detected by RT-PCR or anti-DENV IgM antibody detected by ELISA) dengue patients

enrolled in the Sentinel Enhanced Dengue Surveillance System (SEDSS) site located in Ponce, Puerto Rico. Patients were those presenting with AFI during May 7, 2012 to May 6, 2013 that were hospitalized (n = 262) or were out-patients that returned for follow-up evaluation (n = 120). Of all 382 patients, there were no significant differences in age or sex between hospitalized and non-hospitalized patients. Admitted patients sought care later than non-hospitalized patients (mean day of presentation = 4 vs.2 days), and had a mean hospital stay of 4 days. Clinical findings associated with hospitalization were anorexia ($p = 0.002$), diarrhea ($p = 0.021$) and dengue warning signs of persistent vomiting ($p < 0.001$), abdominal pain ($p < 0.001$) and bleeding ($p = 0.013$). Laboratory findings at presentation associated with hospitalization were leukopenia ($p = 0.021$) and thrombocytopenia ($p < 0.001$). Mean platelet count was significantly different between hospitalized and non-hospitalized patients (mean = 81,000 vs. 151,000) ($p < 0.001$). Patients that presented 4-7 days after illness onset had greater odds of having thrombocytopenia (OR = 2.18; CI: 1.24-3.83) or elevated liver transaminases (OR = 4.74; CI: 1.53-19.45). Enhanced dengue surveillance revealed that hospitalized dengue patients presenting late for clinical care were more likely to present with dengue warning signs, and were hospitalized more frequently. Further analysis will assess correlation between early presentation and ultimate disease severity.

1673

ACCESS MATTERS: AFRICAN-BORN U.S. MILITARY TRAVELERS UTILIZE TRAVEL CARE AT HIGH RATES

Kristina St. Clair[1], Anthony R. Artino[2], Patrick W. Hickey[2]

[1]Naval Medical Center Portsmouth, Portsmouth, VA, United States, [2]Uniformed Services University, Bethesda, MD, United States

Immigrant travelers that visit friends and relatives (VFRs) are less likely to have a pre-travel health encounter and more likely to experience serious illness. Immigrants comprise more than 5% of active duty service members in the United States Department of Defense and have free access to travel medicine care, yet their travel health seeking behaviors are unknown. 351 African-born service members and 470 U.S.-born comparators completed an Internet-based survey to assess pre-travel health care utilization, perceived potential barriers, and health outcomes. While overall use of travel medicine services was equivalent if official duty was included ($p=0.94$), when traveling on leave status (not on official duty) African-born service members were more likely than U.S.-born to see a physician, 65% vs. 35% ($p<0.001$) prior to their most recent travel to a low, low-middle or upper-middle income country. This persisted when stratified by malaria risk at destination with African-born service member VFRs reporting pre-travel health care more than American-born comparators, 65% vs. 45% ($p < 0.001$) Both African-born and American-born service members reported easy access to medical care. African-born military service members perceive less risk of illness when traveling to Africa compared to American born travelers ($p<0.001$) yet, somewhat paradoxically, place more importance on pre-travel medical services ($p = 0.007$). African-born service members are more willing to self-diagnose and treat illnesses such as malaria ($p<0.001$) and rely on locally purchased medications ($p<0.001$). There was no difference in reported adherence to malaria chemoprophylaxis. This United States Military Health System study revealed data that opposes previous civilian studies: African-born VFRs in the military sought pre-travel health care more often than their U.S.-born counterparts. Access to care and positive beliefs about the benefits of travel medicine services contribute to this finding. These findings have implications for the role of national health-care reform and community engagement programs. Disclaimer: The Views expressed are those of the author(s) and do not necessarily reflect the official views of the Uniformed Services University of the Health Sciences, the U.S. Army, or the Department of Defense.

1674

SURVEILLANCE FOR ARTEMISININ, QUININE, AND MALARONE RESISTANCE AMONG IMPORTED *PLASMODIUM FALCIPARUM* MALARIA - CALGARY, CANADA (2013-14)

Asongna Folefoc, Abu N. Mohon, Lydia Lee, **Dylan R. Pillai**

University of Calgary, Calgary, AB, Canada

Calgary has seen an increase in imported malaria cases in recent years with a large proportion coming from sub-Saharan Africa where *Plasmodium falciparum* is hyper-endemic. Surveillance for emerging resistance to antimalarial drugs such as Artemisinin, Quinine, and Malarone is essential in returning travelers. Artemisinin resistance has been reported in Cambodia and mutations within the K13-propeller gene associated with resistance to this drug. Mutations in the cytochrome B (cytB) are linked to Malarone resistance. In this study, we prospectively determined the susceptibility and resistance genotype of imported *P. falciparum* malaria to Calgary from April, 2013 to April, 2014. Travelers were mostly male (n=12, 67%) VFR (n=17, 94%) with mean age of 35.3 years destined for sub-Saharan Africa (n=17, 94%) predominantly West Africa (n=11, 61%). Malarone (oral) was the commonest treatment option (n=11, 61%) followed by artesunate (n=3, 17%) and quinine (n=3, 17%). Positive malaria samples from patients (n=18) were tested with a standardized panel of antimalarials using the ELISA-based HRP2 *ex vivo* drug sensitivity protocol developed by WWARN. DNA was extracted from patient EDTA blood samples (n=18) and primers flanking the K-13 propeller gene and cyt B were used for PCR amplification of this gene. PCR products were bidirectionally sequenced and analyzed for mutations. Our ex-vivo results showed IC_{50} values of 17.36 ± 11.92nM, 4.34 ± 2.34 nM, 4.06 ± 1.66 nM, 4.00 ± 1.39 nM for Artemisinin, Artesunate, Artemether and Dihydroartemisinin, respectively; mean IC_{50}s of 39.04 ± 15.73 nM, 16.33 ± 4.36 nM, 80.44 ± 25.75 nM, 17.23 ± 3.65 nM and 127.38 ± 36.97nM for Chloroquine, Mefloquine, Quinine, Amodiaquine and Piperaquine respectively; and mean IC50 = 27.2 ± 22.26 for Atovaquone. Analysis of the K-13 propeller and CytB gene showed that all imported malaria were wild type to date. Our results confirm that imported *P. falciparum* malaria to Calgary from sub-Saharan Africa remains wild-type at key resistant loci (K13 and cyt B) and susceptible to Artemisinin, Malarone and quinine the commonest treatment options when tested *ex vivo*.

1675

A SURVEY ON KNOWLEDGE, ATTITUDES AND PRACTICES AMONG INTERNATIONAL TRAVELERS IN UGANDA

Zain Chagla[1], Palmyra Immordino[2], Claire Webb[2], Ronald M. Mbwasi[2], Christopher Sanford[3]

[1]McMaster University, Hamilton, ON, Canada, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]University of Washington, Seattle, WA, United States

In an increasingly international environment, global travel rates are increasing. However, significant risk exists amongst travelers. Counseling against preventable morbidity and mortality, such as transit related injuries, vaccine preventable diseases, violent crimes, and tropical illnesses, is essential for international travelers. The majority of data around traveler attitudes and counseling is based on pre-travel assessments, or in evaluation of returned ill travelers, but is biased towards those with health seeking behaviors. This study was designed to look at attitudes and practices amongst international travelers while actively travelling. The study took place Jinja, Uganda, a popular tourist destination near Kampala known for white-water rafting and the Nile River. Participants were recruited at tourist locales in Jinja, and a semi-structured questionnaire was administered to tourists after obtaining voluntary informed consent. A total of 153 travelers were surveyed. The majority was female, with average age 31 years, and predominantly from the USA, Australia, and the Netherlands. Most participants had received pre-travel advice through a travel clinic (64.0%) or a general practitioner (25.4%), and others cited the Internet, friends, and relatives as sources of information. Participants

endorsed malaria (94.7%), vaccines (92.1%), and diarrhea (59.5%) were the most important pre-travel counseling items, but few mentioned traffic accidents (15.0%) or sexually transmitted infections (13.7%). Malaria prophylaxis was prescribed to the majority (80.1%), but only three quarters of those people took prophylaxis, with adherence issues attributed to side effects or a lengthy duration of stay. Finally, when asked about health and safety issues experienced during the trip, nearly a third had encountered an issue, most of which either related to gastroenteritis, malaria, or traffic accidents. Although many travelers seek medical care prior to departure, counseling regarding non-infectious issues such as road traffic accidents, personal safety, and risk behaviors are lacking, despite these being a major cause of morbidity and mortality. Integration of this information into Internet resources and clinical practice may help to decrease mortality amongst global travelers.

1676

FACTORS ASSOCIATED WITH MORTALITY BY DENGUE

Elizabeth Borrero

Fundación Santa Fe de Bogotá, Bogotá, Colombia

Dengue is a public health priority in Colombia due to the significant increase in the number of cases from 26817 in 2008 to 57589 in 2013. In 2010 a 183% increase was noted in respect to 2009 and 9393 cases of severe dengue was reported and 217 confirmed deaths. A case-control study was conducted in the departments of Cundinamarca, Norte Santander, Santander, Cauca Valley and Meta to identify the clinical factors associated with mortality in patients with severe dengue in hospitals of level II and III attention between the periods 2009-2013. A case was considered to be death by dengue and a control pertained to survivors, all were confirmed through IgM or RT-PCR. The sample size was 50 cases and 150 controls. For the analysis logistic regression was utilized. 42% (63) of the patients originated from Cauca Valley, 24% (36) Meta, 18% (27) North of Santander, 10% (15) Santander and 6% (9) Cundinamarca. 60% (90) were less than 16 years and 52% were women. The factors associated independently with mortality were: comorbidities OR 3,18 (IC 95%: 1,33; 7,60), social risk OR 3,33 (IC 95%: 1,21; 9,17), tachycardia OR 8,94 (IC 95%: 2,57; 31,05), tachypnea OR 2,71 (IC 95%: 1,16; 6,28), altered state of consciousness OR 12,09 (IC 95%: 2,72; 53.70), respiratory difficulty OR 5,61 (IC 95%: 2,24; 14,06), pleural effusion OR 2,85 (IC 95%: 1,33; 6,11), main organ damage OR 3,14 (IC 95%: 1,29; 7,64), severe bleeding OR 2,66 (IC 95%: 1,19; 5,95), and previous consultation OR 2,68 (IC 95%: 1,16; 6,19). In the multivariate analysis the factors associated with increased death were: social risk social OR 9.88 (IC 95%: 1,26; 77,11), altered state of consciousness OR 11.48 (IC 95%: 1,34, 97,93), respiratory difficulty OR 9.84 (IC (95%: 1,96; 49,36) main organ damage OR 9.55 (IC 95%: 1,77; 51,41) and severe bleeding OR 8,08 (IC 95%: 1,95; 33,66). Patients with these clinical characteristics should be hospitalized for extended observation and opportune treatment to avoid death.

1677

ATORVASTATIN FOR THE TREATMENT OF RHEUMATOID ARTHRITIS IN IRAQ

Sami Salman[1], Samer Shuker[2]

[1]Rheumatology Unit, College of Medicine, Baghdad University, Baghdad, Iraq, [2]Pharmacology Unit, College of Medicine, Baghdad University, Baghdad, Iraq

Rheumatoid arthritis (RA) is a common chronic inflammatory and disabling disorder that is characterized by synovitis, articular destruction, and many systemic extra articular features. In addition, RA is associated with both morbidity and mortality due to accelerated atherosclerosis and risk of increased cardiovascular disease. Atorvastatin is well known anti dyslipidemic agent that is considered to have potential anti-inflammatory and immune modulatory functions in patients with RA. To explore the possible anti-inflammatory effects of Atorvastain in patients with RA, we designed a study to evaluate the effect of atorvastatin compared to therapy with two more standards RA medications, methotrexate (MTX) and etanercept (EPT). The study group included Iraqi patients with moderate to highly active RA. A double blind, randomized, placebo - controlled clinical trial was conducted in which 100 RA male and female patients were enrolled from a group who were already on MTX or EPT for at least 1 month. This pool of subjects was divided into two groups, one to receive 20 mg atorvastatin tablet and the other to receive placebo capsules for three consecutive months. This study revealed first that only 49 patients completed the 3 months trial, 25 patients in atorvastatin and 24 patients in placebo group. All patients were clinically evaluated by measuring swollen joint count (SJC), tender joint count (TJC), visual analogue scale (VAS) and disease activity score (DAS28). Blood samples of all subjects patients were evaluated for erythrocyte sedimentation rate (ESR), C reactive protein (CRP) at baseline, monthly and at the end of the study. RA patients undergoing 20 mg atorvastatin treatment showed a significant ($P < 0.05$) reduction in CRP, SJC and TJC compared to those who received placebo. In addition, atorvastatin treatment groups trended toward reduced ESR, VAS, and DAS28, but these differences did not achieve statistical significance ($p > 0.05$). In conclusion we believe that 20 mg atorvastatin is a safe and well-tolerated drug that has modest anti-inflammatory effect in patients with moderate to severe active RA.

1678

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY AND DENGUE IN SINGAPOREAN MALES: A CASE-CONTROL STUDY

Tun-Linn Thein, Linda K. Lee, Joshua G. Wong, David C. Lye, Yee-Sin Leo

Tan Tock Seng Hospital, Singapore, Singapore

Clinical presentations of dengue infection range from asymptomatic, non-severe to severe disease. We aim to test the hypothesis that patients with glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency may present with severe disease and hemolysis. We analyzed a cohort of adult dengue patients treated at Tan Tock Seng Hospital, Singapore from January 2005 to December 2008. Dengue infection was confirmed by positive polymerase chain reaction or dengue serology with World Health Organization (WHO) probable dengue definition. Singaporean males with documented G6PD status were defined as cases. For each case, three controls were selected by matching citizenship and year of infection. Hemolysis was defined as low hemoglobin concurrent with low serum haptoglobin, or high reticulocyte or lactate dehydrogenase or bilirubin. Dengue hemorrhagic fever and severe dengue were classified according to WHO 1997 and 2009 dengue guidelines. Compared with cases (n=30), controls (n=120) were significantly younger (median 26 vs. 35 years, p0.05). During their clinical course, cases had significantly higher rates of jaundice (10% vs. 1%, P<0.05), serum bilirubin (median 27 vs. 10 mmol/L, p<0.001), aspartate transaminase (median 148 vs. 91 U/L, p<0.05), and lower hematocrit (45% vs. 46%, p<0.001), haemoglobin level (13 vs. 14 mg/dL, p<0.001). There was no difference in rates of dengue hemorrhagic fever (23% vs. 22%, p>0.05). However, cases had higher tendency to develop severe dengue and hemolysis than controls ([23% vs. 12%] and [14.29% vs. 2.7%] respectively) although the difference was not significant (p>0.05). The two groups had similar rates of blood and platelet transfusions, intravenous fluid and length of hospitalization (p>0.05). The observed differences should be prospectively validated in larger cohorts and in different populations.

1679

A RANDOMIZED, DOUBLE BLIND, CLINICAL TRIAL OF TWO DOSE REGIMENS OF VINS POLYVALENT ANTIVENOM FOR THE TREATMENT OF SNAKEBITE WITH NEUROTOXIC ENVENOMATION IN NEPAL

Emilie Alirol[1], Sanjib K. Sharma[2], Anup Ghimire[2], Antoine Poncet[1], Christophe Combescure[1], Khaled Mostaguir[1], Chabilal Thapa[3], Vijaya Paudel[4], Robert W. Taylor[5], Deb Pandey[6], David A. Warrell[7], Ulrich Kuch[6], **François Chappuis**[1]

[1]Geneva University Hospital, Geneva, Switzerland, [2]B.P. Koirala Institute of Health Sciences, Dharan, Nepal, [3]Dumkauli Primary Health Care Centre, Nawalparasi, Nepal, [4]Bharatpur Hospital, Chitwan, Nepal, [5]Mahidol Oxford Research Unit, Bangkok, Thailand, [6]Forschungszentrum Biodiversität und Klima (Bio+C), Frankfurt am Main, Germany, [7]University of Oxford, Oxford, United Kingdom

Snakebite is an important medical emergency in rural Nepal. Although accurate figures are lacking, over 20 000 cases are recorded annually, with a case fatality rate close to 15%. Important variations exist between hospitals in the management and outcome of snakebite envenomation. In particular, striking disparities are observed in the dosage of antivenom, reflecting poor adherence to the complex national protocol. Although clinical studies have been conducted on viperid snake envenomation, well-designed dose-finding studies are almost non-existent for elapid envenomation. The purpose of this trial was to compare the efficacy and safety of two antivenom dosing schemes in the treatment of neurotoxic envenomation. The trial was conducted between May 2011 and March 2013 in 3 health facilities of southern Nepal. 157 patients presenting with signs of neurotoxic envenomation were randomized either to a high initial dose regimen (intervention) or to the low initial dose regimen as recommended by the Nepalese national protocol (control). The primary composite outcome included death, requirement for manual ventilation and worsening of neurotoxicity. Secondary outcomes included time to recovery, occurrence of adverse reactions, and cost. There was no statistically significant difference between arms in the proportion of patients reaching the primary endpoint (control 48.7% vs intervention 38.5%, p=0.264). No differences were observed in the analysis of safety outcomes. In 51 patients the snake species could be identified. 29 had been bitten by cobras (*Naja spp*) and 22 by kraits (*Bungarus spp*.). Those bitten by kraits experienced more primary outcomes (68.2% versus 27.1%, p=0.004), and recovered less often (40.9% vs 96.5%, p<0.001) or more slowly (mean time 18 hours vs 5 hours, p<0.001) than did patients bitten by cobras. These findings suggest that there is no difference in efficacy and safety between low and high initial dose of antivenom for neurotoxic snakebite, and that envenomation due to krait bites is less responsive to antivenom than that following cobra bites.

1680

FACTORS ASSOCIATED WITH COMPLETE ROUTINE IMMUNIZATION STATUS OF CHILDREN 12-23 MONTHS IN RURAL AREAS OF OSUN STATE - SOUTHWESTERN NIGERIA

Elizabeth B. Adedire[1], Ikeoluwapo O. Ajayi[2], Olufemi Ajumobi[1]

[1]Nigerian Field Epidemiology and Laboratory Training Program, FCT Abuja, Nigeria, [2]University of Ibadan, Ibadan, Oyo State, Nigeria

Immunization is a cost-effective public health intervention to reduce morbidity and mortality associated with infectious disease. Incomplete immunization status especially in rural areas has led to a high burden of VPD in children. The Nigerian Demographic and Health Survey, 2008 showed that only 57.8% of children had received all recommended vaccines in Osun State far below WHO target of 80%. We conducted this study to identify the factors associated with complete immunization status of children in rural areas of Osun State. A total of 750 mothers of children aged 12-23 months were interviewed, using the WHO 30 cluster sampling technique. We collected data on socio-demographic characteristics, history of vaccination and factors associated with immunization status using semi-structured questionnaire, vaccination cards were also reviewed. We defined a completely immunized child as a child who had received one dose of BCG, three doses of oral polio vaccine, three doses of Diptheria-Pertusis-Tetanus vaccine and one dose of measles vaccine by 12 months of age. Bivariate and multivariate data analysis was performed using Epi-info software. Of the 750 mothers interviewed, (36.6%) were fully immunized. Children of mothers with poor knowledge on immunization were less likely to be fully immunized (Odds ratio (OR) =0.55, 95% CI=0.23-0.51). Children whose mothers possessed primary or no formal education were less likely to be fully immunized compared to children of mothers with at least a secondary level education (OR=0.50, 95%CI=0.34-0.73). Children delivered at health facilities were more likely to be fully immunized (OR=1.81, 1.21-2.69). The major determinants of complete immunization status were knowledge level, maternal educational status and place of birth of the children. Raising the level of knowledge and increasing maternal literacy level as well as encouraging health facility births are essential to improve immunization coverage in these rural communities.

1681

VARIATIONS IN PRESENTATION OF *ERYTHEMA NODOSUM LEPROSUM*: REPORT OF THREE CASES SEEN AT A U.S. HANSEN'S DISEASE CLINIC

Kristoffer E. Leon[1], Robert W. McDonald[2], Anandi N. Sheth[2], Jessica K. Fairley[2]

[1]Emory University, Atlanta, GA, United States, [2]Emory University School of Medicine, Atlanta, GA, United States

Hansen's disease (leprosy) remains the leading infectious cause of disability, with 250,000 new cases reported globally yearly. Erythema nodosum leprosum (ENL) reactions, or Type 2 reactions, are humoral responses of the immune system that can cause systemic illness, including fever, skin lesions, joint pains and neuritis. ENL reactions occur in patients on the lepromatous end of the spectrum, classified by the Ridley-Jopling system. In non-endemic countries, Hansen's disease (HD) remains rare and often underrecognized with the literature lacking in clinical descriptions of leprosy complications in the United States. To fill this gap, we report three patients with lepromatous leprosy who were seen at a HD clinic in Atlanta, GA with complicated ENL reactions within the last three years. The first patient was a 33-year-old Bangladeshi woman who presented with high fever, abdominal pain, and arthralgias. She lacked the distinctive skin lesions usually seen in ENL, but was incidentally found to have splenic lesions. She responded well to prednisone and was able to be weaned off after 6 months. Second, a 42-year-old Vietnamese man initially presented with classic ENL lesions, fevers, and lymphadenopathy that progressed in severity despite increasing doses of corticosteroids. He eventually was admitted to the intensive care unit with a severe systemic inflammatory response syndrome. He was subsequently started on thalidomide without recurrences. The last patient was a 68-year-old U.S.-born man, who displayed symptoms representative of both Type 1 and Type 2 reactions as his initial presentation of HD. These included joint pain, severe extremity swelling, skin nodules and a progressive neuropathy. He had been misdiagnosed with a seronegative arthritis prior to this presentation. While all three cases reported are ENL, the differences of clinical courses and presentations highlight the complexity of the disease and the need for increased awareness of unique manifestations of lepromatous leprosy.

1682

SEROLOGICAL STUDY OF ANTIBODIES ANTI-*TOXOCARA CANIS* EVALUATED BY ELISA AND WESTERN BLOT IN PEDIATRIC PATIENTS WITH CRYPTOGENIC EPILEPSY

Maria de Lourdes Caballero-Garcia[1], Jocelyn Simon-Salvador[1], Juan Hernandez-Aguilar[1], Benjamin Nogueda-Torres[2], Enedina Jiménez-Cardoso[1]

[1]Hospital Infantil de Mexico Federico Gomez, Mexico City, Mexico, [2]Escuela Nacional de Ciencias Biologicas, Mexico City, Mexico

It has been suggested a correlation between infection by *Toxocara canis* and epilepsy and it is thought that epileptic seizures can be derived from the immune response generated by the presence of the parasite or direct lesions that can cause in the brain. The objective was to determine antibody anti-*T. canis* against antigen of the parasite through the technique of ELISA and Western blot in pediatric patients with cryptogenic epilepsy attending outpatient consultations in the area of Neurology of the Children Hospital of México Federico Gomez (HIMFG). We analyzed 111 patients from 6 to 16 years of age with confirmed diagnosis of epilepsy with clinical and epidemiological background who attend the external consultation of Neurology of the HIMFG. We analyzed the presence of antibodies anti-*T. canis* by ELISA using excretion-secretion antigens obtained from larvae of L2 *T. canis* cultured *in vitro* and the children who tested positive by this technique were evaluated by the technique of Western blot to determine the molecular weight of excretion-secretion proteins recognized by sera from patients with antibodies anti-*T. canis*. It was found that 12.5%. sera had antibodies against antigens of excretion-secretion for *T. canis*. Nine children were evaluated by the technique of Western blot and only 5 were positive for this technique, recognized two main antigens of 24 and 35 kDa. The analysis in sera from pediatric patients with epilepsy, showed a rate of 6.9% to antigens from *T. canis*, after analysis by ELISA and Western blot.

1683

MAPPING THE POTENTIAL RISK OF MYCETOMA IN SUDAN USING MAXIMUM ENTROPY ECOLOGICAL NICHE MODELLING

Abdallah M. Samy, A. Townsend Peterson

Kansas University, Lawrence, KS, United States

WHO recognized mycetoma as one of 17 neglected tropical diseases (NTDs) worldwide. Studies revealed a soil-borne mediated or thorn prick-mediated origin of mycetoma, but no studies are available to investigate the effect of soil type and Acacia distribution on mycetoma in Sudan. Here, we report efforts to investigate risk factors associated with mycetoma risk in Sudan using ecological niche modeling. Records of mycetoma cases were obtained from the scientific literature, PubMed, and GIDEON. Acacia records were obtained from the Global Biodiversity Information Facility. We developed ecological niche models (ENMs) based on digital GIS data layers summarizing soil, land-surface temperature, and greenness, summarizing environmental variation across Sudan. ENMs calibrated in endemic districts were transferred across all of Sudan, and suggested that greatest risk was in a belt across central and southern Sudan. We visualized mycetoma in environmental dimensions, and the results revealed that mycetoma in ecologically diverse landscapes under wide ranges of environmental conditions. We tested niche similarity between Acacia and mycetoma, and found significant niche similarity. These results revealed contributions of different environmental factors to mycetoma risk, identify suitable environments for disease emergence, raise the concerns for mycetoma-acacia association, and provide steps towards a robust, predictive risk map for the disease.

1684

THE EPIDEMIOLOGY OF ORAL HUMAN PAPILLOMAVIRUS INFECTION AMONG HEALTHY MEN AND WOMEN IN LIMA, PERU

Brian J. Rosen[1], Leora Leora Walter[2], Robert H. Gilman[1], Lilia Cabrerra[3], Patti E. Gravitt[1], Morgan A. Marks[1]

[1]Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States, [2]New York Hospital Queens, New York, NY, United States, [3]Asociacion Benefica Prisma, Lima, Peru

The incidence of head and neck cancers associated with human papillomavirus infection has been increasing in Peru. However, the burden of oral HPV infection in Peru has not been assessed in a healthy population. The objective of the study was to estimate the prevalence and correlates of oral HPV infection in healthy male and female residents from Las Pampas, a shantytown in Lima, Peru. A population-based sample of 1,500 healthy men and women between the ages of 8-85 in low to middle income areas of Lima, Peru was identified through random household sampling between January and August 2010. Adjusted odds Ratios (aOR with 95% CI) were used to assess the association of demographic factors, sexual practices, and oral hygiene on the prevalence of oral HPV infection. The prevalence of any HPV and any high-risk HPV (HR-HPV) was 6.8% and 2.0%, respectively. The three most common types were HPV 55 (3.4%), HPV 6 (1.46%), and HPV 16 (1.09%). Male sex (aOR, 2.32; 95% CI: 1.29, 4.18), age 19-27 (aOR, 2.77; 95% CI: 1.02, 7.56) and 46-55 years (aOR, 3.52; 95% CI: 1.07, 11.5) were significantly associated with prevalent HPV infection after adjustment. The prevalence of oral HPV in this population-based sample of healthy men and women from Peru was similar to estimates observed in the United States. Higher prevalence of oral infections in men were consistent with a male predominance of HPV-associated HNC and may signal a sex-specific etiology in the natural history of infection.

1685

DIAGNOSIS OF LIVER TUMORS USING IMAGE-BASED STATISTICAL FEATURES

Alyaa H. Ali, Intithar Malk, Sabah N. Mazhar

Department of Physics, College of Science for Women, Baghdad University, Baghdad, Iraq

Tumors located in the liver can be detected using CT imaging and comparison of intensity histograms with the associated statistical parameters for characterizing different regions of the liver including normal and cancerous. Identifying whether the tumor is benign or malignant is an important step in image-based liver cancer diagnosis. In this paper we describe an automated system for image-based liver segmentation of CT imagery using a multi-stage process. Each CT liver image is pre-processed to remove noise and enhance image quality to recognize structures within the liver. A key challenge is related to separating the liver from the rest of the abdominal cavity in CT imagery. We used a statistical feature descriptor to characterize healthy tissue versus cancerous regions and then applied a modified K-means classifier to improve the accuracy of the tumor segmentation process.

1686

ASSOCIATION OF HEMOGLOBIN LEVELS AND SELECTED BIOCHEMICAL MARKERS WITH DIABETIC NEPHROPATHY DISEASE

Khalid G. Al-Fartosi, Manal B. Al-Tamimi, Saad H. Al-Badry

Department of Biology, College of Science, University of Thi-Qar, Thi-Qar, Iraq

The present study was designed to investigate the relationship between hemoglobin levels (Hb), serum creatinine, lipid profile and urine albumin excretion in diabetic patients in Thi-Qar province, Iraq with various degrees

of nephropathy. The study cohort included 60 patients and 30 healthy subjects (control group) presented at the Al-Nasiriyah Endocrine Centre in Thi-Qar province, Iraq. The diabetic subjects were divided into three groups each with 20 subjects presenting diabetes mellitus (DM) disease for 1-5 years, 6-10 years and more than 10 years. The clinical results showed a significant decrease in the levels of Hb ($p<0.01$) in patients with DM compared to the control group. Also, there was a significant increase in blood sugar and urine albumin excretion in patients with DM compared with the control group ($p<0.01$). Serum creatinin increased significantly in patients with more than 10 years of DM compared with the control group. These results indicate a dyslipidemia in patients with DM compared with the control group.

1687

THE EFFECT OF METFORMIN ON GHRELIN SERUM LEVEL IN TYPE 2 DIABETES MELLITUS

Mohammed N. Mohammed, **Nahla O. Tawfik**

Department of Dental Basic Sciences, College of Dentistry, University of Mosul, Mosul, Iraq

Ghrelin is an orexigenic peptide hormone. A great deal of evidence suggests that ghrelin is involved in the development of Type 2 diabetes mellitus (DM). The aim of this study was to investigate the effect of metformin on ghrelin serum level in Type 2 DM patients. This clinical control study was carried out at the Al-Wafaa Medical Center for Diabetic and Endocrine Disorder patients in Mosul from October 2011 to March 2012. Fifty-five Type 2 diabetic patients and 20 control healthy subjects were enrolled. Patients and subjects were divided into 4 groups. Blood samples were collected from all subjects and the body mass index (BMI) was calculated for each person. Fasting blood sugar (FBS) level and ghrelin serum level were estimated for each patient. This study demonstrated a non-significant lower mean ghrelin serum level in the diabetic group compared to healthy controls. There were, however, significant differences in ghrelin serum levels between the diabetic group without metformin and the diabetic group treated with 1,000 mg metformin daily ($p < 0.05$). In this study we found that ghrelin serum levels had a negative correlation with age of patients over 30 years and BMI in both healthy and diabetic individuals.

1688

PEDIATRIC MENINGITIS IN THE AL-ABBASEYA FEVER HOSPITAL OF CAIRO

A. M. Al-Abbasi, Amro Shaee, Haifa I. Tawfeek

Infectious Diseases and Clinical Immunology, College of Medicine, Baghdad University, Baghdad, Iraq

Meningitis in children can be a fatal disease. Early discovery and prompt treatment using effective antimicrobials greatly reduces the mortality and resulting complications of the disease. Since meningitis is caused by a number of different microorganisms, detection of the causative agent is essential for proper treatment and to establish any preferential pattern of prevalence in different age groups or geographical regions. We conducted our clinical study on 61 patients admitted to Al-Abbasseya Fever Hospital in Cairo during the period of December 2010 to June 2011. The patients ranged in age from one month up to 17 years. Diagnosis of these cases was based on history, clinical data, laboratory tests, cerebrospinal fluid (CSF) examination and other visual diagnostic tests. There were 61 cases with 35 males and 26 females. Based on the CSF culture, gram stain, cell count and cell type diagnostic information, the patients were grouped into two classes: Acute bacterial meningitis (Group 1) and acute non-bacterial meningitis (Group 2). Group 1 consisted of 31 cases (20 males and 11 females) while Group 2 had 29 cases (15 males and 14 females), and one case of recurrent meningitis. The yield of microbial isolation from Group 1 was only 32.2% with four cases of N. meningitides, two cases of S. pneumonia, three cases of H. influenza and one case of Gram negative rods. The clinical and laboratory information and antimicrobial treatment regimen we used will be described. Pediatric meningitis needs special attention and a high rate of clinical suspicion as the yield of microbial isolation is low primarily due to the use of antibiotics prior to hospital admission. The choice of empirical antimicrobial usage might need to be reviewed from a clinical and public health perspective.

1689

UTILIZING THE COMMUNITY HEALTH WORKER NETWORK FOR LYMPHATIC FILARIASIS (LF) MORBIDITY MONITORING: THE DEVELOPMENT OF AN SMS-BASED SURVEILLANCE TOOL

Michelle C. Stanton[1], Square Z. Mkwanda[2], Alexander Y. Debrah[3], Linda B. Debrah[3], Achim Hoerauf[4], Nana-Kwadwo Biritwum[5], Abigail Best[1], Matthew Cliffe[1], Louise A. Kelly-Hope[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Ministry of Health, Lilongwe, Malawi, [3]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [4]University of Bonn, Bonn, Germany, [5]Ghana Health Service, Accra, Ghana

Lymphatic filariasis (LF) is a parasitic infection that is responsible for over 15 million lymphoedema and 25 million hydrocele cases globally, resulting in LF morbidity being one of the leading causes of long-term disability. Currently, there are no standardised methods available for quantifying and mapping LF morbidity burden. The purpose of this study was therefore to pilot a novel method for collecting LF morbidity data in endemic areas of Malawi and Ghana. The premise of this method is that community health workers are able to quickly identify lymphoedema or hydrocele cases in their villages, but do not currently have a standardised method of collating this information. We have therefore developed an SMS-based tool which enables health workers to submit information on each identified case in their communities using a basic mobile phone. This tool was trialled under two scenarios: in March 2014 the tool was trialled by qualified, salaried health workers in southern Malawi; in May 2014 the study was repeated in Ghana using volunteer community health workers. In both scenarios, each health worker was asked to submit each identified case's village of residence, age, sex, condition and severity of condition (if lymphoedema) via SMS to a smartphone housed in-country. This information was then instantly compiled into a single database. A random sample of cases was visited by a medically qualified person to confirm the health workers' diagnoses, and GPS coordinates of their villages were recorded. The feasibility of the method was assessed in terms of the ease in which health workers were able to correctly identify cases (true positive rate), and the ease of use of the SMS-based tool (data entry error rates). A comparison between the performance of salaried health workers and volunteer health workers was also undertaken. Preliminary results for Malawi indicate that the true positive rate for reported lymphoedema and hydrocele cases using this method were 90% (95% CI [80%, 97%]), and 92% (95% CI [77%, 97%]) respectively.

1690

EPIDEMIOLOGY OF PODOCONIOSIS IN ETHIOPIA: RESULTS FROM A FIRST NATIONWIDE MAPPING

Kebede Deribe[1], Simon J. Brooker[2], Rachel L. Pullan[2], Heven Sime[3], Abeba Gebretsadik[3], Ashenafi Assefa[3], Amha Kebede[3], Asrat Hailu[4], Maria Rebollo Polo[5], Oumer Shafi[6], Moses J. Bockarie[5], Abraham Aseffa[7], Richard Reithinger[8], Fikre Enquselassie[9], Melanie J. Newport[10], Gail Davey[10]

[1]Brighton and Sussex Medical School and School of Public Health, Addis Ababa University, Addis Ababa, Ethiopia, Falmer, Brighton, United Kingdom, [2]Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Ethiopian Public Health Institute, Addis Ababa, Ethiopia, [4]School of Medicine, Addis Ababa University, Addis Ababa, Ethiopia, [5]Center for Neglected Tropical Diseases, Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [6]Federal Ministry of Health, Addis Ababa, Ethiopia, [7]Armauer Hansen Research Institute/ALERT, Addis Ababa, Ethiopia, [8]International Development Group, Research Triangle Institute, Washington, DC, United States, [9]School of Public Health, Addis Ababa University, Addis Ababa, Ethiopia, [10]Brighton and Sussex Medical School, Falmer, Brighton, United Kingdom

Podoconiosis (endemic non-filarial elephantiasis) is a major cause of tropical lymphoedema and is endemic in Ethiopia. To guide targeting and implementation of the National Neglected Topical Diseases control strategy, an integrated nationwide survey of lymphatic filariasis (LF) and podoconiosis was conducted between June and September 2013. Here we present a description of podoconiosis epidemiology in Ethiopia resulting from this survey. District health offices' reports of podoconiosis and LF were used to guide selection of survey sites. Data and cluster level GPS coordinates were collected via smartphones by trained local health workers. Individual level data were available for 129,959 randomly-sampled individuals from 1,315 communities in 659 districts. Blood samples were tested for *Wuchereria bancrofti* antigen using immunochromatographic card tests (ICT). A clinical algorithm was used to diagnose podoconiosis by excluding other potential causes of lymphoedema of the lower limb. Mixed-effects logistic regression was used to identify individual-level correlates, adjusting for dependence within district and municipality. Overall, 8,110 of 129,959 (6.2%, 95%CI; 6.1 to 6.4%) surveyed individuals were identified with lymphoedema with 5253 (4.0%, 95% CI; 3.9 to 4.1%) confirmed as podoconiosis cases. Prevalence among men and women was 3.4% (95%CI; 3.3 to 3.5%) and 4.7% (95%CI; 4.5 to 4.8%) ($p<0.001$), respectively. During the survey 85.2% (95%CI: 84.9 to 85.3%) of respondents were wearing shoes, but only 57.9% (95%CI: 57.6 to 58.2%) of them were wearing protective shoes. Female sex, older age, wearing shoes after 12 years of age, washing feet less frequently than daily were significantly associated with increased odds of having podoconiosis. Attending formal education, living in a house with a covered floor were associated with decreased odds of having podoconiosis. The survey confirmed that podoconiosis remains a significant public health problem and is widely distributed in Ethiopia; it is endemic throughout 30% of the country's landmass, where more than 40% of the population live. Results provide a current benchmark of the burden of the disease, against which future podoconiosis control programmes can be measured.

1691

EFFECTS OF ELIMINATION CAMPAIGN OF LYMPHATIC FILARIASIS SEEN IN NEPAL

Harishchandra Shah

Ministry of Health and Population, Eastern Health Development Region,Dhankuta, Nepal, Dhankuta, Nepal

Effects of Elimination Campaign of lymphatic Filariasis seen in Nepal, 2014* Lymphatic filariasis (LF) is one of the five infectious diseases targeted by WHO for elimination as public health problem in Nepal. They are LF, Kala-azar, Leprosy, Yaws and Chagas disease. WHO launched GPELF with a goal to eliminate LF as public health problem by 2020 A.D. Two key strategies are: Interruption of transmission of LF infection in endemic countries by reducing microfilariae prevalence levels (below 1%) through Mass Drug Administration (MDA); Prevention and alleviation of disabilities and sufferings in individuals already affected by LF. Nepal: A total of 61 districts are considered LF endemic. Some districts with high prevalence are as high as 40% . The population at risk in Nepal are 25 millions. The causative agent are *Wuchereria bancrofti*, and transmission vector is Culex quinquefasciatus. The reported chronic conditions in 2012 are 28,835, majority were hydrocele. The 10 most morbid districts with hydrocele were Morang, Jhapa, Bardia, Banke, Saralahi, Dhading, Nuwakot,Kapilbastu, Bara, and Mahottari.LF Elimination Strategies: Interruption of transmission by Mass Drug Administration (MDA) using two drugs regimen, Diethylcarbamazine (DEC) and Albendazole, once yearly for six years. Morbidity management by self care and with support using intensive but simple, effective and local hygiene technique.MDA 2013: The number of MDA districts were 56. The total population in MDA districts was 25087450. The estimated eligible population for MDA: Phase I : 37 districts of eastern, central and western regions and Phase II : 19 districts of mid western and far western regions.

1692

REAL-TIME PCR AND MELT-CURVE ANALYSIS (QPCR-MCA) AS A REFERENCE LABORATORY TOOL FOR THE DETECTION OF *ONCHOCERCA VOLVULUS* AND ITS IMPORTANCE FOR MONITORING AND EVALUATION ACTIVITIES

Elizabeth A. Thiele[1], Vitaliano A. Cama[1], Thomson Lakwo[2], Sindew Mekasha[3], Paul T. Cantey[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Ministry of Health, Kampala, Uganda, [3]Ethiopian Public Health Institute, Addis Ababa, Ethiopia

The efforts to eliminate onchocerciasis in most of Africa by 2025 necessitate re-examination of current monitoring and evaluation tools. In particular, assessment of hypoendemic zones, stop-treatment determinations, and post-intervention surveillance will require sufficiently sensitive tools to detect low-intensity infections. Mass drug administration with ivermectin (IVM) is expected to decrease microfiladermia and thereby decrease the usefulness of skin snip microscopy, currently the standard assessment tool. Using a pan-filarial qPCR-MCA, we assessed 1) the utility of this single-step assay for detecting evidence of microfilaria (MF) in residual skin snips and 2) the sensitivity of skin snip microscopy relative to our PCR-based assay. Specificity of the qPCR-MCA to *Onchocerca volvulus* was verified using DNA from *O. volvulus* macrofilariae; MF of *B. malayi* and *pahangi*, *L. loa*, *M. ozzardi*, and *W. bancrofti*; and uninfected human controls. Utility of the qPCR-MCA assay and the relative sensitivity of microscopy were evaluated with residual skin biopsies (i.e., after 24-hour incubation in saline) collected from hyperendemic regions of Uganda and Ethiopia (n=500 each) which had received limited rounds of IVM. qPCR-MCA detected over 94% of known positive skin snips (139/147 total microscopy positive), identifiable by consistent, well-defined dissociation curves at 79.35°C (S.D. 0.22) with a minimum 1°C difference from other filarial species. Using qPCR-MCA as the reference test, the sensitivity of the skin snip microscopy was only 74.7% (121/162) and 28.1% (18/64) in Uganda and Ethiopia, respectively. Combined across countries, qPCR-MCA detected an additional 87 positive samples (38.5%), indicating a combined microscopy sensitivity of 61.5% (139/226). When evaluating low-intensity infections (≤2 MF/snip), the sensitivity of microscopy was only 46% (74/154). Thus, skin snip microscopy does not appear to be sufficiently sensitive to assess transmission in areas with low microfiladermia or to make stop-treatment decisions in the absence of other transmission assessments (e.g., vector data). qPCR-MCA can augment sensitivity and provide diagnostic confirmation of skin biopsies and will be useful for validating new monitoring tools that may be developed to support elimination efforts.

1693

FIFTY-EIGHT YEARS OF MAN AGAINST THE WORM IN BUDONGO ONCHOCERCIASIS FOCUS OF UGANDA-INTERRUPTION OF TRANSMISSION IS FINALLY IN SIGHT

Moses N. Katabarwa[1], Tom Lakwo[2], Peace Habomugisha[3], Ephraim Tukesiga[4], Edson Byamukama[3], Edson Byamukama[3], Galex Orukan Ochieng[5], William Mugayo[6], Thomson Isingoma[6], Moris Okumu[7], Martini Mborekere[8], David Oguttu[9], Annet Khainza[3], Edridah Tukahebwa[9], Rolf Garms[10], Tom Unnasch[11], Frank O. Richards[12]

[1]The Carter Center/Emory University, Atlanta, GA, United States, [2]Ministry of Health, Vector Contol Division, Kampala, Uganda, [3]The Carter Center, Kampala, Uganda, [4]Kabarole District Health Services, FortPortal, Uganda, [5]Mbale District Health Services, Mbale, Uganda, [6]Masindi Health Services, Masind, Masindi, Uganda, [7]Bulisa District Health Services, Bulisa, Uganda, [8]Hoima District Health Services, Hoima, Uganda, [9]Ministry of Health, Vector Control Division, Kampala, Uganda, [10]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, [11]Department of Global Health, University of South Florida, Tampa, FL, United States, [12]The Carter Center, Atlanta, GA, United States

The battle against onchocerciasis in hyperendemic Budongo focus (where the baseline microfilaria (mf) rate was 80% and fly infection rate 20%) has lasted over a half a century. Vector control of *Simulium neavei* using DDT commenced in1955, and larviciding was halted when no larval/pupal stages of *S. neavei* were observed on crabs, and no adults flies were found in human landing captures. Six months after stopping larviciding, however, rapid repopulation of *S. neavei* was observed. In August 1956, a new series of DDT larviciding was initiated. In 1958, the early stages of the vector could not be found in the rivers and the adult fly had disappeared by 1962 indicating elimination of *S. neavei* from the focus. Repeated courses of DEC were provided to individuals in the communities who were infected. To avoid vector reinfestation, maintenance larviciding continued until about 1971. Political unrest in Uganda led to the collapse of this work, and by 1989 *S. neavei* had again repopulated the area and onchocerciasis recrudescence had occurred. Annual mass drug administration (MDA) with ivermectin was provided from 1989 to 2007 to all the 184 affected communities and a population of 150,195 people. However, a 2008 serosurvey of 3159 children showed an OV16 antibody rate of 9.5%, indicating continued transmission. After Uganda established a policy for onchocerciasis elimination in 2007, biannual treatment was launched in Budongo in 2008, and continues to date. However, in assessments done in 2011 vector infectivity rates still ranged up to 8.7%. In June 2012, temephos (Abate®) larviciding was added to compliment twice per year ivermectin treatments. By February, 2014, only 2 (0.7%) crabs out of 300 were infested; no adult fly has been collected since September, 2013. Budongo focus is an example of a difficult onchocerciasis 'hot spot' requiring both twice yearly ivermectin MDA and vector control to break transmission.

1694

EVALUATION OF LYMPHATIC FILARIASIS AND ONCHOCERCIASIS IN THREE SENEGALESE DISTRICTS TREATED FOR ONCHOCERCIASIS WITH IVERMECTIN FOR MORE THAN 15 YEARS

Nana O. Wilson[1], Alioune Badara Ly[2], Ngayo Sy[3], Lamine Diawara[4], Abdel Direny[5], Mawo Fall[6], Vitaliano A. Cama[1], Paul T. Cantey[1], Mark L. Eberhard[1], LeAnne M. Fox[1], Daouda Ndiaye[7], Christine Dubray[1]

[1]Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Ministère de la Santé et de l'Action Sociale, Dakar, Senegal, [3]Service de Lutte Antiparasitaire, MSAS, Dakar, Senegal, [4]World Health Organization and African Programme for Onchocerciasis Control Representative, Dakar, Senegal, [5]IMA World Health, Washington, DC, United States, [6]RTI/ENVISION, Dakar, Senegal, [7]Cheikh Anta Diop University, Dakar, Senegal

In Africa, there is significant overlap between onchocerciasis and lymphatic filariasis (LF). Current efforts to eliminate these two diseases are through mass drug administration (MDA) with ivermectin alone for onchocerciasis or with ivermectin and albendazole for LF. Years of ivermectin distribution for onchocerciasis may have decreased or interrupted LF transmission in certain areas. The Kedougou region, Senegal, has been historically known to be co-endemic with LF (immunochromatographic test [ICT] prevalence ≥1%) and onchocerciasis (microfilaria prevalence 9.0-68.4%). MDA for onchocerciasis started in 1988 and as of 2014, albendazole had not been added to target LF. The objective was to assess in an integrated manner the status of LF and onchocerciasis in three districts of Kedougou after ≥15 years of ivermectin MDA. Sixteen villages close to rivers and breeding sites for onchocerciasis vector, *Simulium spp,* were selected. LF antigenemia testing (ICT) was added to skin snip microscopy for onchocerciasis evaluation. Convenience sampling of residents ≥5 years was performed. Dried blood spots were collected to test for antibodies against Wb123 (LF) and Ov16 (onchocerciasis) antigens (results pending). One village refused to participate and one was excluded because it was treated with ivermectin too recently. Forty percent (1154/2925) of residents participated; 50% were males and the median age was 15 years. In two districts, no participants were ICT or skin snip positive. In the third district, 3.4% (6/176) were ICT-positive (village range 1.9-6.4%) and 0.7% (1/150) were skin snip-positive. The mean age of ICT-positive participants was 49 years (range 25-79); the participant with a positive skin snip was 79 years old. After ≥15 years of ivermectin distribution, LF prevalence was still above treatment threshold in one of the three districts included in the evaluation. The integrated evaluation of LF and onchocerciasis provided important information on both diseases that should help program managers make decisions about treatment interventions.

1695

THE USE OF HUMAN SWEAT METABOLITES AS BAIT FOR MONITORING VECTORS OF ONCHOCERCIASIS IN WEST AFRICA AND LATIN AMERICA

Ryan M. Young[1], Nathan D. Burkett-Cadena[1], Tommy W. McGaha[2], Mario A. Rodríguez-Pérez[3], Laurent Toé[4], Bill J. Baker[1], Raymond Noblet[2], Eddie W. Cupp[5], Andrew J. Shilling[1], Javier Garza-Hernández[3], Kristel Rodríguez-Morales[3], José Torres-Estrada[6], Thomas R. Unnasch[1]

[1]University of South Florida, Tampa, FL, United States, [2]University of Georgia, Athens, GA, United States, [3]Instituto Politécnico Nacional, Reynosa, Mexico, [4]Multidisease Surveillance Centre, World Health Organization, Ouagadougou, Burkina Faso, [5]Auburn University, Auburn, AL, United States, [6]Instituto Nacional de Salud Pública, Tapachula, Mexico

Onchocerciasis, a.k.a. river blindness, is a parasitic disease caused by infection from the nematode *Onchocerca volvulus*. The parasite is transmitted to humans by the bite of infected black flies (genus

Simulium). The World Health Organization (WHO) estimates 18 million people suffer from onchocerciasis, the vast majority occurring in central Africa, and isolated foci in the American Tropics. Treatment of the disease relies on accurate epidemiological data, which is best achieved though real-time data of infection prevalence in the vectors. The need for a new monitoring method is crucial. To this end we identified key primary metabolites in human sweat, which putatively attract black flies to humans. Laboratory studies were then conducted in Southern Mexico and West Africa to test which compounds attracted these vectors of onchocerciasis, using electroantennography and y-tube olfactometry. The attractive compounds will be developed into baits to lure black flies to a novel trap for monitoring vector abundance and infection prevalence in both Latin America and Africa. In this study we describe the identification of key human sweat components via GC-MS, the identification of attractive metabolites to the two major species of *Simulium* as well as trap development and bait formulation for the continued monitoring of these important disease vector.

1696

LYMPHATIC FILARIASIS ELIMINATION: ASSESSMENT OF TWO VILLAGES WITH DIFFERENT ENDEMICITY LEVELS IN A PREVIOUSLY HIGHLY ENDEMIC REGION (SIKASSO) OF MALI

Yaya Ibrahim Coulibaly[1], Salif Seriba Doumbia[1], Ilo Dicko[1], Lamine Soumaoro[1], Massitan Dembele[2], Sekou Fantamady Traore[1], Joseph Kubofick[3], Amy Klion[3], Louise Kelly-Hope[4], Thomas B. Nutman[3], Moses J. Bockarie[4]

[1]ICER Mali, Bamako, Mali, [2]National Lymphatic Filariasis Elimination Program, Mali, Bamako, Mali, [3]National Institutes of Health, Bethesda, MD, United States, [4]CNTD, Liverpool School of Tropical Medicine, United Kingdom, United Kingdom

Lymphatic filariasis (LF) is a disfiguring neglected tropical disease that is targeted for elimination by 2020 using annual mass drug administration (MDA) in endemic areas. We previously reported the *Anopheles gambiae* complex frequencies in 2 neighboring villages of Kolondièba, one of the highly LF endemic district of Sikasso, Mali. To assess transmission interruption after 6 annual MDA in the district of Kolondièba and determine the potential impact of vector density on MDA-induced transmission interruption. A cross sectional study with quantitative and qualitative data collection methods in 2 villages of Sikasso region. The village of Boundioba had a lower *Anopheles* density as compared to Bougoula (1,494 versus 251 specimen from July to December 2011). A total of 481 volunteers in Bougoula including 340 female (70.5%) and 332 in Boundioba including 221 female (66.6%) were included in this study. The 6-7 years/15 years and above composition was 113/368 and 127/205 respectively in Bougoula and Boundioba**.** Microfilaremia was significantly more frequent in the15 years and above in Boundioba(1.95%, 4/205) as compared toBougoula (0%, 0/368), (p=0.02, Fisher exact test). Additionally, the 2 villages showed comparable low prevalences in 6-7 years olds with respectively 1/127 and 0/113 for Boundioba and Bougoula. *Anopheles* vector density may be misleading because it is not necessarily associated with a higher endemicity in a village under MDA.

1697

POTENTIAL RE-EMERGENCE OF *WUCHERERIA BANCROFTI* TRANSMISSION IN A PREVIOUSLY CONTROLLED HYPERENDEMIC REGION (SIKASSO) IN SOUTHERN MALI

Siaka Yamoussa Coulibaly[1], Yaya Ibrahim Coulibaly[1], Ilo Dicko[1], Siaka Konate[1], Housseini Dolo[1], Moussa Brema Sangare[1], Michel Emmanuel Coulibaly[1], Lamine Soumaoro[1], Abdallah Amadou Diallo[1], Modibo Sangare[1], Benoit Dembele[1], Massitan Dembele[2], Pj Hooper[3], Eric Ottesen[3], Dominique Kyelem[3], Sekou F. Traore[1], Amy D. Klion[4], Thomas B. Nutman[4]

[1]ICER Mali, Bamako, Mali, [2]National Lymphatic Filariasis Elimination Program, Mali, Bamako, Mali, [3]Neglected Tropical Diseases Support Center, Task Force for Global Health, Atlanta, GA, United States, [4]National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States

Mass drug administration (MDA) for the elimination of lymphatic filariasis (LF) has led to potential transmission interruption in several endemic regions of Mali based on transmission assessment surveys (TAS). To assess the utility of TAS approach, we utilized standard TAS methodology (ICT positive prevalence in 6-7 year olds) and compared it to xenomonitoring, night blood microfilariae counts and IgG4 antibody to Wb123 (for the last 3 years) over a five year period (2009-2014) following the cessation of MDA in 6 villages in the region of Sikasso in southern Mali. In 2009 (at the start of the surveillance period) all 289 children aged 6-7 years were negative for circulating filarial antigen (CFA) by ICT, by calibrated thick smears of blood collected at night, and by IgG4 antibody to Wb123. Despite this, 2/4391 (0.11%) dissected mosquitoes were positive for larvae of *Wuchereria bancrofti* (Wb). In 2011, there was a CFA prevalence by ICT of 2.6% (8/301) in the 6-7 year olds, a prevalence of 1.09% (1/92) for antibody responses to Wb123, but negative xenomonitoring. In the subsequent 2 years (2012 and 2013), there were consistent and significant increases in the prevalence of CFA (Trend Chi^2= 11.49, p=0.0007) to 3.9% (11/285) in 2012, and 4.1% (13/316) and in the prevalence of anti-Wb123 IgG4 to 3.2% (10/316) in 2013. Despite this increase in both ICT and Wb123 IgG4 antibody prevalence, no infected anopheline mosquitoes were found in 2011, 2012 and 2013. These data suggest that despite having met the criteria for cessation at the beginning of the surveillance, that there appears to be low level emergence of Wb transmission and that antibody monitoring may provide a better early warning tool than more standard TAS tools.

1698

SYSTEMIC NON-COMPLIANCE: A POTENTIAL FACTOR INTHE RE-EMERGENCE OF LYMPHATIC FILARIASIS TRANSMISSION IN SIKASSO, MALI

Ilo Dicko[1], Yaya Ibrahim Coulibaly[1], Siaka Yamoussa Coulibaly[1], Housseini Dolo[1], Moussa Brema Sangare[1], Siaka Konate[1], Michel Emmanuel Coulibaly[1], Lamine Soumaoro[1], Abdallah Amadou Diallo[1], Modibo Sangare[1], Benoit Dembele[1], Massitan Dembele[2], Pj Hooper[3], Eric Ottesen[3], Dominique Kyelem[3], Thomas B. Nutman[4], Sekou Fantamady Traore[1], Amy D. Klion[4]

[1]ICER Mali, Bamako, Mali, [2]National Lymphatic Filariasis Elimination Program, Mali, Bamako, Mali, [3]Neglected Tropical Diseases Support Center, Task Force for Global Health, Atlanta, GA, United States, [4]National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States

Recent transmission assessment surveys (TAS) suggest low level re-emergence of *Wuchereria bancrofti* transmission after cessation of mass drug administration (MDA) in 6 previously hyperendemic villages in Sikasso, Mali. Coverage rates in the villages ranged from 67% to 89.6% over the 7 years of MDA and all stopping criteria were met at the beginning of the surveillance period. To begin to identify potential causes for this re-emergence, a questionnaire was administered to randomly selected adult residents of the six villages to assess the prevalence of and

reasons for systematic non-compliance with MDA. A total of 486 subjects (170 men and 316 women) were questioned, of whom 16.1% (79/486) reported never swallowing MDA drugs. The most common reasons given were being unaware of MDA (24/486; 4.9%), being pregnant or breast-feeding (8/486; 1.6%) and not willing to take the drugs (6/486; 1.2%). Although systematic non-compliers were more likely to be younger [OR = 1.7 (1.006-2.921) for individuals 15-30 vs. >30 years of age], compliant and systematically non-compliant subjects were similar with respect to participants' instruction level [OR = 1.2 (0.59-2.51)] and the presence of lymphoedema / hydrocele [OR = 0.5 (0.11-2.63)]. These data suggest that significant rates of systematic non-compliance can be present despite adequate overall coverage rates. Whether persistent infection in systematic non-compliers provided the reservoir for re-emergence of transmission in the 6 study villages requires further study.

1699

NON-*MANSONELLA OZZARDI* ATYPICAL MICROFILARIASIS IN THE PERUVIAN AMAZON BASIN

Salomon Durand[1], Dolores Rimarachin[2], Amy C. Morrison[2], Karen Campos[2], Jorge Nunez[1], Moises Sihuincha[3], Geral C. Baldeviano[1], Andres G. Lescano[1]

[1]U.S. Naval Medical Research Unit - 6, Callao, Peru, [2]U.S. Naval Medical Research Unit - 6, Iquitos, Peru, [3]Hospital Apoyo Iquitos, Iquitos, Peru

Filariasis is a neglected tropical disease around the world. This disease is a vector-borne infection caused by nematodes (roundworms) of the family Onchocercidae. Since 1950s, filariasis caused by *Mansonella ozzardi* has been regularly reported in the Peruvian Amazon basin. Isolated cases have been diagnosed as *M. perstans*, *Bruguia* spp, *Onchocerca* spp. and *Dirofilaria* spp. Since 2010, by examining de-identified blood samples (thick smear and Knott's concentration techniques) we have characterized the prevalence of filarial infections and associated clinical symptoms in both rural (n=383) and urban (n=755) human populations located on tributaries of the Amazon River near and in Iquitos, Peru. In the rural communities, prevalence of microfilariae was 28.5% (109/383) overall; but the majority 99% (108/109) were *M. ozzardi.* Prevalence rates were not heterogeneous ranging from 0 to 72.5% in 11 communities. In contrast, of 755 samples from residents of Iquitos, 2% and 4% were infested with *M. ozzardi* and an atypical microfilaria, respectively. Interestingly, those infected with *M. ozzardi* tended to be febrile adult males with occupations associated with rural areas, whereas those infested with the atypical parasite were rarely febrile and were often children or housewives. Surveillance in local hospitals identified at least one morphologically distinct atypical microfilariae and one co-infection with *M. ozzardi* in seven symptomatic patients; one had subacute skin lesions and the others fever. The atypical microfilariae were macroscopically distinct from *M. ozzardi*, measuring 600 x 8 µm, with no sheath and no nuclei in the tail. In a subset of samples tested by PCR. All *M. ozzardi* were confirmed, but five of the atypical microfilariae tested negative the internal transcribed spacer rDNA sequence of *M. perstans* and *Onchocerca volvulus.* In conclusion, atypical microfilariae with a distinct epidemiology from *M. ozzardi*, and not related to *M. perstans,* or *O. volvulus*, are sufficiently prevalent to warrant investigation of their health impact in the Peruvian Amazon.

1700

INTEGRATED FILARIAL MICRO-MAPPING TO DETERMINE IMPLEMENTATION STRATEGIES IN *LOA LOA* CO-ENDEMIC AREAS: THE ANGOLAN EXPERIENCE

Miguel Brito[1], Rossely Pauloi[1], Tom Unnasch[2], Robert Novak[2], Benjamin Jacob[2], Michelle Stanton[3], David Molyneux[3], Louise Kelly-Hope[3]

[1]CISA - Health Research Centre in Angola, Caxito, Angola, [2]College of Public Health, Department of Global Health, University of South Florida, Tampa, FL, United States, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

The co-distribution of *Loa loa* (loiasis or tropical eye worm) is a significant impediment to the lymphatic filariasis (LF) and onchocerciasis elimination programmes in Angola, due to the potential risk of severe adverse events (SAEs) associated with the drug ivermectin when given to individuals with high *L. loa* microfilarial loads in the blood. This has significant implications for mass drug administration campaigns and alternative strategies may be required in selected areas. One of the highest *L. loa* risk areas is Dande Municipality, Bengo Province in Northern Angola, an area which historically has reported cases of LF and onchocerciasis. To better determine the safest treatment strategy in this area, this study conducted an integrated filarial micro-mapping survey to understand the geographical overlap of the three diseases. GIS-Remote Rapid Eye satellite data was also employed to provide the foundation for empirical information on vector and parasite populations. In total 23 villages, distanced approximately 10-15km apart, across peri-urban and rural areas were surveyed during January-February 2014. In each village, up to 100 individuals were assessed using the rapid assessment procedure for loiasis (RAPLOA) and rapid epidemiological mapping of onchocerciasis (REMO), and two questions on LF morbidity (presence of lymphedema, hydrocele). The study found low levels of endemicity of all three diseases (<20%), with different overlapping distributions, with most villages reporting at least one filariasis case. To confirm the hypo-endemic levels of LF and onchocerciasis, a further seroprevalence survey using rapid diagnostic tests in the same villages is planned for June 2014. This will provide additional micro-epidemiological information to help determine if the recommended alternative strategy of albendazole twice yearly and long-lasting/insecticide treated bednets (LLINs/ITNs) should be used for LF elimination, and if an alternative to ivermectin for hypoendemic onchocerciasis elimination, such as the drug doxycycline or vector control, needs to be considered.

1701

SERO-PREVALENCE AND RISK FACTOR SURVEY FOR LYMPHATIC FILARIASIS IN PAPUA NEW GUINEA

Melinda Susapu[1], Moses Bockarie[2], Lisa Reimer[2], Louise Kelly-Hope[2]

[1]Department of Health, Port Moresby, Papua New Guinea, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Papua New Guinea (PNG) has an estimated population of 7 million inhabitants; of which 4 million are predicted to be at risk of lymphatic filariasis (LF). LF is a debilitating disease caused by lymphatic-dwelling nematodes *Wuchereria bancrofti*, which is transmitted by *Anopheles* mosquitoes in PNG, similar to malaria. Some national and published data exists, however, the geographical distribution, burden of disease and associated risk factors are currently not well defined. This study investigated the prevalence and potential risk factors of LF in one endemic province of PNG through the administration of a prevalence survey and household questionnaire related to the LF transmission and the national LF programme. In April 2013, four villages in a southern remote area of Madang Province were selected. Approximately 100 individuals in each village were interviewed and examined for LF infection, using ICT rapid diagnostic kits, and evidence of clinical disease. This study found that 32 individuals out of 389 surveyed (8.2%) were LF antigen positive, and 3 individuals had lymphodema (elephantiasis) of the leg (0.8%). All of those

interviewed did not know about the disease, what caused it, how it was transmitted or were aware of the national programme to eliminate LF. A follow-up microfilaremia (MF) survey was conducted in the study site with the most ICT positive individuals, and included the majority of community members (n=300). Preliminary results indicate the average Mf prevalence was 41.5%, and ranged from 10.5% in children under 10 years, to 54.6% in adults over 50 years of age. Mf prevalence was found to be higher in males (46.4%) than females (34.9%), and among those living in houses made of bush material (45.8%) compared with other semi-permanent materials (23.1%). The field work is still in progress and expected to be finish with final results by November 2014. This research highlights that LF is endemic in remote areas of the country and the national LF programme has to scale up its efforts to control and eliminate the spread of infection with particular emphasis on LF advocacy and education to those most at risk.

1702

METHODS FOR ASSESSING LYMPHATIC FILARIASIS TRANSMISSION IN LOW ENDEMIC AREAS OF BANGLADESH: ONE STEP CLOSER TO THE ELIMINATION GOAL

A. S. M. Sultan Mahmood[1], Rouseli Haq[1], Mujibur Rahman[1], Shamsur Rahman[1], Louise Kelly-Hope[2]

[1]Directorate General of Health Services, Dhaka, Bangladesh, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Bangladesh had one of the highest burdens of lymphatic filariasis (LF) at the beginning of the Global Programme to Eliminate LF (GPELF), with an estimated 80 million people at risk of infection. Baseline mapping in 2000 using the rapid diagnostic ICT cards, found that 34 of the 64 districts in the country were endemic, however, only 19 districts required mass drug administration (MDA) using the drugs albendazole and DEC to interrupt transmission. The Bangladesh LF Programme has successfully scaled up MDA in these districts, and is moving into the elimination phase and using the WHO recommended Transmission Assessment Survey (TAS) to assess their success in interrupting transmission - so far with excellent results. The outstanding important question for the LF Programme was how to assess the 15 endemic districts that were found to have low prevalence levels (<1%) and not be eligible for MDA. Follow-up night blood microfilaria (Mf) and community clinical surveys undertaken in 2008 -2010 in selected areas of these districts found little or no evidence of infection and disease such as lymphedema and hydrocele. Currently, there is no recommended strategy for assessing low endemic districts, therefore, in order to address this issue and provide more rigorous evidence that LF is not a public health problem, the TAS method is being used as an assessment tool with additional systematic patient searching at household level. The assessments are planned for each month of 2014 and being carried out by trained field teams visiting schools for TAS (targeting children) and using local community clinic workers and volunteers to visit households (targeting individuals with clinical manifestations). To date five districts have been assessed with good results, and if the remaining districts are also found to have little or no LF infection or disease, the national LF programmes can 'shrink the LF map' by approximately 30 million people and move one step closer to their elimination goal, with an increased focus on the new priorities of surveillance and morbidity management.

1703

SYSTEMATIC REVIEW AND META-ANALYSIS OF DOXYCYCLINE IN CONTROL OF LYMPHATIC FILARIASIS

Patricia A. Woodall

Emory University, Atlanta, GA, United States

Lymphatic Filariasis control programs rely on periodic population-wide administration of microfilaricidal agents repeated over several years to decrease transmission. Effective macrofilaricidal agents could decrease the years necessary to achieve elimination. Doxycycline has emerged as a possible agent due to its effects on the Wolbachia endosymbiont. A systematic review identified 9 published randomized controlled trials evaluating the effects of various Doxycycline regimens on *Wuchereria* or *Brugia* microfilaria levels at 12 months post-treatment in 5 countries, with or without interim single-dose microfilaricide (Ivermectin or DEC). For elimination of microfilariae at 12 months (compared to placebo), the pooled Risk Ratio was 3.22 (1.95, 5.32) , with high heterogeneity (I^2=68%). Subgroup analysis showed: Doxycycline 6-8 week regimens, RR= 3.96 (2.07, 7.59); Doxycycline 3-4 week regimens, RR= 2.14 (1.08, 4.26); Ivermectin or DEC 4 months after Doxycycline, RR= 2.29 (1.73, 3.04); no interim microfilaricide, RR= 5.80 (2.36, 14.24). Multi-day Doxycycline regimens effectively eliminate LF microfilariae at 1 year after treatment. Applicability of such multi-day regimens to population-wide control programs is limited. Further studies should evaluate shorter-term treatment.

1704

SIMULTANEOUS DETECTION OF *ONCHOCERCA VOLVULUS* AND *O. OCHENGI* IN INFECTED *SIMULIUM* FLIES USING ANEW MULTIPLEX REAL-TIME PCR

Anna Albers[1], Christina Weier[1], Sandra Arriens[1], Samuel Wanji[2], **Kenneth Pfarr**[1], Achim Hoerauf[1]

[1]University Hospital of Bonn, Bonn, Germany, [2]Research Foundation for Tropical Diseases and Environment, Buea, Cameroon

Onchocerca volvulus parasitic worms infect ~37 million people in sub-Saharan Africa and parts of Latin America, causing dermatitis, skin atrophy and visual impairment. In 27 endemic countries in Africa, 130 million people at risk of the disease. The January, 2014 report of the International Task Force for Disease Eradication has stated the continued need for improved diagnostics to assess when mass drug administration efforts can be halted and to monitor for recrudescence. Currently transmission is monitored by identifying larvae in dissected *Simulium damnosum* species, the vector of *O. volvulus*, which are also able to transmit *O. ochengi*, a parasitic worm of cattle that does not infect humans. We developed a multiplex real-time PCR based on the *ND5* gene of the Onchocercidae genus with specific TaqMan probes to differentiate *O. volvulus* and *O. ochengi* from other Onchocercidae. A blinded study with 217 flies from *O. volvulus* and *O. ochengi* endemic and *O. volvulus*/*O. ochengi* co-endemic areas in Cameroon (n=23) showed 100% specificity in all analyzed *Simulium* flies. Vector monitoring to assess transmission potential in endemic areas is reliable. Our multi-plex real-time PCR offers time and cost savings over species identification via microscopy.

1705

MODELING THE EFFECTS OF MASS DRUG TREATMENTS AND VECTOR CONTROL ON CO-INFECTION WITH MALARIA AND LYMPHATIC FILARIASIS

Sarah Lukens, Brajendra K. Singh, Edwin Michael

University of Notre Dame, Notre Dame, IN, United States

Malaria and lymphatic filariasis (LF) are both transmitted by anopheles mosquitoes and are co-endemic in many regions of the tropics. For ongoing global campaigns to eliminate LF and malaria, it is important to understand interactions between both parasites and hosts where they co-exist. The use of mass drug administrations (MDA) to reduce prevalence and intensity of microfilariae may increase the lifespan of anopheles mosquitoes and thereby potentially increasing the transmission of malaria, while interactions at the host level may affect susceptibility, disease severity, and co-transmission of both diseases. Each parasite system alone exhibits complex dynamics where factors such as vector biting rates and threshold prevalence of human infection contributes to either extinction or stabilization to an endemic level. Knowledge of how interactions between both systems may affect co-infection endemicity and extinction dynamics is important for designing effective disease management programs such as MDA and vector control (VC). We extend a mathematical model of

malaria-LF co-infection to describe how the interplay between these two infections influence threshold behaviors in the system, and how MDA and VC interventions can influence elimination or resurgence of both diseases.

1706

IMPACT OF MEROWE DAM ON ONCHOCERCIASIS VECTORS OF ABU HAMED, NORTHERN SUDAN

Isam Zarroug[1], Arwa Elaagib[2], Sara Abuelmaali[3], Hanan Mohamed[1], Wigdan Elmubarak[1], Kamal Hashim[4], Tong Chor M Deran[1], Nabil Aziz[5], Tarig Higazi[6]

[1]National Onchocerciasis Control Program, Khartoum, Sudan, [2]University of Khartoum, Khartoum, Sudan, [3]National Health Laboratories, Khartoum, Sudan, [4]National Programme for Prevention of Blindness, Khartoum, Sudan, [5]The Carter Center, Khartoum, Sudan, [6]Ohio University, Zanesville, OH, United States

Abu Hamed, the northernmost onchocerciasis focus in the world, is located along the River Nile banks in the Nubian Desert. Hydroelectric dams can alter activity of black flies and may provide breeding sites for black fly. Merowe Dam, the largest hydropower project in Africa, was built west of Abu Hamed focus in 2009. The impact of the Dam on onchocerciasis and its black fly vectors in Abu Hamed focus was measured in this study. Entomological surveys for aquatic stages and adult *Simulium hamedense* were conducted before and after the inception of Merowe Dam in 2007/2008 and 2010/2011. There was no black fly breeding or adult activity in the previously known breeding sites upstream of the Merowe Dam with the western most breeding site found in Al Sarsaf village near the center of the focus. No adult or aquatic stages of black flies were found downstream of the Dam. The artificial lake of the Dam flooded all the breeding sites in the western region of the focus and no aquatic stages and/or adult black fly activity were established in the study area upstream of the Dam. The Dam seems to have positive impact on onchocerciasis and its black fly vectors in Abu Hamed focus. These outcomes of the Merowe Dam might have contributed to the recently declared interruption of onchocerciasis transmission in Abu Hamed focus. Continuous entomological surveys are needed to monitor presence of black fly vectors and its impact on the disease.

1707

THE CURRENT STATUS OF LYMPHATIC FILARIASIS IN COTE D'IVOIRE PRIOR TO IMPLEMENTATION OF A NATIONAL PROGRAM OF MASS DRUG ADMINISTRATION

Aboulaye Meite[1], Andrew C. Majewski[2], Gary J. Weil[2], Peter U. Fischer[2]

[1]Programme National de lutte contre la Schistosomiase, les Géohelminthiases et la Filariose Lymphatique, Ministry of Health, Abidjan, Côte D'Ivoire, [2]Washington University School of Medicine, St. Louis, MO, United States

Côte d'Ivoire is planning to implement a coordinated national program of mass drug administration (MDA) for elimination of onchocerciasis (Oncho) and lymphatic filariasis (LF) in the near future. The country has complex patterns of endemicity for these infections with extensive areas of coendemicity, areas that have received mass drug administration with ivermectin for variable periods, and extensive migration to and from neighboring countries (Liberia, Ghana, and Burkina Faso). For example, some areas in the northern part of the country have received many rounds of ivermectin for onchocerciasis control during OCP and APOC, and LF rates tend to be low in the North. In contrast, Oncho is uncommon in coastal areas (with some exceptions), and little ivermectin has been used in the South. Some 47 of the country's 82 health districts (mainly in Central and Southern districts) are considered to be co-endemic for LF and Oncho. LF mapping circa 2001 was based on antigen testing (Binax Now Filariasis, card test) of 50-100 people in two villages per district. The current study was performed to obtain more current information on the distribution of LF in the country and to identify sentinel sites for monitoring and evaluation of the impact MDA on LF. More than 3,900 people were tested for filarial antigenemia in 40 villages in 6 districts in the central and Southeastern part of the country. Antigen rates ranged from 4-22% in Lakota, 4-21% in Tiebissou, 15-41% in Akoupe, 21-25% in Agboville, 9-14% in Bettie, and 6-35% in Abengourou districts. Microfilaremia rates ranged from 1% in Lakota to 11% in Abengourou and Agboville. Prior ivermectin distribution in areas with coendemic onchocerciasis may partially explain the highly variable Mf rates in these areas. This study has helped to establish the current LF situation in Côte d'Ivoire, and this information will be used to plan and implement the national LF elimination program based on MDA.

1708

PROGRAMMATIC IMPLICATIONS OF EXTENSIVE VECTOR CONTROL ON THE ELIMINATION OF LYMPHATIC FILARIASIS IN ZAMBIA

Mutale Nsakashalo[1], Michelle Stanton[2], Enala Mwase[3], Elizabeth Chizema[1], Victor Mukonka[4], Peter Songolo[5], Brent Thomas[2], Maria Rebollo[2], Moses Bockarie[2], J. Russell Stothard[2], Louise Kelly-Hope[2]

[1]Ministry of Health, Lusaka, Zambia, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]University of Zambia, Lusaka, Zambia, [4]Copperbelt University of Zambia, Ndola, Zambia, [5]World Health Organization Country Office, Lusaka, Zambia

Lymphatic filariasis (LF) is widely endemic in Zambia, and the National LF Programme is planning the first nationwide mass drug administration (MDA) to interrupt transmission in 2014. Overall the LF prevalence is low (<10%) in most regions of the country and it is possible that the well establish vector control programme for malaria has already impacted LF transmission over the past decade. To better understand the distribution of vector control that has occurred across the country and the potential implications for the LF Programme as it moves to scale up MDA, this study developed a spatially modelled vector control map and examined it in relation to the baseline LF prevalence data collected across 108 geo-referenced sites in 2003 and 2011, and the sentinel site data collected across 32 geo-referenced sites in 2014. Information on bed nets, including long-lasting/insecticide treated bed nets (LLIN/ITNs) and indoor residual spraying (IRS) distributions was obtained from the Ministry of Health, and public data sources such as the Demographic Health Survey (DHS) data, President's Malaria Initiative (PMI) reports, and combined in a weighted sum to form a multiple vector intervention score, which was then used to produce district-level maps of vector control intensity. Each district was classified according to LF prevalence and the vector intervention score which included the following combinations i) low LF /high vector control, ii) low LF / low vector control iii) high LF/ high vector control and iv) high LF/low vector control. These groups will help the LF Programme as it scales up MDA to determine if a district has potential for elimination, in need of very high MDA coverage and will require standard or enhanced surveillance.

1709

MODELING IMPACTS OF INTEGRATED VECTOR CONTROL ON LYMPHATIC FILARIASIS TRANSMISSION DYNAMICS AND ELIMINATION PROCESS

Brajendra K. Singh, Edwin Michael

The University of Notre Dame, Notre Dame, IN, United States

Lymphatic filariasis (LF) is a target for global eradication by 2020. Launched in 2000, the Global Programme to Eliminate Lymphatic Filariasis relies mainly on large-scale preventative chemotherapy programs via mass drug administration (MDA) to eliminate this major vector-borne parasitic disease from all endemic settings. Modeling studies of LF transmission and control have been crucial for designing national control programs by establishing and quantifying the presence of infection and vector biting thresholds below which the transmission is interrupted. Renewed malaria control efforts have witnessed large-scale applications of vector control

(VC) through the use of long lasting insecticidal nets (LLIN) alone or in some combination with indoor residual spray (IRS) across the majority of LF endemic regions. LLIN/IRS affects the transmission of mosquito-borne infections either by directly killing, or by preventing mosquitoes from coming into contact with infected hosts through several mechanisms. Recent community trials have shown the substantial impact that VC may have in enhancing LF transmission interruption particularly when infection prevalence has been depressed to low levels using MDA. Despite these observations, theoretical and quantitative modeling of the impact of VC on LF transmission dynamics that takes explicit account of the various effects the different VC options may have on mosquito populations is scarce. Such analysis is vital when chemical insecticides require repeated applications in the affected communities due to their variable durations of effectiveness. This need for frequent insecticide applications introduces a number of factors such as the effects of adherence to the recommended timeframe for the replenishment of LLIN/IRS and the maintenance of the required community coverage, which may contribute to different outcomes from VC between communities. We aim to extend our present Bayesian Melding LF modeling framework by incorporating the specific effects that the application of LLIN and IRS, used separately or in combination, may have on the effective mosquito biting rate to quantify and gain better insights on the role of VC in the MDA-based LF control programs.

1710

AXENICALLY-DERIVED *CAENORHABDITIS ELEGANS* ANTIGEN FOR THE TREATMENT OF AUTOIMMUNITY

Belinda M. Jackson[1], Marina N. Torrero[2], Edward Mitre[1]

[1]Uniformed Services University, Bethesda, MD, United States, [2]National Institutes of Health, Bethesda, MD, United States

The potential use of helminth infections as a protective measure against inflammatory disease has been validated in both animal and clinical studies. However, several obstacles impede the use of parasitic helminths in clinical practice. These include difficulties in obtaining large quantities due to complex lifecycles, challenges in purification from lifecycle hosts, inter-batch variability in product, and the potential of live infections to cause clinical symptoms. To overcome these hurdles, we tested whether a homogenate of soluble antigens prepared from axenically grown *Caenorhabditis elegans* (aCeAg) would protect against autoimmunity. Using a TLR4 reporter cell line, we demonstrated that, in contrast to soluble antigen prepared from *C. elegans* grown on *Escherichia coli* lawns, aCeAg lacks LPS and does not activate TLR4. Twice weekly intraperitoneal injections of 100 mcg of aCeAg protected against the development of type 1 diabetes in non-obese Diabetic (NOD) mice (80% T1DM in PBS-injected controls, vs 10% in aCeAg group). Histological analysis demonstrated twice as many pancreatic islets in aCeAg-treated mice ($p<0.001$) as well as greater numbers of uninfiltrated islets. As observed in studies using antigens from parasitic helminths, aCeAg treatments increased the levels of basophils, eosinophils, and polyclonal and helminth-specific IgE immunoglobulins. Further, we observed increased production of the suppressive cytokine IL-10 ($p<0.05$), but not of the pro-inflammatory cytokine IFN-gamma, from splenocytes of aCeAg-treated animals. This study demonstrates proof-of-concept that antigens obtained from the non-parasitic nematode *C. elegans* can be used to obtain the same immune responses, and same immunoprotective effects, as parasitic helminths. Given that *C. elegans* can be grown axenically in controlled conditions without the need of any intermediate hosts, aCeAg may be able to overcome many of the current obstacles facing helmintic therapies for inflammatory diseases.

1711

DIFFERENCES IN OV-16 ELISA IMMUNE RESPONSES AMONG CHIMPANZEES INOCULATED WITH *ONCHOCERCA VOLVULUS*

Circe E. McDonald, Paul N. Cantey, Vitaliano Cama, Mark L. Eberhard

Centers for Disease Control and Prevention, Center for Global Health, Atlanta, GA, United States

Onchocerciasis, also known as river blindness, is a neglected tropical disease caused by the filarial parasite *Onchocerca volvulus*. Detection of immunoglobulin G4 (IgG4) antibodies to Ov16 recombinant antigen is the basis for serological tests for onchocerciasis. However, the dynamics of this immune response have yet to be thoroughly characterized. A non-human primate model was used to evaluate the temporal evolution of antibody responses under controlled infection conditions. Nine chimpanzees were inoculated with third-stage larvae of *O. volvulus*. Three chimpanzees were inoculated with approximately 100 stage three larva (L3) of Guatemalan origin, either one, three, or five times. Six chimpanzees each were inoculated once with 200, 300, or 400 L3 of Liberian origin. On a monthly basis, serum was collected and the presence of microfilariae (Mf) was determined via skin snip microscopy. Seven of nine chimpanzees developed patent infections, and six were used to evaluate the temporal responses over a median number of 1,660 days post-inoculation (PI). The seventh chimpanzee with patent infection was not evaluated due to health complications and was withdrawn at 535 days PI. Infections were categorized based on average microfiladermia of three consecutive dates as: weak (<10Mf/snip), mild (≥10 and <20) and strong (≥20 Mf/snip). One chimpanzee had a weak infection, two developed mild infections, and three had strong infections. No positive IgG4 responses to Ov16 were detected in the two inoculated but uninfected chimpanzees. The mean time to develop IgG4 responses and detection of Mf were 414 and 485 days PI. Four chimpanzees showed decreases in IgG4 values towards the end of the study. In three of these chimpanzees, decreased IgG4 responses were detected with decreasing microfiladermia. These findings indicate that positive serology to Ov16 occurs only among chimpanzees that developed patent infections, and suggest that anti-Ov16 antibody responses may decrease over time after reductions in detectable Mf loads in skin snips.

1712

CHARACTERIZING REACTIVITY TO *ONCHOCERCA VOLVULUS* ANTIGENS IN MULTIPLEX BEAD ASSAYS

Karla Feeser[1], Vitaliano Cama[1], Elizabeth A. Thiele[1], Thomson Lakwo[2], Sindew Mekasha[3], Jeffrey Priest[1], Paul T. Cantey[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Ministry of Health, Vector Control Division, Kampala, Uganda, [3]Ethiopian Public Health Institute, Addis Ababa, Ethiopia

Onchocerciasis is a neglected tropical disease targeted for elimination in Africa by 2025. Multiplex assays can provide a powerful platform for monitoring and evaluation, as well as integrated surveillance of onchocerciasis and other co-endemic diseases; however, the specificity and sensitivity of *O. volvulus* antigens have not been well-characterized within this context. A multiplex immunoassay was developed and used to evaluate three antigens (Ov16, Ov17 and Ov33) for onchocerciasis. The performance of each antigen was characterized using a panel of 499 specimens. One hundred ten samples were positive for onchocerciasis by skin snip microscopy and PCR, while the 389 controls were from people living in areas where onchocerciasis was not endemic and had infection with *Wuchereria bancrofti, Brugia malayi*, *Loa loa*, *Mansonella spp*, *Strongyloides stercoralis*, *Hymenolepis nana*, cysticercosis, schistosomiasis, or other human pathogenic parasites. All samples were analyzed in duplicate for IgG and IgG4 reactivity. Receiver Operator Characteristics (ROC) analyses were used to determine optimal cutoffs for all antigens. High sensitivity and specificity were detected for Ov16 and Ov33, while the Ov17 assays had specificities below 80%, identifying 75 false positives

among controls with lymphatic filariasis (LF). The Ov16 cutoff values for IgG or IgG4 were 379 and 32 fluorescent units (MFI), with sensitivities of 96.3 and 96.3% and specificities of 98.7 and 99.7%, respectively. For Ov33, a cutoff of 5,216 MFI in IgG reactivity resulted in 90.8% sensitivity and 97.2% specificity. The IgG4 cutoff was 67 MFI with a higher sensitivity of 96.3% and specificity of 98.5%.The IgG4 assay for both Ov16 and Ov33 detected few false positives, although the Ov33 assay detected 5 additional false positives among onchocerciasis-negative samples that were positive for either LF (3) or schistosomiasis (2). While no statistical difference was detected between the IgG and IgG4 assays for Ov16 and Ov33 (p>0.3), assays with the highest specificity and lowest cutoff values will help to ensure the ability of programs to monitor their work towards reaching desired elimination endpoints. Overall, Ov16 and Ov33 are highly sensitive and specific antigens in the multiplex platform. Further analysis of these antigens, either alone or in combination, may be useful for monitoring and evaluating progress towards the elimination of onchocerciasis.

1713

EVALUATION OF HLA IMMUNOINFORMATICS FOR THE IDENTIFICATION OF *BRUGIA MALAYI* PUTATIVE T CELL EPITOPES CONSERVED WITH *WUCHERERIA BANCROFTI* AND *LOA LOA*

Melissa Torres[1], Nils Pilotte[1], Tomabu Adjobimey[2], Sabine Specht[2], Frances Terry[3], Alexander Debrah[4], Linda Batsa[5], Alexander Kwarteng[2], Anne De Groot[3], Steven Williams[1], Achim Hoerauf[2]

[1]Smith College, Northampton, MA, United States, [2]Institute of Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany, [3]EpiVax, Providence, RI, United States, [4]Faculty of Allied Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [5]Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana

The availability of filarial genome sequences and improved immunoinformatics tools promises to accelerate the identification of highly conserved and immunogenic filarial vaccine components. The work done in this study has taken advantage of the functional similarities, rather than genetic diversity, of HLA binding residues in efforts to further identify putative T cell epitopes as potential vaccine antigens to combat lymphatic filariasis (LF). Predictions were previously made using the iVAX websuite containing both the EpiMatrix and ClustiMER immunoinformatics tools. 20-mer peptide sequences were selected for peptide synthesis from proteins within the *Brugia malayi* secretome. The 20 sequences were selected based on predictions to bind up to 8 of the most common HLA alleles represented within the software toolkit: DRB1*0101, DRB1*0301, DRB1*0401, DRB1*0701, DRB1*0801, DRB1*1101, and DRB1*1501. *In silico* filarial conservation analyses were done using basic local alignment tools within BROAD Institute's Filarial Worms Database. These analyses identified cross conservation for 6 out of the 20 sequences that shared sequence identity between *B. malayi* and *Wuchereria bancrofti* and/or *Loa loa.* Proof-of-principle assays for all 20 putative epitopes were performed using rHLA binding assays tested with 4 out of the 8 HLA alleles predicted by the software: DRB1*0101, DRB1*0401, DRB1*1101, and DRB1*1501. Results from these competitive binding assays demonstrated allele-specific binding biases. The 6 putative sequences sharing conservation with *W. bancrofti* and/or *L. loa* were tested on PBMCs from patients living in LF endemic areas that had been exposed to *W. bancrofti.* Upon peptide stimulation, subset CD4+ and CD8+ T cell populations from patients infected with *W. bancrofti* were selected for determination of cytokine-specific responses by ELISpot and flow cytometry. Results demonstrated the predicted peptides derived from the *B. malayi* secretome were capable of inducing T cell responses, which differed dependent on infection and disease status. These results suggested that the cross- conserved peptides were capable of binding to HLA from patients exposed to *W. bancrofti.*

1714

HOOKWORM INFECTION IN SCHOOL-AGED KENYAN CHILDREN IS ASSOCIATED WITH LOWER PHYSICAL FITNESS

Monica Nayakwadi Singer[1], Indu Malhotra[2], Francis Mutuku[3], Jackson Muinde[4], Peter Mungai[4], Dunstan Mukoko[4], Ginny Gildengorin[1], Charles King[2], A. Desiree LaBeaud[1]

[1]University of California San Francisco Benioff Children's Hospital Oakland, Oakland, CA, United States, [2]Case Western Reserve University, Cleveland, OH, United States, [3]Technical University of Mombasa, Mombasa, Kenya, [4]Division of Vector Borne and Neglected Tropical Diseases, Nairobi, Kenya

Children living in parasite-endemic resource-limited areas are heavily burdened by infection and the comorbidities associated with these diseases. Reduced physical fitness can result from anemia, undernutrition and chronic parasitic infections including soil transmitted helminths (STH), *Entamoeba histolytica*, *Giardia lamblia*, malaria and schistosomiasis. Our goal was to determine the prevalence of parasitic infections and their association with physical fitness as measured by the validated multi-stage 20 meter shuttle run (20mSRT) method. From January to March 2014, a cohort of 101children aged 4-7 years in coastal Kenya was evaluated. At the visit, blood, stool, and urine were collected and tested for presence of infection as follows: blood smear for malaria, Ritchie stool examination for STH, *E. histolytica*, and *G. lamblia*, and urine filtration for *S. haematobium*. 20mSRT were scored based on level achieved. Descriptive statistics were used to estimate infection rates. Wilcoxon scores determined the association between each type of infection and 20mSRT level achieved. The cohort included 101 children with a mean age of 5.8 years, 53% male. 43% reached 20mSRT level 1, 48% reached level 2, 5% reached level 3 and 4% reached level 4. Age, sex, and hemoglobin level (mean 10g/dL) were not significantly associated with the shuttle run level achieved. *Trichuris* was the most prevalent parasitic infection (13%), followed by hookworm (7%), *E. histolytica* (7%), giardia (5%), malaria (4%), *Ascaris* (1%), and schistosomiasis (1%). Hookworm infection was associated with a lower level achieved in the 20mSRT (p=0.002, 95% CI 0.014-0.113). Parasitic infections are common in school-aged children in coastal Kenya and may impair physical fitness. Hookworm infection, in particular, is associated with decreased physical fitness as measured by the 20mSRT.

1715

INFERENCE OF PARASITE BURDEN FROM INDIRECT INTENSITY DATA AND IMPLICATIONS FOR STUDY DESIGN

James E. Truscott, Arminder Deol, Roy Anderson

Imperial College London, London, United Kingdom

A substantial proportion of data collected in monitoring and evaluation (M&E) of macro-parasites records secondary indicators of the infection, such as egg counts or microfilaria, as a measure of parasite burden. Arguably, however, the most useful information for diagnosis, estimation of morbidity and for understanding the transmission dynamics of disease are quantities such as mean adult parasite burden and levels of aggregation of this burden within the population. Since adult worm counts are often very difficult to obtain, and we have only a few studies from which to estimate the relationship between adult worms and transmission stages, we need robust statistical descriptions of the distribution of measured quantities, such as egg counts, in terms of basic parameters of parasite biology. These will enable reliable inference of quantities such as mean worm burden, parasite aggregation and density-dependent processes from standard M&E data. We have developed a method for doing this calculation using extensive egg count and worm count data to identify and parameterize models of the distribution of Ascaris lumbricoides egg production as a function of worm burden. Results show that egg counts are best described by a negative binomial distribution with mean egg production from individual subject to an exponentially decreasing fecundity. Models of this kind will allow the optimal use of available data sources to extract reliable estimates of

basic biological parameters and their associated confidence intervals. This has strong implications for the tailoring of study design and choice of diagnostic techniques to optimize information gained against cost incurred. As an example, we discuss the reappraisal of M&E as elimination is approached and egg intensities fall.

1716

EFFECT OF A SINGLE DOSE OF 8 MG MOXIDECTIN OR 150 μG/KG IVERMECTIN ON INTESTINAL HELMINTHS IN PARTICIPANTS OF A CLINICAL TRIAL CONDUCTED IN NORTHEAST DRC, LIBERIA AND GHANA

Simon K. Attah[1], Kambale Kataliko[2], Maurice M. Nigo[3], Mawolo Kpawor[4], Nicholas O. Opoku[1], Eric Kanza[2], Didier Bakajika[3], Hayford Howard[5], Germain Mambandu[3], Kambale Kasonia[2], Michel Vaillant[6], Christine Halleux[7], Piero L. Olliaro[7], **Annette C. Kuesel**[7]

[1]Onchocerciasis Chemotherapy Research Center, Hohoe, Ghana, [2]Centre de Recherche Clinique de Butembo, Université Catholique du Graben, Butembo, Democratic Republic of the Congo, [3]Centre de Recherche en Maladies Tropicales de l'Ituri, C.R.M.T- ITURI, Hôpital Général de Référence de Rethy, Rethy, Province Orientale, Democratic Republic of the Congo, [4]The Liberian Institute for Biomedical Research, Clinical Research Center Bolahun, Monrovia, Liberia, [5]The Liberian Institute for Biomedical Research, Clinical Research Center Bolahun, Lofa County, Liberia, Monrovia, Liberia, [6]CCMS CRP-Santé, Luxembourg, Luxembourg, [7]World Health Organization, Geneva, Switzerland

In the Phase 3 study comparing the effects of a single dose of 8 mg moxidectin vs. ivermectin standard dose (150μg/kg) on *O. volvulus* skin microfilariae levels and participant well-being in 941 males and 531 females, including 79 children 12-17 years, , participants were randomized in a 2:1 ratio to moxidectin : ivermectin. 1465 participants underwent a single sample Kato-Katz test at screening. 876/1465 (60%) were infected with ≥1 type of intestinal helminth: *Ascaris lumbricoides* 44 (3%), *Trichuris trichuria* 22 (1.5%), hookworm 783 (53.4%), *Schistosoma mansoni* 233 (15.9%), *Strongyloides* 4 (0.3%). 835 with ≥1 type of intestinal helminth were treated with either moxidectin or ivermectin and tested again 1 month later with a single sample Kato-Katz test. Results obtained are expressed as cure rate (CR) and egg reduction rate (ERRam) using arithmetic mean egg counts post treatment relative to arithmetic mean egg counts pretreatment (EPG) for each species. Results for ivermectin treated subjects: *A. lumbricoides* n=10, EPG=408, CR=100%, ERRam=100%; *T. trichuria* n=6, EPG=2856, CR=83%, ERRam=76%; hookworm n=259, EPG=842, CR=29%, ERRam=52%; *S. mansoni* n=67, EPG=236, CR=54%, ERRam=73%. Results for moxidectin treated subjects: Ascaris lumbricoides n=34, EPG=386, CR=97%, ERRam=97%; *T. trichuria* n=11, EPG=1409, CR=91%, ERRam=99%; hookworm n=491, EPG=601, CR=48%, ERRam=82%; *S. mansoni* n=143, EPG=168, CR=64%, ERRam=66%. Co-administration of either ivermectin or moxidectin with drugs like benzimidazoles and/or praziquantel may help achieve high efficacy in preventive chemotherapy programmes for soil-transmitted nematodes and schistosomiasis.

1717

EPIDEMIOLOGY OF ANTHELMINTHIC TREATMENT FAILURE IN HOOKWORM (*NECATOR AMERICANUS*) INFECTIONS IN CHILDREN IN THE KINTAMPO NORTH MUNICIPALITY, GHANA. II. BASELINE PARASITOLOGY DATA

Michael D. Wilson[1], Josephine Quagraine[1], Gertrude Ecklu-Mensah[1], Joseph Otchere[1], Yvonne Ashong[1], Millicent Opoku[1], Lisa Harrison[2], Debbie Humphries[3], Kwadwo A. Koram[1], Michael Cappello[2]

[1]Noguchi Memorial Institute for Medical Research, Legon-Accra, Ghana, [2]Yale School of Medicine, New Haven, CT, United States, [3]Yale School of Public Health, New Haven, CT, United States

Previous studies in multiple communities in the Kintampo North Municipality (KNM), Ghana in 2007 revealed a high prevalence of hookworm infection (45%) and albendazole (single dose, 400mg) treatment failure (39%) school age children. Subsequent study in 2010 confirmed the high prevalence of hookworm (39%) and an even higher rate of albendazole treatment failure (56%) in this population. The overall goal of an NIH-funded longitudinal study is to fully characterize the epidemiology and molecular basis of benzimidazole treatment failure in KNM. Parasitological, anthropometric, socioeconomic, nutritional and immune status data were obtained from a cohort of consented 273 school-aged children (48.1% females and 51.9% males). Fecal samples, demographic data were also obtained from three consented household members of participants in two communities. We report here, the baseline parasitology results. Stool and blood samples were analyzed for intestinal helminths (Kato-Katz) and malaria (RDT and microscopy) infections, and PCR used to identify the hookworm species. All the participants gave fecal samples and 260 donated blood, and those found infected treated with albendazole. Fifty-eight (21.2%) were infected and 16 of 46 (34.7%) failed treatment and the overall cure rate was 61.7%. The geometric mean of intensity infection was 376.1epg (± 890.5) at pre-treatment and was 57epg (± 46.3) post treatment. The fecal egg count reduction rate was 81.2%. Sixty nine hookworm specimens were all identified as *Necator americanus*. 76.54% (199/260) and 67.7% (176/260) were positive by RDT and microscopy respectively. Geometric mean of *Plasmodium falciparum* intensity was 1377.3 (± 3623.9) parasites/ml of blood. 16.6% (45/173) participants were co-infected with both parasites. Hookworm and malaria co-infection rate was 17.3% (45/260). The cross sectional survey revealed hookworm prevalence of 33.3% (46/138) among household members, and 56.9% (29/51) of households, and the percent positive child with at least one household member positive was 73.3% (11/15).

1718

EPIDEMIOLOGY OF HOOKWORM (*NECATOR AMERICANUS*) INFECTIONS IN CHILDREN AND ANTHELMINTHIC TREATMENT FAILURE IN THE KINTAMPO NORTH MUNICIPALITY, GHANA. III. EVALUATION OF NUTRITIONAL RISK FACTORS AT BASELINE

Sena Apeanyo

Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana

An ongoing NIH-funded longitudinal study of school-age children aims to fully characterize the epidemiology of hookworm in the Kintampo North Municipality (KNM) where previous studies have found high albendazole treatment (400mg) failures in children. This arm of the study is to investigate the influence of modifiable host factors including overall nutritional status,food security and dietary diversity as risks factors of hookworm infestationat baseline. Structured questionnaires and food frequency questionnaires were used to collect data from 271 study participants. Haemoglobin concentration, weights and height z- scores were used to determine nutritional status of the study participants, WHO criteria was used to determine food security and food diversity. Fifty-eight participants (21.2%) and sixteen out of 46 studied participants (34.8%) were hookworm infected pre- and post-treatment respectively.

The overall mean Hb levels(anemia defined as <12.0g/dl) was 11.47g/dl (±1.28), 11.50g/dl (±1.25)and 11.35g/dl (±1.38) for negative and positive cases respectively, which were not significantly different between them. The mean weight z-scorefor the study participants was -0.02 (±0.97), 0.65 (±1.19) and -0.39 (±0.89) for negative and positive cases respectively, which were significantly between the two groups (P=0.02). The mean height z-score overall, was -0.01 (±1.0), -0.01 (±0.97)and -0.12 (±1.07) for negative and positive cases respectively, which were not significantly between the two groups (P=0.087). 74.2% of the study participants experienced food insecurity and so were 72.4% and 74.6% of the negatives and positives cases respectively. Above-average dietary diversity was observed in 46.5% of the study participants, and was 48.8% and 37.9% for negative and positive cases respectively. In conclusion, most of the study participants were anemic, underweight, stunted and consumed less diverse food. Moreover, infected children were significantly underweight than their non-infected counterparts.

1719

COMPARING METHODS TO ASSESS *SCHISTOSOMA* RESPONSE TO PRAZIQUANTEL TREATMENT

Piero L. Olliaro[1], Michel Vaillant[2], Aïssatou Diawara[3], Jean T. Coulibaly[4], Amadou Garba[5], Jennifer Keiser[5], Charles H. King[6], Stefanie Knopp[5], Aly Landouré[7], Eliézer K. N'Goran[4], Giovanna Raso[5], Alexandra U. Scherrer[8], José Carlos Sousa-Figueiredo[9], Katarina Stete[5], Xiao-Nong Zhou[10], Jürg Utzinger[5]

[1]World Health Organization, Geneva, Switzerland, [2]Methodology and Statistical Unit, Centre for Health Studies, CRP-Santé, Luxembourg, Luxembourg, [3]New York University Abu Dhabi, Abu Dhabi, United Arab Emirates, [4]Unité de Formation et de Recherche Biosciences, Université Félix Houphouët-Boigny, Abidjan, Côte D'Ivoire, [5]Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Basel, Switzerland, [6]Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH, United States, [7]Institut National de Recherche en Santé Publique, Bamako, Mali, [8]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zürich, University of Zürich, Zürich, Switzerland, [9]Department of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom, [10]National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai, China

To diagnose *Schistosoma* infection, stool or urine samples are examined for worm eggs, but there is no definitive agreement as to how best express treatment efficacy. We analyzed data from 24 trials conducted in Africa, Asia and Latin America enrolling overall 4740 individuals infected with *S. mansoni* (Sm, n=1804; 38.1%), *S. haematobium* (Sh, n=2633; 55.5%), or *S. japonicum* (Sj, n=303; 55.5%), and treated with praziquantel at doses of 40 (n=3713), 60 (n=690) and 80 (n=337) mg/kg. Efficacy was measured using cure rate (CR) and egg reduction rate (ERR) calculated using either geometric or arithmetic means (ERRgm, ERRam). We compared efficacy outcome measures: 1) ERRam vs. ERRgm, 2) ERR vs CR, and 3) ERR and CR based on quadruplicate vs single Kato-Katz thick smear examination for Sm. We found that: 1) ERRam and ERRgm can be used interchangeably only if treatment efficacy is very high (>95%); as efficacy falls, estimates are higher with ERRgm than ERRam. Modeling data shows that consistency between means is better for Sh and Sj than for Sm; 2) poor correlation between ERRgm/am and CR except when ERRs are very high (>97%). 3) using a single rather than quadruplicate Kato-Katz thick smear excluded 19% of Sm-infected individuals; the effect on estimating ERR was negligible by individual studies; however, on aggregate ERRam and CR were 8-9% higher (no effect on ERRgm.) A valid complement for drug efficacy monitoring is to study the distribution of individual responses to identify suboptimal responders. Of the 2358 Sh-infected individuals with complete data records 61.3% were negative post-treatment (cure rate, CR), 32.4% had reduced egg counts (rEC), 6.3% had no change/increased egg counts (nEC). For Sm (n=1699) individuals CR was 75.4%, rEC 20.5%, nEC 4.1%. For Sj (n=300) CR was 90%, eEC 8.3%, nEC 1.7%. The response achieved by the 5th centile (the 5% worse responders) was 79.1%, 77.9%, and 23.6% for Sj, Sh and Sm; for the 10th centile it was 100%, 88.2%, and 70.3%, and for the 25th centile 100%, 97.8%, and 100%, respectively.

1720

EPIDEMIOLOGY OF HOOKWORM (*NECATOR AMERICANUS*) INFECTIONS IN CHILDREN AND ANTHELMINTHIC TREATMENT FAILURE IN THE KINTAMPO NORTH MUNICIPALITY, GHANA. I. BASELINE INDICATORS OF EPIDEMIOLOGY AND SOCIOECONOMIC RISK FACTORS

Hector L. Samani[1], Sena Apeanyo[1], Karen Zhang[2], Debbie Humphries[2], Kwadwo A. Koram[1], Michael Cappello[3], Michael D. Wilson[1]

[1]Noguchi Memorial Institute for Medical Research, Legon-Accra, Ghana, [2]Yale School of Public Health, New Haven, CT, United States, [3]Yale School of Medicine, New Haven, CT, United States

In 2007 and 2010, high prevalence rates of hookworm infections (45%) and (39%) respectively in school age children was observed in the Kintampo North Municipality (KNM), Ghana. Also observed were high rates of albendazole (single dose 400mg) treatment failure; (39%) and (54%) respectively. As part of an NIH-funded study which aims to fully characterize the epidemiology and molecular basis of benzimidazole treatment failure in KNM, a census/enumeration survey was conducted in eight communities in KNM, then 271 children aged between 8 and 12 years were randomly selected for a longitudinal study. To define the epidemiology of hookworm infection at baseline and the specific host factors associated with albendazole treatment failure, demographic, socioeconomic and environmental information was collected using structured questionnaires. The cohort comprised of 138 males and 133 females. The cohort's mean age was 9.49 (± 1.69). The overall hookworm prevalence was 21.4% (58/271), of which 67.2% of infected children were the group 10 years and above. Significant associations were found between hookworm infections and possession of cattle and dogs (P=0.049 and P=0.029), ownership of shoes (P=0.015) and wearing shoes daily (P=0.020). No significant associations were found with gender, age, access to agricultural land, and types of water sources and toilet facilities (P>0.05). No associations were also found between treatment response and any of the socio-economic parameters (P>0.05).

1721

RICE FORTIFIED WITH IRON AND OTHER MICRONUTRIENTS IMPACTS HOOKWORM INFECTION RISK IN SCHOOLCHILDREN

Brechje de Gier[1], Maiza Campos Ponce[1], Marlene Perignon[2], Marion Fiorentino[2], Khov Kuong[3], Chhoun Chamnan[3], Megan Parker[4], Kurt Burja[5], Jacques Berger[2], Katja Polman[6], Frank Wieringa[2]

[1]VU University Amsterdam, Amsterdam, Netherlands, [2]IRD, Montpellier, France, [3]DFPTQ, Fisheries Administration, Phnom Penh, Cambodia, [4]PATH, Seattle, WA, United States, [5]WFP, Phnom Penh, Cambodia, [6]ITG, Antwerp, Belgium

Soil-transmitted helminth (STH) infections often co-exist with micronutrient deficiencies in the developing world. There is evidence that these major health issues can influence and exacerbate each other. Fortification of staple foods such as rice can be an effective tool to improve micronutrient status of vulnerable groups, but its impact on STH infection is currently unclear.In a cluster randomized, placebo-controlled, double-blinded trial, 3 different types of fortified rice were introduced through the World Food Program (WFP) School Meal program in Cambodia. The 3 types of fortified rice differed in micronutrient compositions and production method. Children (6-15 y) received 1 type of rice, unfortified rice (placebo), or no school meal (control) for 7 months. Stool samples were collected and analyzed by Kato Katz method at baseline, 3 months and 7 months. After baseline, all children received a single dose of 400mg albendazole.

The effects of consumption of fortified rice on hookworm infection were analyzed by multiple logistic regression. Baseline prevalence of STH was 17.0%, which were mainly hookworm infections (16.6%) of light intensity. A risk factor for hookworm infection was being a boy (P=0.011). After 7 months (n= 1236 children), hookworm infection prevalence was between 17-24% in control, placebo and in the NutriRice fortified rice groups. In the children receiving 2 types of UltraRice fortified rice, hookworm prevalence was 33-34% (P<0.001). The new infection rate was highest in the UltraRice group with the highest iron content (24.6%), intermediate in the UltraRice group with less iron (21.8%), and lowest in the control and placebo and groups (12.5% and 11.9%, P=0.001 for difference among groups). Fortifying rice with micronutrients, especially iron, can increase risk of hookworm infection. Type of fortificant appears to be a major effect modifier. These findings have big implications for policies aiming to improve child health and nutritional status in tropical regions.

1722

HOW DOES THE SCALE OF DEWORMING PROGRAMS AFFECT THEIR COST-EFFECTIVENESS?

Hugo C. Turner, James M. Truscott, Roy M. Anderson

Imperial College London, London, United Kingdom

The predominant control strategy for the soil-transmitted helminths (STHs) is regular periodic mass drug administration (MDA), targeting school-aged children. The World Health Organization (WHO) and London declaration on Neglected Tropical Diseases (NTDs) has set goals to scale up MDA, so that by 2020, 75% of the pre-school and school-aged children in need, will be treated regularly. It has been observed that increasing the number treated can reduce the per capita costs of MDA programmes (economies of scale). This is because a number of the costs associated with MDA delivery are fixed (i.e. do not depend on the number treated), and therefore increasing the number treated reduces the average fixed cost per treatment. However, the implications this has on the cost-effectiveness of scaling up control for STH infections, and the optimum treatment strategy have not been explored. We developed costing functions which account for the changes in the per capita costs of treatment with scale, and incorporated them into STH dynamic transmission models. We found that, due to these economies of scale, the cost effectiveness of STH control programmes markedly increased with the number treated. This has notable implications for programmes considering scaling up MDA, in line with the current goals set by the London declaration.

1723

THE EFFECT OF SEASONALITY ON THE PREVALENCE OF *ASCARIS LUMBRICOIDES* AND IMPLICATIONS FOR THE OPTIMAL TIMING OF MASS TREATMENT PROGRAMS

Emma L. Davis[1], Rachel Pullan[2], Leon Danon[3], Deirdre Hollingsworth[1]

[1]University of Warwick, Coventry, United Kingdom, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Queen Mary University of London, London, United Kingdom

Previous studies have shown that changes in temperature affect maturation times, development proportions, and mortality rates of *Ascaris lumbricoides* and *suum* eggs, which suggests that climate could impact both overall prevalence and reinfection rates after treatment. Depending on the size of this effect, this could have implications for the optimal time of year for mass treatment in order to get the maximum impact out of the large number of donated drugs. However, there has been little quantitative investigation of the influence of seasonal timing of mass chemotherapy on treatment programmes. Using historical data from experimental studies on *A. suum* eggs – which have been shown to display equivalent behaviour to *A. lumbricoides* eggs – to characterise relationships between temperature and egg maturation and survival, models were developed which investigated the effect of temperature dependent development of eggs on mean worm burden in the human population. These reveal fast maturation and low egg mortality at high temperatures, but also a drop in proportion of eggs reaching maturity above 30°C. To demonstrate implications for the optimal timing of mass treatment campaigns, models were applied to districts with differing levels of *A. lumbricoides* transmission across Kenya. District-level prevalence estimates were generated using predictive risk maps developed by the Global Atlas of Helminth Infection and monthly temperature patterns were derived from MODIS. Results suggest timing of treatment could have important consequences for programme impact. Depending on region, changing the treatment date resulted in an estimated 18-55% comparative decrease in prevalence after four yearly treatment rounds. This highlights the potential importance of appropriate timing of the established Kenya National Deworming Programme. More generally, this approach provides insight into the epidemiology of *A. lumbricoides* infection, methods for testing and validating these predictions, and can help guide optimal long-term helminth control strategies in diverse settings.

1724

KEY RESIDUES OF CRY5B STRUCTURE AND FUNCTION: MUTAGENESIS BY ALANINE SCANNING

Jillian Sesar, Yan Hu, Hui Fan, Partho Ghosh, Raffi Aroian

University of California San Diego, La Jolla, CA, United States

Soil-transmitted helminthes infect more than 2 billion people worldwide and only one drug (albendazole) is able to show a high enough efficacy against parasite worms under conditions for mass drug administration. However, recent studies have shown an increase in resistance to this drug, stressing the importance of finding a new treatment option. Crystal (Cry) proteins produced from the soil bacterium Bacillus thuringiensis have been used for decades as a means to control insects that destroy crops and transmit human diseases, and studies have shown these proteins to be safe to humans. Our lab has shown that crystal proteins, specifically Cry5B, are able to kill both the free-living nematode Caenorhabditis elegans, as well as parasitic roundworms (eg. *Ancylostoma ceylanicum*, hookworm). Cry proteins intoxicate invertebrates by acting as pore-forming toxins. Several defined steps in their mechanism of action have been suggested from insect studies, but there is still great uncertainty as to the importance of these various steps. We believe that the nematode - Cry5B system has great potential to unlock mysteries surrounding Cry proteins and to be a potential therapeutic agent. Here, I have mutated all of the 698 amino acids in the toxin domain of Cry5B, and subsequently tested these mutants on C. elegans to assess for changes in toxicity levels, screening for variants with an increase in activity as compared to the wild type. From this screen and subsequent quantitative LC_{50} killing assays to confirm the screen results, I have identified several key variants of interest that are additionally more active against *A. ceylanicum* both *in vitro* and *in vivo*. Additionally, these residues most likely play a key role in Cry5B protein function, with the eventual goal being to correlate these changes in activity with specific changes in protein functionality. These improved Cry protein variant candidates have the potential to be used in therapeutics for treating one of the most neglected diseases of our time, parasitic worms.

1725

A NOVEL NEXT-GENERATION SEQUENCING APPROACH TO DEVELOP IMPROVED MOLECULAR DIAGNOSTICS FOR THE DETECTION OF SOIL TRANSMITTED HELMINTH INFECTIONS

Nils Pilotte, Marina Papaiakovou, Lou Ann Bierwert, Steven A. Williams

Smith College, Northampton, MA, United States

The soil transmitted helminths (STHs) are a group of parasitic worms responsible for causing extensive morbidity in many of the world's most economically depressed locations. With an estimated 880 million children infected with one or more species of STH parasite, accurate and cost-effective diagnostic measures are of premier importance to global control and elimination efforts. Accordingly, the use of molecular

diagnostic measures, such as real-time PCR, has shown great promise for the improved detection of STH infections. To date, such molecular assays have utilized ribosomal and mitochondrial target sequences, as previously reported. While effective, such sequences are frequently not the highest copy number target and will not yield the most sensitive assay possible. The most sensitive assay will utilize the most highly repetitive, unique, non-coding DNA sequences found within the genome of each species. Consequently, we have coupled next-generation sequencing technology with the Galaxy-based software *RepeatExplorer*, to identify the most numerous, non-coding DNA sequences within multiple species of STH parasites including *Trichuris trichiura*, *Necator americanus*, *Ancylostoma duodenale*, and *Ascaris lumbricoides*. Following the application of this approach to each STH species, we designed TaqMan-based primer-probe combinations for each candidate sequence. Species-specificity was verified for each assay and repeatable detection of genomic DNA isolated from each parasite was demonstrated at concentrations ranging from 1.0ng to 1.0fg. Through this novel approach to the identification of species-specific, high copy-number target sequences, we have developed a new strategy for the design of a PCR-based diagnostic assay with improved sensitivity.

1726

THE ROAD TOWARDS EFFICIENT CONTROL OF SCHISTOSOMIASIS IN THE DEMOCRATIC REPUBLIC OF CONGO: PRE-ASSESSMENT OF STAFF PERFORMANCE AND MATERIAL RESOURCES IN ENDEMIC REGIONS

Sylvie Linsuke[1], Liliane Mpabanzi[2], Joules Madinga[3], Sabin Nundu[1], Faustin Mukunda[4], Pascal Lutumba[1], Katja Polman[2]

[1]Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo, [2]Institute of Tropical Medicine, Antwerp, Belgium, [3]University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [4]Programme National de Lutte contre la Bilharziose et Parasitoses Intestinales, Kinshasa, Democratic Republic of the Congo

Schistosomiasis is a disease affecting approximately 200 million people worldwide. Since a long time, schistosomiasis has been known to be endemic in certain provinces of the Democratic Republic of Congo. However, the most recent national data available on schistosomiasis prevalence and distribution were published in the early sixties. . Recently, the Ministry of Health adopted a national plan against schistosomiasis aiming at to distribute Praziquantel (PZQ), the treatment of choice for schistosomiasis, to all individuals infected. For effective introduction of control strategies, data on national prevalence and distribution of schistosomiasis in the DRC urgently need to be updated. The present study assessed the knowledge of health workers on schistosomiasis as well as the availability of the facilities needed for adequate diagnosis and management of the disease in the endemic provinces of Kinshasa and Bas-Congo in the DRC. This study was conducted in 9 health zones (HZ) of Kinshasa and 2 HZ in Bas-Congo. Health workers could name all symptoms of schistosomiasis. Kato-Katz, urine filtration or sedimentation were not available as diagnostic methods in any health facilities. Diagnosis therefore almost solely relied anamnesis. The knowledge on schistosomiasis did not differ between the rural Bas-Congo and urban Kinshasa. The fees for consultation, diagnostics and treatment were three times higher in Kinshasa than Bas-Congo. Health workers in Kinshasa and Bas-Congo are able to name the symptoms related to schistosomiasis. However there is a lack of availability of adequate diagnostic tools and treatment. The fees of diagnostics and treatment are high for a population often living in extreme poverty.

1727

HELMINTH INFECTIONS DURING PREGNANCY MAY DECREASE NUTRITIONAL FITNESS OF THE OFFSPRING

Emily A. McDonald[1], Sunthorn Pond-Tor[1], Blanca Jarilla[2], Marianne J. Sagliba[2], Analisa Gonzal[2], Remigio Olveda[2], Luz Acosta[2], Jonathan D. Kurtis[1], Jennifer F. Friedman[1]

[1]Rhode Island Hospital, Providence, RI, United States, [2]Research Institute of Tropical Medicine, Manila, Philippines

Helminth infections represent a significant disease burden in endemic regions of the world, and polyparasitism may have a bigger impact on overall health than any individual infection. We have previously shown that *Schistosoma japonicum* results in a profound pro-inflammatory response at the maternal-fetal interface during pregnancy and decreased invasion characteristics of placental trophoblast cells *in vitro*. Another critical function of the placenta is to regulate nutrient exchange between mother and fetus. Herein, we have shown that treatment *in vitro* of primary trophoblasts with schistosome soluble egg antigens (SEA), resulted in a significant drop in gene expression of specific amino acid transporters. These include the sodium-coupled neutral amino acid transporter 1 (SNAT1; 80% reduction) and large neutral acid transporter (LAT1; 70% reduction). To investigate the metabolic impact of helminth infections during pregnancy, we utilized samples from a cohort of pregnant women from Leyte, the Philippines. Most subjects had polyparasitic infections, including schistosomiasis and geohelminth infections, with prevalence rates of 70%, 79%, and 40% for *Ascaris lumbricoides, Trichuris trichiura,* and hookworm, respectively. Given the relatively low intensity of schistosome infection and the high prevalence rates of geohelminths, we assessed the relationship between the number of helminth infections and metabolic parameters *in utero*. After controlling for SES and gestational age, leptin levels were found to be lower in the cord blood of infants born to mothers with one or more helminth infections. In addition, cord blood leptin levels were positively associated with birth weight (107g heavier on average in those infants in the highest tertile of leptin levels), and increased leptin levels were associated with a reduced risk of fetal growth restriction. These data suggest that helminth infections can impact the transport of nutrients across the maternal-fetal interface, providing a possible link between fetal metabolic hormones and growth *in utero*.

1728

GUT INSTINCTS: EVALUATING PARENTAL ATTITUDES TOWARD INTESTINAL WORM TREATMENT IN RURAL CHINA

Louise Lu[1], Chengfang Liu[2], Alexis Medina[1], Darvin Scott Smith[1], Scott Rozelle[1]

[1]Stanford University, Stanford, CA, United States, [2]Center for Chinese Agricultural Policy, Beijing, China

Nearly forty percent of elementary schoolchildren in rural provinces of Southwest China are infected with soil-transmitted helminths. However, parasitic worm infection is neglected as a major public health problem in these villages and deworming treatment is rarely sought, despite its efficacy and low cost (one treatment dosage costs less than 3 cents USD). Surveys and interviews were conducted in six rural villages in Guizhou, China to evaluate what factors influence parental decisions to seek or not seek deworming among rural Chinese schoolchildren. It was found that knowledge about helminth infection and prevention was severely lacking and often influenced by deep-rooted myths, such as the local belief that deworming medicine can harm a child's future fertility. The majority of household interviewees were highly skeptical of high worm prevalence in their children, despite the nearly universal practice of regularly deworming their pigs. A comprehensive deworming program involving biannual administration of deworming treatment, household health education, and village health system strengthening is necessary to effectively mitigate the disease burden of helminth infection in rural China.

1729

RANDOMIZED CONTROLLED TRIAL OF TWO IVERMECTIN REGIMENS FOR *STRONGYLOIDES STERCORALIS:* EARLY FINDINGS

Guadalupe Jimenez[1], David Goodman-Meza[1], Herbert B. Tanowitz[2], Christina M. Coyle[2]

[1]Jacobi Medical Center, Bronx, NY, United States, [2]Albert Einstein College of Medicine, Bronx, NY, United States

Humans can be chronically infected with *Strongyloides stercoralis* for decades because of the mechanism of autoinfection. Although most infected individuals are asymptomatic, *S. stercoralis* is capable of transforming into a fatal illness in setting of HTLV-1 and steroids. Chronic infection with *S. stercoralis* presents both diagnostic and therapeutic challenges. Parasitologic diagnosis of chronic infection is difficult, because the larval output can be low and irregular. The diagnostic accuracy of serologic testing has a sensitivity and specificity of 93% and 95%, respectively. Ivermectin is the treatment of choice for this parasitic infection, although optimal dosing is yet to be determined. This study evaluated the serologic response to *S. stercoralis* infection after treatment with ivermectin 200 mcg/kg given 2 weeks apart based on the auto-infective cycle (Group A) vs. given on two consecutive days (Group B). Patients were referred from outpatient clinics or identified on the in-patient services and invited to participate in the study. Participants were randomized to either treatment arm and repeat serologies were performed at 3 months intervals for 9 months after treatment. Forty-seven cases were enrolled, mean age was 54.1 (SD 15.2), 61.7% male, 55.3% Hispanic, mean eosinophil count 0.55/nl (SD 0.53), IgE 608.0 mg/dl (SD 680.4) and HTLV-1 was negative in all cases. There were no significant differences in baseline demographic or clinical variables between the two groups. Of the 47, 51.0% had completed the 9 months follow-up. Mean eosinophil count (p=0.002) and IgE value (p=0.045) both decreased after treatment of cases in both treatment groups. Of the 47 patients, 9 cases remained sero-positive on follow-up; five (22.7%) in Group A and four (16.0%) in Group B (p=0.751). In this randomized controlled trial, there was no difference in serologic outcome in the two treatment arms, but treatment resulted in decreased eosinophil and IgE values.

1730

TRYPANOSOMA CRUZI INFECTION PREVALENCE AND BLOOD MEAL ANALYSIS IN VECTORS OF CHAGAS DISEASE IN SOUTHWEST TEXAS, 2013-2014

Rodion Gorchakov[1], Edward J. Wozniak[2], Teresa P. Feria[3], Melissa N. Garcia[1], Peter J. Hotez[1], Kristy O. Murray[1]

[1]Baylor College of Medicine, Houston, TX, United States, [2]Texas Department of State Health Services, Uvalde, TX, United States, [3]University of Texas Pan American, Edinburg, TX, United States

Protozoan pathogen *Trypanosoma cruzi* is an etiologic agent of Chagas disease, which affects millions of people in Latin America and is an emerging public health threat in the United States. Transmission cycle of the parasite involves alternating infection of its vertebrate hosts and insect vectors. We identified the current vector infection burden and potential range of natural reservoirs of *T. cruzi* in 11 southwestern counties of Texas by analysis of insects of genus *Triatoma* collected during the period from June 2013 to January 2014. Out of 40 submitted specimens, the vast majority of the insects were *T. gerstaeckeri*, with only four samples of *T. sanguisuga*, two samples of *T. lecticularia*, and one sample of *T. rubida*. We found 73% of the insects positive for *T. cruzi*. Blood meal analysis was performed on the infected triatomines. Blood sources were determined for all but one of the insects, and included 13 different species of mammals (mouse, woodrat, squirrel, porcupine, armadillo, cottontail, raccoon, fox, coyote, dog, pig, cow, human). Interestingly, 36% of the bugs were identified as having multiple blood sources. Since most of the insects were collected in or around residential houses, the most prevalent type of blood meal was human (50% of the insects). High infection rate of the triatomine vectors combined with high incidence of feeding on humans underscore the importance of Chagas disease surveillance in Texas and prompt for urgent measures for vaccine development, vector control, and increasing public awareness.

1731

POPULATION GENETIC STRUCTURE OF THE TSETSE FLY: TARGETING REPRODUCTIVE REFRACTORY INTERVENTIONS FOR WILDLIFE AND LIVESTOCK TRYPANOSOMIASIS IN KENYA

Benard Kulohoma

International Centre for Insect Physiology and Ecology, Nairobi, Kenya

Tsetse flies (*Glossina* species) are major vectors of both human and livestock trypanosomiasis, and efficiently transmit the causative parasites trypanosomes. Tsetse flies infest up to 10 million km^2 of land stretching across 40 countries in sub-Saharan Africa. Multiple drugs that treat African animal trypanosomiasis exist, and have substantially improved veterinary management of livestock with both susceptible and resistant trypanosome strains. Although, multi-drug resistant trypanosome strains have lower fitness and are therefore thought to be less persistent, the effect of increased communal use of multiple antibiotics on transmission rates of these pathogenic species is still not fully clear. Moreover, widespread multi-drug resistance due to prolonged usage or under-dosing could also have adverse repercussions on public health, further complicating management disease management. Our study, will exploit genomic approaches to understand the population structure of tsetse flies in circulation in disease endemic regions of Kenya so as to determine the impact of drug use on the prevalence of multi-drug resistance. It's effect on tsetse infectivity and transmissibility of multi-drug resistant trypanosome strains. This study also aims to identify genes essential for successful reproduction as potential long-term vector control targets. This study holds the promise of identifying socio-demographic independent vector control strategies, and will enable the judicious use of appropriate drugs to which trypanosome strains are not resistant. Thereby enabling prolonged trypanosome and tsetse control while avoiding widespread multiple-drug resistance.

1732

EMERGENCE OF A TROPICAL DISEASE IN U.S. DOGS: A PROSPECTIVE STUDY OF *LEISHMANIA* IN U.S. FOXHOUNDS

Angela J. Schneider, Robert Schaut, Christine Petersen

University of Iowa, Iowa City, IA, United States

Leishmania is the causative agent of leishmaniasis, a deadly protozoan disease which affects approximately 12 million people worldwide. Generally associated with tropical and subtropical regions, *Leishmania* can be found as far north as the United States. Similarly, the vector for *Leishmania* transmission, the sand-fly, has been found within areas as far north as Missouri and Ohio. *Leishmania infantum,* infects canines as well as humans. In endemic areas dogs serve as the domestic reservoir. Within the United States, foxhound hunt populations have developed endemic disease. The first documented foxhound case of *Leishmania* in the US was reported in 1980. *Leishmaniasis* is a chronic disease with long latency, diagnosis can be difficult with gold standards limited to invasive techniques including bone marrow aspirate with culture. Serological testing has been used as a diagnostic technique but has cross reactivity with *Trypanosoma cruzi* also found in the southern U.S. To help with diagnosis, a highly sensitive and specific real time quantitative polymerase chain reaction (RT-qPCR) assay was developed. While this assay is capable of identifying parasite DNA within the peripheral blood it lacks the ability to determine whether the *Leishmania* is actively proliferating. Over the course of the last 8 years, diagnostic testing using PCR increased. Cases of *Leishmaniasis* can be tracked throughout regions of the US within the foxhound hunt populations. This study is the first to report changes in *Leishmania* prevalence and incidence over a six-year span (2007-2012) within US foxhound hunts. Trends in infection over time and across regions

were examined. *Leishmania* infection has stayed consistent over time with a point prevalence in 2007 of 3.54 per 1000 foxhounds and 3.23 per 1000 foxhounds in 2012. Incidence rates over the 5-year period began at 4.77 per 1000 foxhounds in 2007 and ended at 3.08 per 1000 foxhounds in 2012. The consistent prevalence and incidence of this infection stresses the need for appropriate risk management and disease prevention techniques in this community.

1733

MATERNAL *TRYPANOSOMA CRUZI* INFECTION AND INFANT GROWTH

Elizabeth J. Howard[1], Xu Xiong[1], Maria Luisa Cafferata[2], Gabriela Cormick[3], Alvaro Ciganda[2], Pierre Buekens[1]

[1]Tulane University School of Public Health, New Orleans, LA, United States, [2]La Unidad de Investigación Clínica y Epidemiológica Montevideo, Montevideo, Uruguay, [3]Institute for Clinical Effectiveness and Health Policy, Buenos Aires, Argentina

Maternal *Trypanosoma cruzi* infection and subsequent congenital transmission is a serious, yet neglected, global health issue. Infant growth assessment can provide an understanding of the health of children and an indirect evaluation of the quality of life for an entire population afflicted with Chagas disease. The objective of this study was to determine if maternal *T. cruzi* infection indirectly affects a newborn's physical development. Infected mothers and their infants (n=153) were followed at birth, 4-8 weeks and 10 months post-partum in Tucuman, Argentina from April 2011 until April 2013. Age- and sex-specific estimates of infant weight, length, weight-for-length, and head circumference were compared to an international child growth standard. All mean z-scores were between ±1 standard deviations (SD) of the standard. However, the prevalence of infants falling below -2 SD of the WHO standard peaked at 16% for weight-for-age for females, 10.9% for length-for-age in males and 10.8% for weight-for-length in females, all at visit 1. Infants who experienced growth faltering were more likely to be female and weighed 0.8 kg less at birth, 0.9 kg less at 4-8 weeks and 0.5 kg less at 10 months of age. They also were 3 cm shorter and had a 2 cm reduction in head circumference. This analysis provides evidence of progressive stunting over the 10 month period and early failure to thrive with improvement by 10 months. Nutrition and health interventions, as well as socioeconomic changes, may be helpful in improving the growth and development of infants from Chagas affected populations.

1734

COMPARISON BETWEEN PATIENTS WITH CLASSICAL AND ATYPICAL PRESENTATIONS OF CUTANEOUS LEISHMANIASIS, FROM AN AREA OF *LEISHMANIA (VIANIA) BRAZILIENSIS* TRANSMISSION IN NORTHEAST BRAZIL

Luiz Henrique Guimarães, Adriano Queiroz, Juliana A. Silva, Silvana C. da Silva, Ednaldo L. Lago, Paulo Roberto L. Machado, Edgar M. Carvalho, **Albert Schriefer**

Hospital Universitário Prof. Edgard Santos, Salvador, Brazil

The purpose of this study was to compare clinical, immunological and parasitological aspects between patients with atypical cutaneous leishmaniasis (ACL) and classical cutaneous leishmaniasis (CL) of a region with high endemicity for American tegumentary leishmaniasis (ATL) in northeast Brazil. Fifty one ACL and an equal number of CL patients were enrolled. ATL was confirmed and parasite species was determined by PCR of lesion biopsies. For each patient clinical data was annotated, peripheral blood was collected, and parasite isolation was attempted. Cultured parasites were genotyped according to the sequence of a locus in chromosome 28, previously shown to be polymorphic in this population of *L. (V.) braziliensis*. All cases had their living sites geographic coordinates acquired by GPS then distributions of ACL and CL cases were compared by the Cusick and Edward's test (CE). Among ACL included there was no pregnant women or HIV positive subjects. ACL presented the same distribution as CL patients in the affected region (CE p = 0.26), but had a greater proportion of lesions above the waist line (94% in ACL x 33% in CL, p = 0.0001) and of failure to antimony treatment (41% in ACL x 0% in CL, p = 0.0006) than CL individuals. Immunologically, ACL showed lower production of TNFα (average 316.5 pg/ml in ACL x 1906.1 pg/ml in CL, p=0.0001) and IFNγ (average 747.1 pg/ml in ACL x 4445.9 pg/ml in CL, p=0.0002), but higher IL-10 (average 392.8 pg/ml in ACL x 171.9 pg/ml in CL, p=0.0006) and IL-17 (average 218.4 pg/ml in ACL x 69.4 pg/ml in CL, p=0.0008) after *in vitro* stimulation of peripheral blood mononuclear cells with leishmania antigen than CL patients. All subjects were infected with *L. (V.) braziliensis*, but parasites from ACL presented genotypes that were not found in isolates from CL individuals. Therefore, in the region studied, patients with ACL consist in a more homogeneous group of individuals than originally suspected, and are distinct from classical CL regarding treatment outcome, immune response and causative strain of *L. (V.) brazilensis*.

1735

MODELING ECO-BIO-SOCIAL DETERMINANTS FOR HOUSEHOLD INVASION OF SYLVATIC *TRIATOMA DIMIDIATA* IN NORTHERN BELIZE

Angela T. Caranci[1], John P. Grieco[2], Nicole L. Achee[3]

[1]Uniformed Services University, Kensington, MD, United States, [2]Uniformed Services University, Bethesda, MD, United States, [3]University of Notre Dame, Notre Dame, IN, United States

Initial reports have confirmed presence of *Triatoma dimidiata*, an important Chagas disease vector throughout northern Belize. To date, *T. dimidiata* remains the sole vector species reported from this Central American country, yet much of the disease transmission dynamics remain unclear. Here, we report updated infection rates of the vector population as well as infestation rates for villages in north and central Belize. In order to further characterize the epidemiological risk of human-vector contact, Over 225 households have been surveyed and characterized with respect to 30 key determinants related to the probability of household infestation by *T. dimidiata*. These key variables included: presence of domestic animals, distance of household to village periphery, and proximity of community light sources. The infestation behavior of *T. dimidiata* in Belize is confirmed to be distinct from what would classically be designated a domiciliated vector population. Risk factors reported here can be used to guide integrated control efforts to reduce infestation and limit human-vector contact.

1736

CHAGAS DISEASE IN MEXICO: SURVEILLANCE AND PERCEPTIONS OF BURDEN

Ellen Shelly[1], Rodolfo Acuna-Soto[2], Charles Sterling[1], Kacey Ernst[1], Heidi E. Brown[1]

[1]University of Arizona, Tucson, AZ, United States, [2]National Autonomous University of Mexico, Mexico City, Mexico

Chagas disease, a parasitic disease caused by *Trypanosoma cruzi*, disproportionately affects the poor throughout Latin America. We describe the spatial and temporal distribution of officially reported Chagas disease incidence and mortality in Mexico. The greatest burden appears to occur in southern states. Incidence rates and deaths were highest in adults (25-44 years and ≥45 years, respectively). We show increasing temporal trends for incidence (AR(2) p=0.002, 95% CI: 0.040-0.061) and mortality (MA(1) p < 0.0001, 95% CI: 0.012-0.021). While these results provide insight to the changing burden of Chagas in Mexico, under-reporting likely compromises our capacity to understand the epidemiology of this disease. The reported 500 new cases and 20 deaths in 2010 are in stark contrast to estimates of 69,000 new cases and 25,000 deaths per year from seroprevalence studies. As changes in Chagas surveillance improve our understanding of the full burden of this disease, it is likely that the reported and estimated

incidence will align more closely. This will facilitate understanding the epidemiology of this disease and result in more focused and successful control and prevention strategies.

1737

TRYPANOSOMA CRUZI AND OTHER TRYPANOSOMATIDS IN COMMONLY HUNTED WILD MAMMALS FROM REMOTE LOCATION OF THE PERUVIAN AMAZON

E. Angelo Morales[1], Pedro Mayor[2], Mark Bowler[3], Esar Aysanoa[1], Julio A. Ventocilla[1], Jocelyn Perez[4], G. Christian Baldeviano[1], Andres G. Lescano[1]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Autonomous University of Barcelona, Barcelona, Spain, [3]San Diego Zoo Global, Iquitos, Peru, [4]University of Prince Edward Island, Charlottetown, PE, Canada

Chagas disease is generally transmitted by contact with the feces of triatomine bugs infected with *Trypanosoma cruzi*, but oral transmission has also been documented. We evaluated the prevalence of *T. cruzi* and other trypanosomatids in four orders of mammals hunted for subsistence to better understand the risk of infection associated with human consumption and contact. Blood samples from wild mammal species were collected on filter papers by subsistence hunters from a remote small community in Loreto, Peru, bordering Brazil. DNA was isolated from filter papers and amplified using a nested-PCR targeting the 24S alpha subunit rRNA gene. Primers D75/D76 and D71/D72 were used to amplify specific regions of trypanosomatids and *T. cruzi*, respectively. Comparisons of prevalence between orders were performed using Chi-Square and Fisher's exact tests. A total of 142 mammalian blood samples from four orders (10 species) were tested: Carnivora (n=34), Edentata (n=24), Artiodactyla (n=28) and Rodentia (n=56). The prevalence of *T. cruzi* in Carnivora (18%) was significantly higher (p=0.008) compared to other orders (0% - 4%). The prevalence of trypanosomatids ranged from 7% in Artiodactyla to 27% in Rodentia with no significant differences (p=0.180), possibly due to the small sample size. *Nasua nasua* (ring-tailed coati), *Dasypus novemcinctus* (nine-banded armadillo), *Agouti paca* (spotted paca) and *Tayassu tajacu* (collared peccary) accounted for 89% of the samples and all positive animals. Among these four species the prevalence of *T. cruzi* was 19%, 4%, 2% and 0%, respectively (p=0.016); and the prevalence of trypanosomatids ranged from 9% to 31%. The high prevalence of *T. cruzi* in Nasua nasua, a type of raccoon, suggests the importance of carnivores in sylvatic *T. cruzi* transmission. The multiple hunted species infected with trypanosomatids highlights the risk of human infection by consumption of improperly cooked meat.

1738

PREVALENCE OF TRYPANOSOMATIDS AND *TRYPANOSOMA CRUZI* IN WILD AND CAPTIVE NON-HUMAN PRIMATES FROM PERÚ

Esar Aysanoa[1], Pedro Mayor[2], A. Patricia Mendoza[3], E. Angelo Morales[1], Carlos M. Zariquiey[3], Jocelyn G. Perez[4], Mark Bowler[5], Julio A. Ventocilla[1], G. Christian Baldeviano[1], Andres G. Lescano[1]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Autonomous University of Barcelona, Barcelona, Spain, [3]Wildlife Conservation Society, Lima, Peru, [4]University of Prince Edward Island, Charlottetown, PE, Canada, [5]San Diego Global Zoo, Iquitos, Peru

Non-human primates (NHPs) can be infected with *Trypanosoma cruzi*, the etiological agent of Chagas disease, and other trypanosomatids. Primates may act as reservoirs and close contact between humans (traders, owners, hunters, zookeepers, etc.) and NHPs is a potential risk of accidental infection. We compared the prevalence of *T. cruzi* and other trypanosomatids in wild and captive Peruvian NHPs to assess this risk. Blood samples were obtained from captive NHPs (n=192) at zoos, wildlife rescue centers, wet markets and households in six Peruvian cities, and wild NHP hunted for subsistence (n=126) in two remote communities in the Peruvian Amazon. Blood smears from 88 captive NHPs were stained with Giemsa and examined by microscopy. Samples were collected from 318 NHPs on filter paper, FTA cards or EDTA tubes and tested with a nested PCR protocol using primers for the 24S alpha subunit rRNA gene. Primers D75/D76 target the conserved flanking sequences of the D7 alpha domain in trypanosomatids, while primers D71/D72 target a region in the same domain that is specific to *T. cruzi*. PCR was used as gold standard to calculate the sensitivity and specificity of microscopy. Trypanosomatid and *T. cruzi* prevalences were compared using Chi2 and Fisher's exact tests. We studied captive NHPs from five families (14 species) and wild NHPs from three families (11 species). Wild NHPs had significantly higher prevalence of both trypanosomatids (56% vs 27%, p<0.001) and *T. cruzi* (9% vs 3%, p=0.034), compared to captive NHPs. Pitheciidae had the highest trypanosomatid prevalence (18/20, 90%) and Cebidae had the highest *T. cruzi* prevalence (14/116, 12%). Captive NHPs from wet markets (n=38) had very high trypanosomatid (53%) and *T. cruzi* (13%) prevalence. Compared to PCR, microscopy was 83% sensitive and 98% specific. *T. cruzi* and trypanosomatids are common in Peruvian NHPs and pose a risk to human and animal health that has not been properly studied. Although microscopy is poorly sensitive compared to PCR, it may still be useful for screening in the field.

1739

CHAGAS DISEASE, POVERTY AND BIODIVERSITY IN MEXICO

Alejandro Cruz-Reyes, Blanca Camargo-Camargo

Instituto de Biología, Universidad Nacional Autonoma De Mexico, Mexico City, Mexico

Since 1928 Chagas disease (ChD) has been studied in Mexico. Official data from the National Council for the Evaluation of the Politics for Social Development (Consejo Nacional de Evaluación de la Política de Desarrollo Social) [CONEVAL] in 2012 shows that poverty is 50% in Mexican population out of the 110 M people (2010 national census: National Institute of Statistic and Geographical Information) [INEGI], from this, 15% is under extreme poverty. In the Southern region, Mexico has high level of poverty and the highest biodiversity richness in the country, which is currently under serious ecological threating. There is evidence that reduced biodiversity affects the transmission of infectious diseases in humans and other animals. This situation matches perfectly in maps with the high prevalence of ChD in that region. The importance of this work is related to the role of multinational control initiatives against ChD. In some way ChD is a neglected disease in Mexico. This situation is also relevant giving the immigrant phenomenon between Mexico and the US. In this work we present data and geographical evidence that even the great number of Mexican scientists working on ChD, this zoonotic parasitic disease is still under estimation. Back in 2006 we published information about ChD in Mexico form our data base "CHAGMEX", now we are working on a new data base (2004-2014), and so far, the new bibliographic information shows that the number of human cases is increasing considering all clinical and epidemiological forms of the disease: vectorial transmission, blood transfusion, congenital transmission. From the 32 species of Triatominae identified in Mexico, more than 10 are reported with domestic habits. Also *Trypanosoma cruzi* is becoming quite common in domestic dogs, from urban and rural areas. We think that besides academic research, is urgent to implement vector control programs by each climatic region of Mexico, along with a wide epidemiological and socio-economical approaches of ChD in Mexico.

1740

MAPPING THE PREVALENCE AND CASE DETECTION RATES OF GAMBIENSE HUMAN AFRICAN TRYPANOSOMIASIS

Nick Golding[1], Simon I. Hay[1], Giuliano Cecchi[2], Massimo Paone[3], José R. Franco[4], Pere P. Simarro[4]

[1]University of Oxford, Oxford, United Kingdom, [2]Food and Agriculture Organization of the United Nations, Addis Ababa, Ethiopia, [3]Food and Agriculture Organization of the United Nations, Rome, Italy, [4]World Health Organization, Geneva, Switzerland

Recorded human cases of gambiense human African Trypanosomiasis (HAT) have undergone a remarkable decline over the past decade, prompting plans for elimination of the disease. Effective planning for this elimination programme will require accurate and reliable spatial information on the contemporary distribution of the disease. The Atlas of HAT, recently established by WHO and FAO, aims to map the locations of all known HAT cases. These data provide an unparalleled resource for spatial risk assessment. However, under-reporting of cases and spatial variation in reporting rates complicate their interpretation and reduce their utility for continental-scale planning. To overcome these issues, a collaboration between the Spatial Ecology and Epidemiology Group (Oxford), WHO and FAO is developing a spatial modelling framework to simultaneously map the prevalence of gambiense HAT cases and the probability of detection of cases through the passive reporting system. In order to construct this framework, a novel Bayesian spatio-temporal joint statistical model has been developed to integrate data from both active and passive case detection. The modelling framework will be outlined and preliminary results presented for the first time.

1741

THE HEALTH IMPACT OF VISCERAL LEISHMANIASIS AND HUMAN AFRICAN TRYPANOSOMIASIS WHEN REACHING THE 2020 WHO CONTROL AND ELIMINATION TARGETS

Epke le Rütte, Wilma Stolk, Nico Nagelkerke, Jan H. Richardus, Sake de Vlas

Erasmus MC, Rotterdam, Netherlands

Visceral leishmaniasis (VL) and human African trypanosomiasis (HAT) are neglected tropical diseases (NTDs). The London Declaration was established in 2012 to support the WHO control and elimination targets for ten NTDs by 2020. This initiative intends to globally generate a positive health and socioeconomic impact by decreasing and eliminating the disease burden caused by these NTDs. In our study the global health impact for VL and HAT is calculated for the ideal situation that the 2020 targets are met. The global burden of disease (GBD) study provides prevalence data for VL and HAT in 1990 and 2010. The 2020 targets, as formulated in the WHO Roadmap, provide the ideal situation: control for VL (100% detection and treatment at global level, and 1/10,000 new cases at subdistrict level per year on the Indian subcontinent) and elimination of HAT by 2030. Linear trends between 1990, 2010, 2020 and 2030 provide a simplification of the real situation, representing the number of remaining cases with disease, per country, age group and sex. Continuing the 1990 prevalence until 2030, corrected for demographic changes based on UNPOP data, serves as baseline situation without interventions. The difference between the baseline and the remaining cases results in the number of averted cases. The total number of averted years lived with disability (YLD) is calculated by multiplying the number of averted cases with the GBD disability weights. The total number of averted disability adjusted life years (DALYs) between 2010 and 2030 results in app. 140 and 100 million DALYs for VL and HAT, respectively. The DALYs are almost completely determined by the number of years of life lost (YLLs). The number of averted deaths over these two decades is 2.4 million and 1.7 million for VL and HAT, respectively. Although there have been many successful interventions for VL and HAT, it is important to emphasize the need for continuation and even increase of these efforts, especially when recognizing the sizeable health impact that can be gained when achieving the 2020 targets.

1742

ANTI-*LEISHMANIA* ANTIBODIES IN BLOOD DONORS FROM BRAZIL USING RECOMBINANT *L. INFANTUM* PROTEIN K39 ELISA

Maria Carmen A. Sanchez[1], Marina P. Dornelas[1], Silvano Wendel[2], Silvia M. Di Santi[1], Adeiana G. Lima[1], Beatriz J. Celeste[1], Hiro Goto[1]

[1]São Paulo University, São Paulo, Brazil, [2]Sirio Libanês Hospital, São Paulo, Brazil

In Brazil, visceral leishmaniasis (VL) caused by *Leishmania infantum* has a wide geographic distribution throughout the country with 3,894 reported cases in 2011, 47% in the Northeast region. Most of *L. infantum*-infected individuals (around 95%) are asymptomatic and may be undetected and accepted as blood donors in endemic areas. Since leishmaniasis can be transmitted through blood transfusion, this study aimed to investigate the presence of anti-*L. infantum* rK39 antibodies in blood samples from Brazil endemic areas. We used ELISA-rK39 (rK39 antigen kindly provided by Infectious Disease Research Institute, USA) that yielded 95.45% sensitivity, assaying 44 sera from parasitologically confirmed symptomatic VL patients with positive Direct Agglutination Test (DAT) and 100.0% specificity assaying 44 healthy endemic DAT negative control samples. The present study was carried out with 916 blood samples from Brazilian Northeastern states, Bahia (N=604) and Ceará (N=312). Anti-rK39 antibodies were detected in 26 out of 916 samples (2.8%): Bahia (2.8%) and Ceará (3.0%). The reactivity index (RI = absorbance/cut-off) varied from 1.010 to 6.756. Immunochromatographic rK39 test (ICT) applied to the 26 reactive samples showed one positive (from Ceará). Using *L. major*-like promastigote antigen (Lm), ELISA-Lm and indirect immunofluorescence test (IFT-Lm) detected respectively seven and one out of 26 positive samples. The sample showing RI of 6.756 in ELISA-rK39 was positive also in ELISA-Lm and ICT. Of note, the studied samples had been screened for Chagas' disease using antigen that cross-reacts with *Leishmania* and has been approved for transfusion; however, the present results showed that this assay using cross-reactive antigen missed those 26 samples with anti-*Leishmania* antibodies that likely result from an asymptomatic *L. infantum* infection. As in endemic areas, it is not easy to differentiate transfusion- or vector-mediated transmission; the occurrence of transmission by transfusion is probably underestimated and raises concerns on blood transfusion safety.

1743

THE RELATIONSHIP BETWEEN CLIMATIC AND OTHER ENVIRONMENTAL FACTORS AND ANNUAL FLUCTUATIONS IN INCIDENCE OF VISCERAL LEISHMANIASIS IN GEDAREF STATE, EASTERN SUDAN

Dia-Eldin A. Elnaiem[1], Alexandra Sweeney[2], Andrew Kruczkiewicz[2], Caitlin Reid[2], François Chappuis3[3], Koert Ritmeijer[4], Omran Osman[5], Madeleine Thomson[2], Ceccato Ceccato[2]

[1]University of Maryland Eastern Shore, Princess Anne, MD, United States, [2]International Research Institute for Climate and Society, Columbia University, Palisades, NY, United States, [3]MSF-Switzerland, Geneva, Switzerland, [4]MSF-Holland, Amsterdam, Netherlands, [5]University of Khartoum, Khartoum, Sudan

Visceral leishmaniasis (VL, kala azar), in Gedaref State, eastern Sudan, is caused by *Leishmania donovani*, which is transmitted by *Phlebotomus orientalis* sand flies. The endemicity of the disease in this region is characterized by marked annual fluctuations and occasional severe epidemics that claim the lives of many people. Since no significant interventions are undertaken against the disease in this region, the fall and rise in the incidence of VL in Gedaref state may be due to climate

variability, which characterizes the Sahelian region. Previous studies conducted by our groups and other research teams in Sudan and the Republic of South Sudan (RSS) showed marked variation in spatial distribution of kala azar that can be related to a number of environmental and socio-economic factors that may be acting together or independently to increase the vulnerability of specific populations to the disease. Our findings supported the previous notion that the vector and the disease are associated with *Acacia seyal - Balanites* woodland and chromic vertisol soils. We used this knowledge to produce a general kala azar risk map based on environmental prediction of the distribution of *P. orientalis*, the VL vector in Sudan and RSS. However, no attempt has yet been made to correlate annual incidence of kala azar with climatic factors and it is not known whether the flare up in disease incidence is associated with dry or wet years. In this study we analyzed VL records of MSF-Switzerland and MSF-Holland, form 1996-2004 and 2010-2012, in relation to a number of climatic and environmental variables, including temperature, humidity, rainfall and normalized difference vegetation index (NDVI). Our results indicated that the incidence of kala azar in this region is related to late onset of the rainy season. Results are discussed in relation to the epidemiology of the disease. Findings from the study may be used in the future to develop an Early Warning System and construct high resolution Geographical Information System (GIS) risk-maps for the disease.

1744

CO-STIMULATORY MOLECULES ARE INVOLVED IN ANERGY IN SYMPTOMATIC VISCERAL LEISHMANIASIS

João Firmino Rodrigues-Neto

Federal University of Rio Grande do Norte, Natal, Brazil

The proliferation and differentiation of naive T cells require signals provided by co-stimulatory molecules on antigen presenting cells (APCs), in addition to antigen-induced signals. In the absence of co-stimulatory signals, T cells become anergic. We hypothesized that co-stimulatory molecules might be involved in part in the reversible anergy observed during symptomatic visceral leishmaniasis. We analyzed the profile of co-stimulatory molecules in lymphocytes and in CD14+ monocytes in whole blood collected from subjects with symptomatic visceral leishmaniasis (sVL) and after their clinical recovery (rVL). An increase in CD8+ T cells expressing CTLA-4 after stimulation with soluble Leishmania antigens (SLA) was observed ($p<0.01$) in sVL. An increase in percentage of CTLA-4 in CD4+ and CD8+ in *ex vivo* condition was observed, when compared sVL versus rVL ($p<0.05$). No difference in lymphocytes expressing CD28 after SLA stimulation was observed in sVL or rVL, but rVL showed a high percentage of CD8+CD28+ in *ex vivo* condition when compared with sVL ($p<0.05$). An increase in the percentage of OX-40 in CD4+ T cells after SLA stimulation in sVL ($p<0.05$) was observed, as well as, an increase of ICOS in CD4+ and CD8+ T cells after SLA stimuli in sVL ($p<0.01$). Furthermore, a high percentage of ICOS in CD4+ in *ex vivo* condition was observed in sVL ($p<0.01$). There was no difference in CD40, CD86, CD80, ICOSL and HLA-DR in CD14+ monocytes after SLA stimulation in sVL or rVL. There wasn't also difference in the median fluorescence intensity (MFI) of CD40, CD80, ICOSL or HLA-DR after SLA stimulation in sVL or rVL observed in CD14+ monocytes. But, CD86 showed a high expression in rVL after SLA stimulation ($p<0.05$). These data support the role of co-stimulatory molecules in the reversible anergy observed during symptomatic VL and might indicate pathways to be explored for immunotherapy against leishmaniasis.

1745

DEFICIENCY OF PROLACTIN-INDUCIBLE PROTEIN LEADS TO IMPAIRED TH1 IMMUNE RESPONSE AND SUSCEPTIBILITY TO AN INTRACELLULAR PATHOGEN

Jintao Li[1], Dong Liu[1], Anne Blanchard[2], Ping Jia[1], Yvonne Myal[2], Jude Uzonna[1]

[1]Institute of Tropical Medicine, Third Military Medical University, Chongqing, China, [2]Department of Pathology and Deprtment of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, MB, Canada

The prolactin-inducible protein (PIP) is a secretory protein strategically located at several ports of pathogen entry into the body suggesting it might play a role in host defense. To date, no study has addressed the contributions of PIP in immunity against infectious agents. Here, we assessed the phenotype and responsiveness of immune cells from PIP KO mice to polyclonal T cell stimulators and model antigens *in vitro* and *in vivo*. We found comparable numbers of immune cells, (including T, B, natural killer and dendritic cells) in the primary and peripheral lymphoid organs of wild type and PIP KO mice. Further in-depth phenotypic analysis revealed that PIP KO mice had slightly but significantly lower numbers of $CD4^+$ T cells in their spleens and lymph nodes. $CD4^+$ T cells from PIP KO mice showed significantly decreased proliferation, IL-2 production and impaired Th1 differentiation *in vitro*. The impaired *in vitro* Th1 response was confirmed *in vivo* where $CD4^+$ T cells from OVA-immunized PIP KO mice showed significantly impaired proliferation and IFN-γ production following *in vitro* restimulation. Furthermore, PIP KO mice were highly susceptible to *Leishmania major* infection as evidence by inability to control lesion progression and parasite proliferation. This impaired resistant was associated with dramatic impairment in IFN-γ and nitric oxide production by splenic and draining lymph node cells from infected mice. Collectively, our findings implicate PIP as an important regulator of $CD4^+$ Th1 cell response, and play a critical role in resistance to intracellular pathogens.

1746

T CELL ACCUMULATION IN THE SPLEEN DURING CHRONIC PROGRESSIVE VISCERAL LEISHMANIASIS

Audrie A. Medina, Elvia Y. Osorio, Omar A. Saldarriaga, Peter C. Melby

University of Texas Medical Branch, Galveston, TX, United States

Visceral leishmaniasis (VL), caused by the protozoan, *Leishmania donovani*, is a chronic systemic infection that contributes to half a million new cases each year. During progressive VL, there is profound expansion of immune cells in the spleen. Type 1 and type 2 cytokines are increased during VL in patients, but the T cell population in the spleen during VL has not been fully characterized. In the Syrian hamster model of progressive VL, which mimics human VL, we found a significant increase in expression of the T cell transcription factors Tbet, GATA3 and Foxp3 in spleen cells over the course of chronic infection. This suggests the presence of a mix of Th1, Th2 and Treg cells. In purified splenic CD4 T cells from 28 day infected hamsters we found an increase in Tbet ($p<0.001$) and GATA3 ($p=0.0087$) and Th1-associated chemokine receptors CXCR3 ($p<0.0001$) and CCR5 ($p<0.001$). There was no significant difference in Foxp3 or the Th2-associated chemokine receptor CCR4 in purified CD4 T cells from uninfected and infected animals. These data suggest both Th1 and Th2 cells are present in the spleen during chronic infection, although one would expect Th2 cells to prominently express CCR4. Notably, we also found a significant population of CD4 T cells that expressed both Tbet and GATA3. The increase in Th1 and Th2 cells in the spleen during chronic infection could be due to local proliferation or splenic recruitment by T cell attracting chemokines. We found an array of chemokines (CCL2, CCL4, CCL5, CCL17, CCL22) increased in the spleen of hamsters over a course of disease. This suggests that T cell attracting chemokines may be playing a role in T cell accumulation at the site of infection.

1747

EVALUATION OF THE EFFICACY OF ANTIGEN DELIVERY BY THE TRANSCUTANEOUS IMMUNIZATION ROUTE IN A MURINE MODEL OF CUTANEOUS LEISHMANIASIS

Ines Elakhal Naouar[1], Nancy Koles[1], Elaine Morrison[2], Mangala Rao[2], Carl Alving[2], Naomi Aronson[1]

[1]Uniformed Services University, Bethesda, MD, United States, [2]Walter Reed Army Institute of Research, Laboratory of Adjuvant and Antigen Research, Silver Spring, MD, United States

Transcutaneous immunization (TCI) is a novel attractive vaccination method which offers advantages over traditional vaccination routes, exploiting the abundance of antigen presenting cells in the skin. We provide the first report of Transcutaneous immunization (TCI) is a novel attractive vaccination method which offers advantages over traditional vaccination routes, exploiting the abundance of antigen presenting cells in the skin. We provide the first report of TCI induced immune responses to Leishmania antigens. Leishmania major soluble antigens (SLA) and Phlebotomus papatasi salivary gland homogenates (SGH) were delivered transcutaneously with cholera toxin (CT), a potent adjuvant for developing mucosal immunity. Sixty inbred BALB/c mice were immunized three times at weeks 0, 3 and 6 with the vaccine formulations (different doses of SLA or SGH, SLA+SGH). TCI was well tolerated. Two weeks after the last vaccine boost, we assessed humoral (IgG titer to antigens and CT) and cellular immune responses (IFN-γ ELIspot and cytokine levels from splenic cell culture). In contrast to SGH alone, we showed that transcutaneous immunization of mice with SLA resulted in high titers of anti-SLA IgG that increased when SLA was combined with SGH antigen. Immunization was also associated with high anti-CT IgG titers. A Th1-type immune response was demonstrated with high levels of IFN-γ production and lower levels of IL-10 resulting in a significantly higher IFN-γ/IL-10 ratio compared to the control groups. A high frequency of IFN-γ secreting cells was also seen in groups of mice immunized with SLA. Altogether, these data are consistent with reported protective immune responses and indicate the strong potential of our TCI strategy to protect against Leishmania major infection, with the combined antigen SLA and SGH showing the strongest responses. Experiments using the same regimen of immunizations followed by parasite challenge are in progress. Results of lesion evolution and parasite load along with immune responses pre and post challenge will be presented.

1748

MECHANISMS OF DISEASE-PROMOTING MACROPHAGE PROLIFERATION IN VISCERAL LEISHMANIASIS

E. Yaneth Osorio[1], Audrie A. Medina[1], Omar A. Saldarriaga[1], Bruno L. Travi[1], Alda M. da-Cruz[2], Peter C. Melby[1]

[1]University of Texas Medical Branch, Galveston, TX, United States, [2]FIOCRUZ, Rio de Janeiro, Brazil

Macrophages have classically been viewed as mature non-proliferative cells, which originate from bone marrow monocytes released to peripheral blood to infiltrate tissues in response to injury. However, recently it was discovered that macrophages can be locally self-maintained without major contribution of infiltrating monocytes, and that M2 macrophages are able to proliferate locally within a Th2 environment. The mechanism(s) that drive this proliferation have not been defined. In a model of chronic progressive visceral leishmaniasis, we found huge expansion of disease promoting macrophages in the spleen, with signs of Th2-amplified M2 activation, increased STAT-6 activation, and pathological arginase expression. We discovered that growth factors IGF-I and FGF-2 were key contributors to both STAT-6 activation and arginase expression in *L. donovani* infected macrophages, and inhibition of growth factor signaling blocked arginase expression and parasite replication. Since that these growth factors also drive cellular proliferation and differentiation, and arginase contributes to the cell growth through polyamine production, we explored the possibility that these factors had a role in macrophage expansion in visceral leishmaniasis. *L. donovani* infection of bone marrow and splenic macrophages resulted in increased cell number, DNA synthesis (BrDu incorporation) and mitosis (ki-67 antigen expression). The combination of growth factor FGF-2 or IGF-I with IL-4 significantly increased macrophage proliferation, suggesting that they interact to control the cell cycle. Inhibition of FGFR, IGFR and PI3K significantly reduced mitosis indicating that growth factor signaling through PI3K was a major contributor to macrophage proliferation. The local amplification of macrophages in response to chronic infection through the expression of type 2 cytokines and growth factors may have broad significance to other chronic infectious diseases.

1749

FACTORS CONTRIBUTING TO TISSUE DAMAGE IN HUMAN CUTANEOUS LEISHMANIASIS

Sara T. Passos, Taís Menezes, Patrícia Cisneiros, Paulo R. Machado, Juliana Almeida, Viviane Magalhães, Viviane Magalhães, Lucas P. Carvalho, Edgar M. Carvalho

Federal University of Bahia, Salvador, Brazil

Human cutaneous leishmaniasis (CL), due to *Leishmania braziliensis* infection, is characterized by intense immune mediated tissue inflammation and skin ulceration. For many years immunological studies in CL patients have focused on the evaluation of host response mainly using peripheral blood cells assays. In this context, high levels of IFN-gamma and TNF and low levels of regulatory cytokine, IL-10, is observed in cultures of peripheral blood mononuclear cells stimulated with soluble *Leishmania* antigen. Histopathology studies show mononuclear cells infiltration and low number of parasites. Our hypothesis is that the inflammatory environment helps to control parasitemia but mediates tissue destruction. In the present study we evaluated cytokine and chemokine profile at lesion site. Cells from CL lesion produced high levels of pro-inflammatory cytokines, TNF, IL-6 and IL-1b in absence of stimuli. To determine the contribution of skin epithelial cells to the production of these cytokines we cultured epidermis and dermis separately. TNF was only produced by cells composing the dermis, while IL-6 and IL-1b were produced by dermis and epithelial cells from epidermis. High levels of CCL2, a chemokine that recruits mononuclear phagocytes, and CXCL9 and CXCL10, involved in lymphocyte recruitment, were also observed. Metalloproneinase-9 (MMP-9) is a zinc-dependent enzyme that degrades collagen type 4 (present in basal membrane) and has been associated with tissue damage in skin inflammatory diseases. We found increased production of MMP-9 in CL lesion when compared to healthy skin. TNF is known to induce MMP-9 production. To determine the role of TNF in MMP-9 production in CL, we cultured CL cells in presence of monoclonal antibodyes anti-TNF. Blockage of TNF decreased MMP-9 production in CL. Ou study contributes to the understanding of immunopathology in CL and revele possible targets for immunotherapy.

1750

IMMUNOGENICITY AND PROTECTIVE EFFICACY OF PVAX-NH36 AS A DNA VACCINE AGAINST CUTANEOUS LEISHMANIASIS IN A CANINE MODEL

Vladimir Cruz-Chan[1], Amaru Aguilar-Cetina[1], Liliana Villanueva-Lizama[1], Pedro Martinez-Vega[1], Maria Jesus Ramirez-Sierra[1], Miguel Rosado-Vallado[1], Leonardo Guillermo-Cordero[1], Jaime Ortega-Lopez[2], Eric Dumonteil[1]

[1]Universidad Autonoma de Yucatan, Merida, Yucatan, Mexico, [2]CINVESTAV, Mexico, D.F., Mexico

Leishmaniasis is a major tropical disease affecting 2 million people annually, against which there is no effective treatment. Vaccine development for humans requires preclinical testing in animal models including canines. One of the best vaccine candidates is NH36, a *Leishmania donovani* 36 kDa protein. As DNA vaccine NH36 provides better protection than the recombinant protein or purified fucose-manose

ligand. In this study, we evaluated the immunogenicity and protection of pVAX-NH36 for the prevention and therapy of *L. mexicana* infection in dogs. We first established a model of infection in Beagle dogs. Then, Beagles received 3 doses of 250 microg of pVAX-NH36 as prophylaxis or therapy, while dogs from control group received saline solution. *L. mexicana* promastigotes were used for infection via intradermal. Immune response was evaluated measuring antibodies, IFN and IL-10 production, and DTH. Ulcer diameters and parasite burden were evaluated to assess protection. Canines receiving pVAX-NH36 showed higher IgG levels against NH36 in comparison with the control group. High IFN and low IL-10 levels were produced by PBMC stimulated with NH36 from vaccinated canines. In addition, only vaccinated animals were DTH positive against recombinant NH36. Finally, some protection was observed based on skin parasite burden as two vaccinated animals showed negative results by qPCR. In conclusion, pVAX-NH36 is safe and immunogenic in dogs, and can confer some protection against *L. mexicana*.

1751

LEISHMANIA SPECIFIC CD4 T CELLS RELEASE IFNΓ THAT LIMITS PARASITE REPLICATION IN PATIENTS WITH VISCERAL LEISHMANIASIS

Om Prakash Singh[1], Rajiv Kumar[1], Neetu Singh[1], Shalini Gautam[1], Kamlesh Gidwani[1], Madhukar Rai[1], David Sacks[2], Shyam Sundar[1], Susanne Nylen[3]

[1]Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, [2]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [3]Karolinska Institutet, Department of Microbiology Tumor and Cell Biology, Stockholm, Sweden

Visceral leishmaniasis (VL) is associated with increased circulating levels of multiple pro-inflammatory cytokines and chemokines, including IL-12, IFNγ, and TNF-alpha, and elevated expression of IFNγ mRNA in lesional tissue such as the spleen and bone marrow. However, an immunological feature of VL patients is that their peripheral blood mononuclear cells (PBMCs) typically fail to respond to stimulation with leishmanial antigen. Unexpectedly, it was recently shown that Leishmania specific IFNγ, can readily be detected when a whole blood stimulation assay (WBA) is used. We sought to define the conditions that permit whole blood cells to respond to antigen stimulation, and clarify the biological role of the IFNγ found to be released by cells from VL patients. CD4+ T cells were found to be crucial for and the main source of the IFNγ production in Leishmania stimulated whole blood (WB) cultures. Complement, antibodies and red blood cells present in whole blood do not play a significant role in the IFNγ response. The IFNγ production was reduced by blockade of human leukocyte antigen (HLA)-DR, indicating that the response to leishmanial antigens observed in WB of active VL patients is a classical HLA- T cell receptor (TCR) driven reaction. Most importantly, blockade of IFNγ in ex-vivo splenic aspirate cultures demonstrated that despite the progressive nature of their disease, the endogenous IFNγ produced in patients with active VL serves to limit parasite growth.

1752

ISOLATION AND INITIAL CHARACTERIZATION OF *TRYPANOSOMA CRUZI* ISOLATES FROM A POPULATION OF CYNOMOLGUS MACAQUES NATURALLY INFECTED IN THE UNITED STATES

Phil Yao

University of Georgia, Athens, GA, United States

The protozoan parasite *Trypanosoma cruzi*, causative agent of Chagas disease, is capable of infecting not only humans, but also virtually any mammalian species. Non-human primates housed in outdoor facilities in the southern United States are known to acquire *T. cruzi* infection, as is the case for a group of 64 cynomolgus macaques from Texas who are seropositive for *T. cruzi* infection. This group provided a unique opportunity to investigate natural *T. cruzi* infection in an entire population from a locale. Similar to endemic human infection, infection in these macaques is thought to have been acquired early in life (age range at infection 1-15 years) and to persist chronically with an average infection time of 6.4 years. The *T. cruzi* isolates were obtained from hemocultures of 43 out of 64 (67%) macaques. An additional 8 hemocultures were positive for *T. cruzi* DNA by PCR, but failed to yield a culturable line, resulting in an overall hemoculture detection rate of 80%. This figure is comparable to the frequency of detection of infection by serial PCR (up to 3 samples) of whole blood (81%). Furthermore, parasite isolates were obtained by hemoculture from 8 of the 12 PCR-negative animals. The combination of whole blood PCR and hemoculture +/- PCR confirmed active infection in 94% of the seropositive animals. Genotyping of hemoculture-isolated *T. cruzi* revealed the presence of two lineages: TcI and TcIV, which are the most common lineages identified by previous studies in infections originating in North America. To date, all tested hemoculture-isolated *T. cruzi* could be converted to metacyclic trypomastigotes and were orally infective in C57Bl/6 and IFN-gamma knockout mice with the TcIV lineage isolates exhibiting less virulence in both mouse models. All isolates appeared to establish chronic infection in mice and to induce immune responses consistent with those observed in infections by long-maintained laboratory-adapted strains of *T. cruzi*. Future studies will assess the susceptibility of these fresh isolates to clearance by treatment with benznidazole.

1753

IMMUNOREGULATORY NETWORKS AND IMMUNOPATHOGENIC PATHWAYS IN VISCERAL LEISHMANIASIS

Omar A. Saldarriaga, Fanping Kong, Heidi Spratt, Bruce A. Luxon, Elvia Y. Osorio, Bruno L. Travi, Peter C. Melby

University of Texas Medical Branch, Galveston, TX, United States

Visceral Leishmaniasis (VL) caused by *Leishmania donovani*, is an important problem in tropical and subtropical areas of the world. VL is characterized by a progressive increase in visceral parasite burden, cachexia, splenomegaly, pancytopenia and ultimately death. We have studied the pathogenesis of VL in Syrian hamsters *(Mesocricetus auratus*) since it closely mimics active human disease. We demonstrated previously that the simultaneous expression of Th1 (IFN-γ) and Th2 (IL-4) cytokines, parasite activation of STAT6, and decreased production of nitric oxide are associated with susceptibility to the infection. To fill the gaps in our understanding of the pathogenesis of VL we examined global changes in gene expression in spleen tissue and splenic (adherent) macrophages. We used a novel approach of deep sequencing (RNAseq) coupled with *de novo* assembly of full-length transcripts because the Syrian hamster does not have an annotated reference genome. Differentially expressed transcripts in *L. donovani* infected (28 days) vs. uninfected hamsters were determined by alignment back to the *de novo* constructed transcriptome and cross-species BLAST, after the removal of contaminating parasite sequences. Transcriptome analysis confirmed that adherent cells were enriched for expression of macrophage (CD14, CD64 and Mertk) but not T cells markers (CD4, Gata3 and Tbet). Differentially expressed genes analyzed with IPA software revealed a number of highly enriched canonical pathways in spleen and splenic macrophages, including hepatic fibrosis, pathogenesis of multiple sclerosis, atherosclerosis signaling, communication between innate and adaptive immune cells, and the glucocorticoid receptor signaling. Notably, within the differentially expressed transcripts we identified mixed expression of genes associated with classical (M1) and alternative (M2) activation of macrophages that was confirmed by qPCR. This approach provides a valuable tool to overcome the obstacles of working with a non-model organism without a reference genome. With it we can begin to understand the complex immunopathogenic mechanisms at the site of visceral infection.

1754

ENHANCEMENT OF MURINE VISCERAL LEISHMANIASIS DUE TO CROSS REACTIVE *LEISHMANIA MAJOR* ANTIBODIES

Heidi Anderson, Blaise Dondji, **Gabrielle A. Stryker**

Central Washington University, Ellensburg, WA, United States

Leishmaniasis is a global disease found in regions with compatible temperatures for the phlebotomine sandfly vector to survive and lacking rigorous vector control programs. An estimated 1.3 million new cases and 20,000 - 30,000 deaths occur annually due to this parasitic protozoan. More than twenty different species of *Leishmania* infect humans with multiple species occurring in the same geographic areas. Symptoms range from a minor cutaneous lesion at the bite site due to dermotropic species such as *L. major*, to life threatening disseminated disease with multiple organ involvement, caused by viscerotropic species such as *L. infantum*. We have previously shown susceptible BALB/c mice infected with a low/self-healing dose of cutaneous *L. major* and challenged with *L. infantum*, develop a markedly worsened disease with higher parasite burden, relative to naïve mice. There was little notable difference in the cytokine profiles between *L. major* exposed and naïve mice in response to *L. infantum*. Cross-reactive antibodies were seen in both groups of *L. infantum* infected mice regardless of their immune history. Opsonizing antibodies have been shown to lead to increased disease in visceral leishmaniasis. The present studies focus on exploring the role cross-reactive antibodies may play in exacerbation of visceral disease seen in mice previously exposed to *L. major*. Mice receiving passively transferred serum from *L. major* infected mice, 48 hours prior to challenge with *L. infantum,* developed equivalent organ parasitemia to age/*L. major*-infected matched control mice. Naïve mice inoculated with control serum did not suffer any disease enhancement with *L. infantum*. We speculate that cross-reactive antibodies are augmenting visceral disease in mice with immunological memory to *L. major*. While *L. major* is known to produce long lasting immunological memory and protect against recurrent cutaneous disease, antibody enhancement due to inter-*Leishmania* infection may enhance disease in regions with multiple circulating *Leishmania*-species and suggests leishmanization might be riskier than previously thought.

1755

HUMAN SEROPREVALENCE OF LEPTOSPIROSIS AND RICKETTSIOSIS IN FOUR REGIONS OF PERU

Gabriela Salmon-Mulanovich[1], Mark Simons[1], Maria E. Silva[1], Bruno M. Ghersi[2], Matthew R. Kasper[3], Hugo R. Razuri[4], Daniel G. Bausch[5], Allen L. Richards[6]

[1]U.S. Naval Medical Research Unit - 6, Callao, Peru, [2]Tulane University, New Orleans, LA, United States, [3]Armed Forces Health Surveillance Center, Silver Spring, MD, United States, [4]McGill University Health Centre, Montreal, QC, Canada, [5]Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States, [6]Naval Medical Research Center, Silver Spring, MD, United States

Rickettsia and *Leptospira* are obligate intracellular bacteria with global distributions and a wide variety of animal hosts. Two disease-causing groups of *Rickettsia* are noted: the spotted fever group *Rickettsia* (SFGR) and the typhus fever group *Rickettsia* (TFGR). We explored the prevalence of exposure and risk factors for human *Rickettsia* infection in four ecologically distinct regions of Peru (Lima, Cusco, Puerto Maldonado and Tumbes) and for *Leptospira* in Puerto Maldonado and Tumbes. In January 2012 we randomly collected 2165 serum samples from participants in a surveillance cohort for respiratory disease in the aforementioned sites and tested them for IgG by ELISA (*Rickettsia*) and microscopic agglutination test (*Leptospira*). Overall antibody prevalence across the four sites was 10.6% for SFGR (ranging from 6.2-14.0%, with the highest prevalence in Tumbes) and 3.3% for TFGR (ranging from 2.6-6.4%, with highest prevalence in Puerto Maldonado). Factors associated with positive IgG for SFGR on multiple logistic regression analysis were male sex (OR 2.2, 95% CI 1.5-3.3), increasing age (OR 1.02, 95% CI 1.01-1.04 per year), contact with backyard birds (OR 2.1, 95% CI 1.4-3.0), and working in agriculture or with livestock (OR 4.3, 95% CI 1.7-10.8). However, exposure to any kind of animal within the household decreased the odds ratio by half (OR 0.50, 95% CI 0.31-0.81), perhaps indicating that arthropod vectors on birds preferred non-human hosts when they were present, thus diminishing exposure to humans. Age was the only variable associated with antibody positivity to TFGR (OR 1.03, 95% CI 1.02-1.05). The antibody prevalence to *Leptospira* was 11.3% in Puerto Maldonado and 5.8% in Tumbes, with a borderline association with keeping animals in the household (OR 2.5, 95% CI 1.0-6.2). Exposure to *Rickettsia*, especially SFGR, and *Leptospira* appears to be frequent in Peru. We plan now to perform testing in domestic animals in some of these sites to determine the specific reservoirs and vectors for these agents and to obtain pathogen isolates for identification of the specific species.

1756

PROXIMITY TO PIG POPULATIONS AS A KEY RISK FACTOR FOR JAPANESE ENCEPHALITIS DISEASE; RESULTS OF A FIVE-YEAR SURVEILLANCE STUDY FROM NORTHWESTERN BANGLADESH

Henrik Salje[1], Salah Uddin Khan[2], Abu Hannan[3], M. Atiqul Islam[4], A. A. Mamun Bhuyan[4], M. Ariful Islam[4], M. Ziaur Rahman[4], Mahmadur Rahman[5], Nazmun Nahar[4], M. Jahangir Hossain[4], Stephen P. Luby[6], Emily S. Gurley[4]

[1]Johns Hopkins School of Public Health, Baltimore, MD, United States, [2]University of Florida, Gainesville, FL, United States, [3]Fisheries Ministry, Dhaka, Bangladesh, [4]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [5]IEDCR, Dhaka, Bangladesh, [6]Stanford University, Stanford, CA, United States

Japanese encephalitis (JE) is a mosquito-borne virus that causes severe disease in humans with 10-20% case fatality. It is the commonest cause of encephalitis in Bangladesh. Humans are dead-end hosts. Pigs, by contrast, are viral amplifying hosts, so the distribution of pig populations may be particularly important for human disease risk. Previous studies have shown that the highest incidence of human JE infections occur in northwestern Bangladesh, where JE is also endemic among pigs. The objective of this study was to explore proximity to pigs as a risk factor for human JE disease in Bangladesh. We first geocoded the locations of residence for all JE patients identified through hospital-based surveillance in Naogaon, Chapainawabganj, and Rajshahi districts in northwestern Bangladesh between 2007 and 2011. Next, we used data from a 2009 pig census in these areas to map all pig raising households. To explore the impact of proximity to pigs as a risk factor for JE disease we compared the odds of a human JE case living within a set distance of a pig-raising household to that of a randomly selected control population. We identified 81 human JE cases from throughout the region, with a mean age of 32 years (range: 0 - 75 years); 11% died. Disease patterns were highly seasonal with 90% of cases occurring between the months of August and November. Humans infected with JE were 2.7 times more likely to live within 500m of a pig-owning household compared to controls (95% confidence interval [CI] 1.3 - 4.6) and 1.7 times more likely to live within 5km (95% CI: 1.0 - 3.7). Results from this analysis suggest that proximity to pig populations is an important risk factor for human JE disease in northwestern Bangladesh. JE vaccination is not currently included in the Bangladesh immunization program; therefore, interventions to reduce infections among pigs could be an important strategy for reducing human risk in these areas and should be explored.

1757

ECOLOGICAL NICHE MODELING FOR SYLVATIC RABIES TRANSMITTED BY *DESMODUS ROTUNDUS* IN PERU

M. Claudia Guezala Villavicencio[1], William D. Valerrama[2], Gabriela Salmon-Mulanovich[1], Daniel G. Bausch[3]

[1]U.S. Naval Medical Research Unit - 6, Callao, Peru, [2]Servicio Nacional de Sanidad Agraria, La Molina, Peru, [3]Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States

Rabies is a viral infection endemic in many parts of the world that causes severe and usually fatal encephalitis. In Latin America, a sylvatic cycle exists in which rabies virus is maintained in several species of wild animals, especially the hematophagous bat *Desmodus rotundus*. Peru and Brazil report the highest number sylvatic rabies cases, mainly transmitted by bats. Vaccination of humans and livestock is an effective prevention method, but vaccination is not usually undertaken until a human exposure our outbreak in livestock occurs. The ecologic and topographic factors underlying the distribution of rabies vectors are not well understood. Specifically for sylvatic rabies, there is little data on the factors influencing the distribution of hematophagous bats. Such information would help define areas and populations at risk for rabies and inform effective prevention campaigns. We therefore modeled the potential geographic distribution of *D. rotundus* using the ecological niche modeling algorithm MaxEnt. Incorporating climatic, environmental and anthropogenic factors that may relate to the geographic distribution of *D. rotundus* and rabies-infected farm animals, we developed a risk map for bat-associated rabies transmission in Peru. *D. rotundus* occurrence was found to be associated with the colder, drier months of the year. In addition, land classification data show that bats prefer firmer, non-flooding low lands. Variables associated with occurrence of animal rabies included livestock population density, mean diurnal temperature range, and precipitation in the drier months of the year. This study offers a first glimpse of the environmental and bioclimatic factors associated with the distribution of hematophagous bats and animal rabies using novel techniques that extract the maximum information and offer robust results from the little data available.

1758

THE PROBLEM OF LEPTOSPIROSIS IN AFRICA: REVEALING A NEGLECTED 'ONE HEALTH' CHALLENGE THROUGH A SYSTEMATIC REVIEW

Kathryn J. Allan[1], Holly M. Biggs[2], Jo E. Halliday[1], Rudovick R. Kazwala[3], Venance P. Maro[4], Sarah Cleaveland[1], John A. Crump[2]

[1]University of Glasgow, Glasgow, United Kingdom, [2]Duke University Medical Center, Durham, NC, United States, [3]Sokoine University of Agriculture, Morogoro, United Republic of Tanzania, [4]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania

Recent evidence from Tanzania indicates that leptospirosis is an important cause of non-malarial febrile disease. However, relatively little is known about the incidence and geographic distribution of leptospirosis in Africa as well as the diversity of infecting *Leptospira* spp. in human and animal populations. To examine current knowledge, we performed a systematic review of acute human leptospirosis and confirmed *Leptospira* infection in animals. We searched eight international and regional scientific databases using the terms '*Leptospira*' OR 'leptospirosis' AND 'Africa' for articles published between 1930 and June 2013. Of 630 unique articles identified and reviewed against predetermined inclusion and exclusion criteria, 89 (14.1%) were considered eligible. Eligible articles described human and animal *Leptospira* spp. infection in 26 (44.8%) of 58 countries included in the UN continental definition of Africa. Prevalence of acute leptospirosis in hospital-based cohort studies of patients with non-malarial febrile illness ranged from 2.3% (n=43) to 47.5% (n=59). Estimates of annual human leptospirosis incidence ranged from 4.1 to 101 cases per 100,000 based on surveillance studies of island populations. *Leptospira* spp. infection was also reported in a wide range of animal hosts. 11 out of 15 human-infecting *Leptospira* serogroups were isolated from one or more animal host species in Africa. For several important human-infecting serogroups, multiple animal host species were identified. *L. borgpetersenii, L. interrogans* and *L. kirschneri* were the predominant genetic species reported in human and animal populations across Africa, although some local variation was observed. In conclusion, this systematic review highlights the importance of acute leptospirosis in febrile patients in Africa and reveals many areas of uncertainty that remain in our understanding of this complex, multi-host disease. A 'One Health' approach is advocated to integrate human and animal studies in future work, and to explore local and regional variation in leptospirosis epidemiology in Africa.

1759

CHARACTERIZING EXPOSURE TO BATS AND BAT GUANO AMONG MEN, WOMEN AND CHILDREN IN LAO PDR TO INFORM INTERVENTIONS FOR REDUCING THE RISK OF ZOONOTIC DISEASE

Laura Seckel[1], Khounkham Xaymounvong[2], Sara Woldehanna[1], Susan Zimicki[1]

[1]FHI 360, Washington, DC, United States, [2]FHI 360, Vientiane, Lao People's Democratic Republic

Bats host more viruses per species than other mammals, and are reservoirs of numerous pathogens that pose significant risk to humans. Several attention-grabbing emerging diseases, such as SARS, Ebola, Nipah and MERS-CoV have been linked to bats. Transmission routes for these, and other zoonotic diseases, are extremely varied, and bats are thought to transmit diseases through urine, feces, saliva or via intermediate hosts. To inform development of interventions to prevent the spread of disease from bats to humans, human exposure to bats and their excrement must be better understood. In Lao PDR, a country of great biodiversity, people are regularly exposed to bats in a variety of ways, including hunting, consumption, and the collection and use of guano. In addition, both humans and domestic animals are often exposed to bats and their excreta through environmental exposure. We used rapid appraisal and participatory research methods to characterize bat exposure among men, women and children in four sites in Vientiane and Bolikhamxay provinces, Lao PDR. We will report findings about human interactions with bats/excreta, environmental exposure of humans and domestic animals to bats/excreta, and the seasonality of exposure, mediated by gender, location, ethnicity (Lao-Tai, Hmong, Kammu), age, and occupation and will discuss possible preventive interventions.

1760

RISK FACTORS FOR HUMAN EXPOSURE TO WILDLIFE ZOONOSES IN NIGERIAN HUNTING COMMUNITIES

Sagan Friant, Tony Goldberg, Sarah Paige

University of Wisconsin Madison, Madison, WI, United States

Bushmeat hunting clearly increases the risk of zoonotic pathogen transmission. However, limited information exists on the nature and frequency of contact with wildlife in communities that practice bushmeat hunting, especially with respect to socioeconomic drivers of hunting behavior. We surveyed 188 hunters and 139 non-hunters in five rural communities in Cross River State, Nigeria. Responses were used to: 1) quantify contact rates with wildlife, 2) identify socioeconomic factors predisposing individuals to hunt, and 3) identify specific hunting behaviors that increase frequency of contact with wildlife. Among hunters, 95% hunted rodents, 91% ungulates, 90% carnivores, 78% primates and 35% bats. Hunters used traps (75%), guns (71%), machetes (71%), and dogs (18%) to hunt animals both day (78%) and night (69%). We constructed generalized linear mixed models to examine socioeconomic predictors of individual hunting behavior and frequency of contact with wildlife, especially primates. We found that lower education level ($<.01$), having a father who hunts ($p<.0001$), and larger household sizes ($p<.05$) were all associated with becoming a hunter. Among hunters, high rates of wildlife contact were associated with high hunting frequency ($p<.05$), hunting

both night and day (p<.05) and with a gun (p<.05); while sleeping in the forest (p<.0001), hunting night and day (p<.0001) and with a dog (p<.05) were associated specifically with high rates of primate contact. Results demonstrate that hunters have risky contact with a diversity of wildlife, and that the decision to become a hunter is deeply rooted in family history and modified by economic necessity. Improved education, reduced family sizes, and alternative livelihoods may reduce the risk of zoonotic disease exposure in rural hunting communities in Nigeria. Public health programs aimed at reducing zoonotic transmission of wildlife pathogens in such settings will be most efficient when they target root socioeconomic drivers that lead to hunting behavior and risky wildlife contact.

1761

ENVIRONMENTAL RESERVOIRS OF ANTIBIOTIC RESISTANCE ASSOCIATED WITH SMALL SCALE POULTRY FARMING IN NORTHWESTERN ECUADOR

Nikolay P. Braykov[1], Joseph Eisenberg[2], Marissa Grossman[1], Lixin Zhang[2], William Cevallos[3], Karla Vasquez[4], Diana Muñoz[4], Carl F. Marrs[2], Betsy Foxman[2], Gabriel Trueba[4], Karen Levy[1]

[1]Emory University, Atlanta, GA, United States, [2]University of Michigan School of Public Health, Ann Arbor, MI, United States, [3]Universidad Central del Ecuador, Quito, Ecuador, [4]Universidad San Francisco de Quito, Quito, Ecuador

Non-therapeutic use of antibiotics in agriculture poses a threat to human health by contributing to an environmental reservoir of antibiotic-resistant (AR) bacteria. Small-scale "backyard" broiler chicken production involving use of antibiotics for feed promotion is becoming increasingly common in developing countries. We use E. coli antibiotic susceptibility profiles to assess the potential for transmission of AR from broiler chickens to the surrounding environment (water, soil, surface) in the context of small-scale poultry farming in Northwestern Ecuador. Field work spanned 190 households in 17 villages visited between 08/2010-08/2012. We collected a total of 529 samples from drinking water, soil and food preparation surfaces, and surveyed water, sanitation and antibiotic use practices. From a subset of 91 households involved in broiler production, we collected 131 cloacal samples from production chickens and 66 soil and surface samples from chicken coops. In addition, 153 non-production ("free-range") birds were sampled across all villages, and 54 water samples from local rivers were collected. Up to five E. coli isolates from each sample were tested against 12 antibiotics using disc diffusion. Zones of inhibition and their categorical interpretations were compared using mixed-effect models. AR was more common in broilers than free-range chickens for every antibiotic tested (p<0.01), particularly tetracycline (76.8% vs 33.1%), sulfisoxazole (66.9% vs 19.2%) and streptomycin (61.1% vs 26.7%). A pattern of AR to gentamicin, fluoroquinolones and beta-lactams that was unique to broilers was also found in coop surfaces and soils, but not in household samples. The prevalence of this phenotype declined with bird age, implying importation form hatcheries outside the study system. AR was more common in coop than household samples. Farming and non-farming households showed no difference in AR profiles. These results suggest broiler chickens carry AR and pass it to their immediate environment. However, transmission to households may operate at different scales.

1762

FACTORS ASSOCIATED WITH *GIARDIA* IN HUMANS AND ANIMALS RESIDING IN RURAL AND URBAN AREAS OF COASTAL ODISHA, INDIA AND ENVIRONMENTAL LOADING ESTIMATES FROM ANIMALS

Miles Daniels[1], Woutrina Miller[1], Arpit Shivastava[2], Priyadarshi Sahu[2], Mitsunori Odagiri[1], **Marion W. Jenkins**[1]

[1]University of California Davis, Davis, CA, United States, [2]Kalinga Institute of Industrial Technology, Bhubaneswar, India

Globally, *Giardia lamblia*, is one of the most commonly detected intestinal parasites and is particularly relevant in developing countries such as India, where inadequate sanitary conditions, high population densities, and frequent contact with animals capable of carrying zoonotic pathogens can exist in both urban slums and rural areas. We present a study to estimate and compare the prevalence of *Giardia* infection in rural and urban settings for both human and animal populations residing in coastal Odisha, India, evaluate if gender or age is associated with *Giardia* infection in humans, and estimate fecal loading rates of *Giardia* cysts from animal host populations residing in the study area. From April to May of 2012, human fecal samples from 85 diarrhea patients presenting at three diarrhea wards and 111 pooled animal fecal samples across seven host species (cattle, buffalo, goat, sheep, chicken, cat, and dog) were collected across urban and rural residential areas served by the wards. Samples were screened and fluorescent microscopy used to enumerate *Giardia* cysts and a subset of dog and human samples analyzed by molecular methods to identify isolate genotypes. *Giardia* cysts were detected in 12% of tested diarrhea patients, while 32% of pooled animal samples were positive. No evidence for difference in the presence of *Giardia* cysts among humans was observed between urban and rural settings, gender, or age groups (<5 years, 5-59 years, >59 years). There was substantial support for a location effect on *Giardia* shedding among animals, with rural animals shedding higher numbers of parasites. Of the seven animal host groups screened, dogs and cattle, both reported to shed zoonotic genotypes of *Giardia* in India, shed decisively more *Giardia* cysts per gram of feces, as much as 2-3 orders of magnitude greater than other animal types (adjusting for location). Molecular characterization of isolates identified host specific Assemblages in dog samples and a possible zoonotic Assemblage in a human sample. Using current animal populations and observed *Giardia* shedding rates, cattle were estimated to contribute >99% of *Giardia* animal cysts into the study area environment, followed by dogs as the next largest source. This study shows *Giardia* prevalence is similar for humans living in rural and urban settings, but different for animals and that exposure from infected cattle and dogs may be an important public health concern in Coastal Odisha.

1763

COMPARATIVE GENOMIC ANALYSIS OF *COCCIDIOIDES* AND RELATED SPECIES

Christian Olsen, Kashef Qaadri

Biomatters, Newark, NJ, United States

Dimorphic fungi *Coccidioides immitis* and *C. posadasii* are primary pathogens of immunocompetent mammals, including humans. *Coccidioides* infection results from environmental exposure, which is believed to grow as a soil saprophyte in arid desert environs. To investigate hypotheses about the evolution of *Coccidioides*, the genomes of several Onygenales, a close, nonpathogenic relative, and a more diverged pathogenic fungus, were compared with those of 13 more distantly related Ascomycetes. This poster aims to identify shifts in gene family size associated with a host/substrate shift from plants to animals in the Onygenales. Comparison among Onygenales revealed distinct evolutionary changes in *Coccidioides* that may underlie its infectious phenotype, coccidioidomycosis. Phylogenetic analysis suggest that *Coccidioides* species are not soil saprophytes as previously hypothesized. Data indicate that they have evolved to remain associated with their dead animal hosts in soil. Using a bioinformatics workflow, we show that metabolic pathway genes, membrane-related proteins, and putatively antigenic compounds have evolved in response to interaction with an animal host.

1764

MODELING *BRUCELLA* INFECTION DYNAMICS IN PASTORALIST COMMUNITIES: THE ROLE OF HERD MANAGEMENT AND SPECIES COMPOSITION IN SUSTAINING *BRUCELLA* TRANSMISSION

Peter S. Larson, Johannes Foufopoulos, Rebecca Hardin, Annie Wang, **Joseph N. Eisenberg**

University of Michigan, Ann Arbor, MI, United States

Zoonotic bacterial pathogens present significant threats to human health and compromise the economic well-being of pastoralist herding communities. The dynamics of transmission of pathogens such as *Brucella* sp., the causative agent of Brucellosis, however, are poorly understood. Prior studies have suggested that transmission of Brucellosis cannot be sustained in the small to medium size herds characteristic of most pastoralist systems. These analyses, however, did not consider the joint effects of animal trading between herds and inter-species mixing within herds. Using quantitative and ethnographic data collected in a pastoralist community in Laikipia, Kenya, we create a deterministic SIR model of *Brucella* sp. transmission to examine how these transmission heterogeneities may facilitate disease persistence. We explore dynamics of infection given various herd sizes and management strategies observed in Laikipia Kenya. Specifically, we model a community that engages in multispecies livestock raising, assuming different types and levels of interaction between herds. We find that transmission is unsustainable in small herds of all species when in isolation, confirming prior research. Multi-species herds which include a high proportion of goats compared with large stock such as cattle can sustain itself for a longer time, but not indefinitely. Links through sales and purchases or other between-herd contact, however, create conditions where *Brucella* can transmit indefinitely. Though it is likely that herding strategies historically accommodate potentially devastating risks to herd health, it is possible that herders are unaware of threats, both to health and economic wellbeing, presented by bacterial infections such as Brucellosis. In addition to education programs on specific diseases, herders should be encouraged to cull animals showing obvious signs of illness as soon as possible and should be discouraged from selling such animals to minimize risks to human health.

1765

MARKETS AS HUBS OF RISKY CONTACT BETWEEN HUMANS AND WILD/DOMESTIC ANIMALS: CASE STUDIES FROM REPUBLIC OF CONGO (ROC) AND DEMOCRATIC REPUBLIC OF CONGO (DRC)

Jariseta Rambeloson Zo[1], Henri Banga-Mboko[2], Roger Albert Mbété[2], Lonna Shafritz[1], Tula Michaelides[1], Jean-Luc Hornick[3], Susan Zimicki[1]

[1]FHI 360, Washington, DC, United States, [2]Veterinarian and Zoonotic Research Center, Brazzaville, Republic of the Congo, [3]University of Liege, Liege, Belgium

Traditionally, market studies have focused on elements of market dynamics such as the volume of wildlife and domestic animals being sold. We are examining markets through a different lens: to assess them as locations where humans - consumers, vendors, managers - are at increased risk of transmission of zoonotic infections via exposure to live animals, animal products, and poor biosecurity practices. USAID's PREVENT Project is studying eight urban markets in Brazzaville and two in Dolisie, ROC, as well as eight markets in Kinshasa, DRC. We conducted key informant interviews, consumer exit interviews and then a household survey in the market catchment areas (as determined by exit interviews) to learn where people shop, what they buy and why. In addition, since February 2014 we have carried out monthly one-week full-day observations of vendor stalls selling bush meat and/or poultry. This presentation will describe what we have learned to date about hygiene conditions, infrastructure, and biosecurity practices in these markets and about the importance, diversity and seasonal variation of the bush meat trade as well as how the forms of animals sold (live, freshly dead, large pieces, small pieces, smoked, raw) and change with the length of time they are in the market. We will discuss the implications of all of these factors for human exposure and risk

1766

MASSTAG PCR DETECTION OF EV-D68, RSV-A AND B, AND MORE, IN CLUSTERS OF UNEXPLAINED ACUTE FEBRILE ILLNESS IN CAMBODIA

Nary Ly[1], Rafal Tokarz[2], Nischay Mishra[2], Stephen Sameroff[2], Komal Jain[2], Agus Rachmat[1], Sam An Ung[3], Touch Sok[3], Steven Newell[1], Ian Lipkin[2], **Dustin J. Harrison**[1]

[1]U.S. Naval Medical Research Unit – 2, Phnom Penh, Cambodia, [2]Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, NY, United States, [3]National Institute of Public Health, Cambodian Ministry of Health, Phnom Penh, Cambodia

Fevers of unknown origin constitute a substantial disease burden on patients in Southeast Asia with the majority of the cases remaining undiagnosed. To expand the breadth of possible infectious pathogens, we used MassTag PCR to test for the presence of 20 bacterial and viral respiratory agents from 85 patients with unexplained respiratory illness representing six disease clusters that occurred in Cambodia between 2006 and 2012. We detected potential pathogens in 62 (73%) of 85 total cases, identifying a virus in 37 patients (44%) and a bacterium in 53 (62%) cases. In a cluster from Kandal province from August 2009, we detected a high frequency of enterovirus 68 and human rhinoviruses. Among 22 cases that occurred during October 2009 in Kampong Speu province, we detected human respiratory syncytial virus B. Finally, a cluster of children < five years of age from the Ratanakiri province previously diagnosed with pneumonia, revealed infection from human respiratory syncytial virus A. These findings provide insight into the etiologies of previously undiagnosed acute febrile illness in Cambodia and point to the utility of multiplexed diagnostics during disease outbreaks.

1767

DIAGNOSTIC VALUE OF CLINICAL FEATURES FOR DIAGNOSING PNEUMONIA IN CHILDREN UNDER FIVE YEARS OF AGE: A SYSTEMATIC REVIEW AND META-ANALYSIS

Clotilde Rambaud Althaus[1], Fabrice Althaus[2], Blaise Genton[1], Valérie D'Acremont[1]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Department of Ambulatory Care and Community Medicine, Lausanne, Switzerland

The aim of this review is to assess the diagnostic value of clinical signs and symptoms in identifying radiological pneumonia in children under the age of five and to review the accuracy of WHO criteria for diagnosing clinical pneumonia in developing countries. Electronic databases (Medline and Embase) and reference lists of relevant studies were searched to identify articles assessing clinical predictors of radiological pneumonia in children. 1697 potentially relevant studies were identified. Selection was based on: design (diagnostic accuracy studies), target disease (pneumonia), participants (children below 5 years), setting (ambulatory or hospital care), index test (clinical features), reference standard (chest radiography). Quality assessment was based on the 2011 Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) criteria. For each index test, sensitivity and specificity were calculated. Meta-analyses with the Bivariate model and hierarchical SROC plots were done for index tests assessed in four or more studies. Eighteen articles were included in the analysis. Age-related fast breathing (six studies, pooled sensitivity: 0.62 [95%CI 0.26-0.89]; specificity: 0.59 [0.29-0.84]) and lower chest wall indrawing (four studies, 0.48 [0.16-0.82]; 0.72 [0.47-0.89]) showed poor diagnostic performance in the meta-analysis. Features with the highest pooled, positive likelihood ratios were: respiratory rate above 50/min (1.90 [1.45-2.48]), grunting (1.78 [1.10-2.88]), chest indrawing (1.76 [0.86-3.58]), and nasal flaring (1.75 [1.20-2.56]). Features with the lowest pooled negative likelihood

ratio were: cough (0.30 [0.09-0.96]), history of fever (0.53 [0.41-0.69]), and respiratory rate above 40/min (0.43 [0.23-0.83]). No single clinical feature was sufficient for definitively diagnosing pneumonia. Combining clinical features in a decision tree may improve diagnostic performance, but the addition of new point-of-care tests for diagnosing bacterial pneumonia would help to reach an acceptable level of accuracy.

1768

COMMUNITY ACQUIRED PNEUMONIA IN ADULT HOSPITAL ADMISSIONS NORTHERN VIETNAM; CLINICAL FEATURES AND ETIOLOGY

Behzad Nadjm[1], Ta Thi Dieu Ngan[2], Vu Quoc Dat[2], Rogier van Doorn[1], Peter Horby[1], Nguyen Van Kinh[2], Heiman Wertheim[1]

[1]Oxford University Clinical Research Unit, Hanoi, Vietnam, [2]National Hospital for Tropical Diseases, Hanoi, Vietnam

Community acquired pneumonia (CAP) causes an estimated 1 million deaths in Asia each year including 160 000 amongst those aged 15 - 60 years. Knowledge of local etiology is critical to formulating treatment guidelines and vaccination priorities. Despite this studies of the etiology of CAP in South East Asia have been limited. We conducted a study of the causes of CAP in adult patients admitted to 3 hospitals in Ha Noi, Vietnam. Adults with an infiltrate on chest radiography and one of: cough, dypnoea, fever (≥38.3C) or hypothermia (<36.0C), purulent respiratory secretions, bronchial breathing or rales on auscultation, leucocytosis or leucopenia that had not been residing in hospital or a long term care facility in the 14 days prior to onset were eligible for admission. Consenting patients were enrolled and recieved a standardized clinical evaluation. All patients had routine haematology and biochemistry test results recorded and received blood culture, sputum culture and PCR for the following bacterial pathogens: *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *M. amphoriforme*, *Chlamydophila pneumoniae*, *C. psittaci*, *Legionella pneumophila* and *L longbeachae*. PCR was also performed for 14 respiratory viruses on nasal/throat swabs and/or sputum. Urine was tested for pneumococcal antigen and L pneumophila serogroup 1. In a selection of cases where acute and convalescent serum was available serology for *C. pneumoniae, M. pneumoniae, Orientia tsutsugamushi*, *Rickettsia typhi* and *R. prowazekii* was also performed. Preliminary results only are available at this time, full results should be available in time for presentation. Preliminary results show a case fatality rate (died in hospital or palliative discharge) of 12/116 (10.3%). The rate of positivity for blood culture was low (5/112, 4.5%). Sputum PCR was positive for *S. pneumoniae* in 80/125 cases (64%), *M. pneumoniae* in17/125 (13.6%), *C. psittaci* in 10/125 (8%), *M. amphoriforme* in 5/125 (4%), *C. pneumoniae* and *L. pneumophila* in 1/125 (0.8%) each and there were no cases of *L. longbeachea*. Clinical findings and outcomes will also be explored.

1769

PROFILE OF *MYCOBACTERIUM TUBERCULOSIS* DRUG RESISTANCE IN A TROPICAL REGION OF PERU 2007-2013

J. Antonio Grandez-Urbina, J. Gonzalo-Rodriguez, S. Bedoya-Vidal, G. Grandez-Castillo, M. Sotelo, A. Santillan-Romero, R. Pichardo-Rodriguez, J. Oscar Tarazona-La Torre, Enver Terrazas Nuñez

Universidad Ricardo Palma, Lima, Peru

Tuberculosis in Peru is a public health problem . In terms of South America is the second country in prevalence after Bolivia, and in America is the third after Haiti and Bolivia . In 2013 , WHO reports that in Peru Multidrug-resistance Tuberculosis (MDR - TB) exceeds the reported cases that year by Colombia , Ecuador , Argentina , Chile and the United States throughout its territory , and ranks first in report for MDR - TB and Extreme drug resistance tuberculosis(XDR - TB) . In Peru the regions with high prevalence of TB are Lima, Callao , Madre de Dios , but the profile of Mycobacterium tuberculosis drug resistance is not known in the region of Madre de Dios . Determine the profile of Mycobacterium tuberculosis drug resistance in patients admitted to retreat in Madre de Dios , Peru , 2007-2013 Observational, cross sectional study . Logbook and monitoring of TB patients in retreatment Regional Health Direction - Madre de Dios of 2007-2013 , included 111 patients diagnosed with tuberculosis smear (+) , they were tested for sensitivity at the National Institute of Health (NIH) . Frequencies established variables, measures of central tendency and dispersion in qualitative variables were used. The frequency of monoresistance 33.63 % (n = 37) , poliresistencia 9.09 % (n = 10) , 45.45 % MDR - TB (n = 50) and 12.72% sensitive cases (n = 14) . The overall frequency of isoniazid resistance was 90.18 % (n = 92) , rifampicin 79.09 % (n = 87) , Streptomycin 18.18 % (n = 20) Etambutol 11.81 % (n = 13) and pyrazinamide 7.27% (n = 8).The initial cultures were basciloscopias negative and 44.6% (n = 50) and 32.1% (n = 36) respectively. Only 1.8% (n = 2) showed HIV positive reaction. . In the present study we found that there is a high prevalence of resistant TB in patients admitted to retreat in the Madre de Dios Region where HIV positive reaction is not related to the presentation. MDR-TB were the most frequent type of resistance and isoniazid is the drug resistance most often generated in monoresistencia patients .

1770

INFLUENZA VACCINE EFFECTIVENESS IN THE TROPICS: MODERATE PROTECTION IN A SURVEILLANCE POPULATION IN BANGKOK BETWEEN AUGUST 2009 AND JANUARY 2013

Jens W. Levy[1], Sriluck Simasathien[2], Veerachai Watanaveeradej[2], Piraya Bhoomiboonchoo[1], Stefan Fernandez[1], Richard G. Jarman[3], Chonticha Klungthong[1], Robert V. Gibbons[1], Piyarat Suntarattiwong[4], **In-Kyu Yoon**[1]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Phramongkutklao Hospital, Bangkok, Thailand, [3]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [4]Queen Sirikit Institute for Child Health, Bangkok, Thailand

Influenza in the tropics occurs year round with peaks that correspond variably to temperate regions. However, data on influenza vaccine effectiveness (VE) in the tropics is sparse. We report on the effectiveness of influenza vaccine to prevent medically attended laboratory confirmed influenza from a sentinel surveillance study conducted at a Thai military medical facility in Bangkok, Thailand from August 2009 to January 2013. Patients ≥6 months old presenting with influenza-like illness underwent nasal/throat swabs which were tested by influenza RT-PCR. A case test-negative study design was used to evaluate VE. Of 2992 samples available for analysis, 1058 (35.4%) were PCR-positive (cases) and 1934 (64.6%) were PCR-negative (test-negative controls). Five hundred and eight (16.9%) of these patients reported being vaccinated within the previous 12 months. Periods of high and low influenza activity were defined based on publicly available Thai Ministry of Public Health data. Overall adjusted VE was found to be 51.6% (95%CI: 36.8, 63.1%). Adjusted point estimate for VE was highest in the 18-49 year age group (77.0%) followed by 6-23 months (55.1%) and 2-17 years (44.6%). Adjusted estimates were not done for those ≥50 years of age due to small numbers. VE in patients with underlying disease was 75.5% compared to 48.6% in those without. VE appeared to be much higher during high versus low influenza activity periods. Among those who reported receiving vaccine 14 days-3 months prior to illness, VE was 55.8% (95% CI 26.6 to 74.1%), and tended to decrease as the interval between vaccination and illness increased (46.8% at >3 to 6 months; 48.9% at >6 to 9 months; 31.5% at >9 to 12 months). Our findings demonstrate moderate protection by influenza vaccination and support the utility of influenza vaccination in the tropics including in very young children and those with underlying disease. Our study also suggests that booster vaccination may be useful within several months even when the formulation does not change.

1771

SEASONAL INFLUENZA'S ASSOCIATION WITH SPECIFIC HUMIDITY IN THREE TROPICAL CENTRAL AMERICAN COUNTRIES: HONDURAS, NICARAGUA AND COSTA RICA

Radina P. Soebiyanto[1], Wilfrido A. Clara[2], Jorge Jara[3], Maria L. Matute[4], Angel Balmaseda[5], Jenny Lara[6], Mariel Lopez Moya[6], Marc-Alain Widdowson[7], Eduardo Azziz-Baumgartner[7], Richard K. Kiang[1]

[1]NASA Goddard Space Flight Center, Greenbelt, MD, United States, [2]Centers for Disease Control and Prevention, Regional Office for Central America Region, Guatemala City, Guatemala, [3]Universidad Del Valle de Guatemala, Guatemala City, Guatemala, [4]Ministry of Health of Honduras, Tegucigalpa, Honduras, [5]Ministry of Health of Nicaragua, Managua, Nicaragua, [6]Costa Rican Institute for Research and Education on Nutrition and Health, San Jose, Costa Rica, [7]Centers for Disease Control and Prevention, Atlanta, GA, United States

Studies have demonstrated the association between seasonal influenza & meteorological factors. We previously showed that seasonal influenza in Guatemala, El Salvador & Panama was associated with specific humidity. In this work, we investigated the association in 6 departments from three other Central America countries: Cortes & Francisco Morazan in Honduras; Managua in Nicaragua; Alajuela, Cartago & San Jose in Costa Rica. As an indicator for influenza activity, we used the weekly proportion of samples which tested positive for influenza in the period 2008-2013 from each country's National Influenza Centers. Respiratory samples were collected from case-patients presenting with influenza-like illness or severe acute respiratory infection. Meteorological factors - rainfall, temperature & specific humidity (SH) - were obtained from NASA's satellites & models. We used logistic regression and adjusted for previous influenza activity & co-circulating viruses (respiratory syncytial virus, adenovirus and parainfluenza virus). We found that SH was proportionally associated ($p<.05$) with influenza activity in all departments (Odds Ratio (OR)=1.2-1.6). Temperature was inversely associated with influenza activity in Alajuela of Costa Rica (OR and 95% Confidence Interval=0.7(0.6-0.8)) & Cortes of Honduras (OR=0.8(0.7-0.9)). There was no statistical association ($p<.05$) with rainfall in any locations. Among the meteorological factors, SH had the highest contribution (2-15%) to the model in all locations except in Cortes. The model estimated influenza activity accurately (R=0.6-0.9) for the final 6 months in all countries except Honduras. Time-frequency analysis using Hilbert-Huang Transform showed that seasonal components of influenza activity was positively correlated with SH (R=0.2-0.6, $p<.05$), further corroborated the SH findings from logistic regression. Our results highlighted influenza's proportional association with SH in these countries, which was consistent with other studies in the tropics. Understanding of climate role in influenza may help in estimating epidemic timing and intensity.

1772

THE ECONOMIC BURDEN OF VALLEY FEVER IN CALIFORNIA

Leslie Wilson[1], Jie Ting[1], Michael MacLean[2], Michael Peterson[3], Nathan Stockamp[3], Robert Libke[3], Paul Brown[4], Herbert Boro[5], Harold Lin[5]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Kings County Health Department, Kings County, CA, United States, [3]University of California San Francisco Fresno, Fresno, CA, United States, [4]University of California Merced, Merced, CA, United States, [5]Kaiser Permanente-Fresno, Fresno, CA, United States

Coccidioidomycosis or Valley Fever (VF) is a mycotic disease endemic to southwestern US. In California, where 33% of VF cases nationwide occur, VF incidence increased from 4.2-10.8/100,000 in the period 2002-2012 (157% increase). There is no complete study of the economic burden of VF to guide clinical care and public health planning. We estimated the total and per person lifetime direct and indirect cost of VF in 2012 US $. The 4,904 incident VF cases reported in 2012 in California were symptomatic VF infections, which we followed for lifetime costs. Unreported cases were assumed to be asymptomatic infections with no costs. We included early treatment costs of VF misdiagnosed as community acquired pneumonia. We costed VF by disease categories; (uncomplicated pneumonia [UP], chronic/diffuse pneumonia with [CD] and without dissemination [CN], chronic pulmonary nodule [PN], and chronic pulmonary cavity [PC]). Direct costs were VF diagnosis, treatment, and follow-up including physician visits, ER, hospitalization, tests, procedures, and medications. VF epidemiology data were from literature and expert interviews. Treatment and utilization were from published guidelines and 2 hour prepared interviews with 5 expert VF physicians. Hospitalizations were from the 2012 California Patient Discharge Dataset and HCUP prices, medication costs from average wholesale price minus 17% for contract pricing, physician visit were costed using CPT based Medicare estimates. Total lifetime costs of 2012 VF incident cases in CA was $212 million (M), $51,743/person. UP accounted for 85% of our population and 31% of direct lifetime costs ($65.4M), CD 2.5% and 42% costs ($89.2M), CN 2.5% and only 11% of costs, pulmonary nodules and cavities 10% and 16% of costs, respectively, primarily due to cost of differential diagnosis of cancer. Short term work loss costs were $6.4 M, and mortality another $126 M. VF causes a large cost burden, especially for disseminated cases. Variation exists by geographic regions, insurance status and practice patterns. Areas for cost control exist.

1773

THE NEED FOR ENHANCED LABORATORY BASED DIAGNOSIS CAPACITY IN THE TESTING AND SURVEILLANCE FOR OTHER RESPIRATORY VIRUSES FROM PATIENTS REPORTING WITH INFLUENZA LIKE ILLNESSES

Nicholas Owor, Barbara Namagambo, Timothy Byaruhanga, John T. Kayiwa, Richard Chiza, Irene Nabukenya, Barnabus Bakamutumaho, Julius Lutwama

Uganda Virus Research Institute, Entebbe, Uganda

The capacity of Laboratories to promptly identify particular strains or subtypes of organisms using modern diagnostic techniques has become essential for rapid and efficient response to disease outbreaks and preventing potential epidemic or pandemic spread. In Influenza surveillance, patients present with symptoms that match the case definition of Influenza Like Illnesses (ILI) and Severe Acute Respiratory Infection (SARI). Up to 24% of the samples collected and tested for influenza at the National Influenza Centre are positive for influenza while the biggest percentage are negative. There are various respiratory viruses and bacteria that affect humans which include adenovirus, human rhinovirus A, coronavirus OC43, parainfluenza virus 1, parainfluenza virus 3, respiratory syncytial virus B, human metapneumovirus, respiratory syncytial virus A, parainfluenza virus 2 and coronavirus 229E. Nasopharyngeal and oralpharyngeal swab specimens collected from patients presenting with ILI and SARI in 8 sentinel sites between December 2011- April 2014 in Uganda were tested for Influenza by RT- PCR, subtyping and isolation. Of a total of 5931 samples tested; 645(10.9%) were positive for Influenza with 2(0.3%) co-infections, 405(62.8%) Influenza A and 238(36.9%) Influenza B; though, 5286(89.1%) were negative yet the patients presented with symptoms that match the case definition for ILI and SARI. This raises a need to establish baseline information on the prevalence of other respiratory pathogens that cause upper and lower respiratory disease in populations through strengthening laboratory diagnostic capabilities for the identification and characterization of infectious agents likely to cause public health emergencies. There is need for more-simplified testing systems that enable researchers and clinicians to perform multiplexed molecular diagnostics quickly and easily. The results would be useful to guide future surveillance and case management strategies involving other respiratory infections in Uganda.

1774

EPIDEMIOLOGY OF RESPIRATORY VIRAL PATHOGENS FROM SENTINEL SURVEILLANCE IN WESTERN CAMBODIA NEAR THE BORDER WITH THAILAND

Ilin Chuang[1], Ans Timmerman[1], Melanie Melendrez[2], Youry Se[3], Nou Samon[3], Nichapat Uthaimongkol N[1], Stuart Tyner[4], Rith Sareth[5], Rick Jarman[2], Delia Bethell[6], Nitima Chanarat[1], Julie Pavlin[7], Tippa Wongstitwilairoong[1], Piyaporn Saingam[1], But Sam El[3], Sok Touch[8], Ly Sovann[8], Lon Chanthap[9], Stefan Fernandez[1], Phillipe Buchy[5], David Saunders[1]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Armed Forces Research Institute of Medical Sciences, Battambang, Cambodia, [4]U.S. Army Institute of Surgical Research, Houston, TX, United States, [5]Institute of Pasteur, Cambodge, Phnom Penh, Cambodia, [6]Department of Paediatrics, Oxford, United Kingdom, [7]Armed Forces Health Surveillance Center, Silver Spring, MD, United States, [8]Communicable Disease Control Department, Phnom Penh, Cambodia, [9]Armed Forces Research Institute of Medical Sciences, Phnom Penh, Cambodia

Little is known about influenza and other common respiratory viruses in remote populations along the Thai-Cambodia border in Western Cambodia. Real-time PCR for influenza was performed on combined nasal and throat specimens from outpatients presenting with influenza-like-illness (ILI) at 4 sentinel sites in Western Cambodia between May 2010 and December 2012; a subset was further characterized by antigenic analysis, antiviral susceptibility testing and full genome sequencing for phylogenetic analysis. PCR-negative ILI-specimens for influenza were cultured; cultured negative specimens were then tested with RT-PCR for enteroviruses and rhinoviruses (EV/RV) and enterovirus EV71. Among 586 ILI-patients (median age 5 year, range 1-77 years), 168 (29%) tested positive for influenza by RT-PCR and at least 1 respiratory virus was detected in 258 (44%) patients. Influenza strains were highly related and matched circulating strains and although vaccination coverage was low, most strains matched the vaccine strains. No intrasubtype reassortment was detected. Our Western Cambodian H1N1(2009) isolates were more closely related (based on full genome analysis) to 10 earlier isolates from Cambodia (94.4% genome conservation) compared to 13 Thai isolates (75.9% genome conservation. Aside from adenovirus (5.74%) and parainfluenza virus (3.8%), detection of non-influenza viruses by viral culture was low (<10%), with no detection of coronavirus, human bocavirus, human metapneumovirus and respiratory syncytial virus. We detected 5.9% of non-polio enteroviruses among our culture-negative specimens: human Coxsackievirus types A4, A6, A8, A9, A12, B3, B4 and human echovirus types E6 and E9. We conclude that influenza epidemiology in this sample of isolates is following similar trends as observed elsewhere in Cambodia. Further research to clarify the burden of adenovirus and non-polio enteroviruses as etiologic agents for acute respiratory infections is needed in Cambodia.

1775

DIFFERENT FROM EQUATORIAL BRAZIL, SOUTHERN HEMISPHERE WHO VACCINATION RECOMMENDATIONS ARE ADEQUATE FOR MOST OF SOUTHERN PARTS OF BRAZIL

Wladimir J. Alonso[1], Priscila S. Born[2], Paola C. Resende[2], Gonzalo Bello[3], Fernando C. Motta[2], Marilda M. Siqueira[2]

[1]Division of International Epidemiology and Population Studies, Bethesda, MD, United States, [2]Respiratory Viruses and Measles Laboratory, Rio de Janeiro, Brazil, [3]AIDS and Molecular Immunology Laboratory, Rio de Janeiro, Brazil

Influenza vaccination is the most important public health measure to prevent severe cases and deaths due to influenza infection. However, influenza viruses are constantly evolving, forcing continuous vaccine reformulation. In this study we investigated the annual genetic matching between circulating influenza viruses and recommended A(H3N2) vaccine components for over a decade (1999-2012) in three regions of a southern hemisphere country with continental dimensions (Brazil). A total of 237 hemagglutinin sequences from Northeast (NE), Southeast (SE) and South (S) Brazilian regions were compared against the corresponding vaccine strains recommended annually by the WHO. We used MEGAv5.1 to infer nucleotide and amino acid distances between sequences and annual vaccine prototypes. PhyML was used for phylogenetic reconstructions by Maximum Likelihood (ML) to infer the antigenic relationship between viral samples and vaccine composition. We next compared the putative effectiveness of the influenza vaccination in the three regions using hypothetical vaccination scenarios where alternative vaccine delivery timing and vaccine compositions (either Southern or Northern Hemisphere WHO recommendations) were considered (comparison following method of Mello et al 2009). We found that, although influenza circulates in most (NE) or all (S,SE) months of year, the current Southern Hemisphere recommendation in Brazil is adequate for these regions. This was less expected in the NE region, but we attribute it to the fact that most of the samples of the NE actually came from the its southernmost part (Bahia, at approximately 13oS), where influenza seasonality differ from the equatorial pattern of circulation. Our results show that WHO hemisphere vaccine recommendation decisions must consider differences in influenza circulation patterns even within regions of a country.

1776

CONCORDANCE BETWEEN SOLID AND LIQUID CULTURE FOR ANTITUBERCULOSIS DRUG SUSCEPTIBILITY TEST (DST) IN PERU

Giancarlo A. Valle[1], Renato Quispe[1], Tatiana Cáceres[1], Eduardo Gotuzzo[2], Carlos Zamudio[1], Carlos Seas[2]

[1]Instituto de Medicina Tropical "Alexander von Humboldt", Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Departamento de Enfermedades Infecciosas, Tropicales y Dermatológicas, Hospital Nacional Cayetano Heredia, Lima, Peru

Löwenstein Jensen (LJ) has been the most widely technique for MTB detection and DST. However, its long incubation and time to detection has led to develop alternative methods. Automated Mycobacteria Growth Indicator Tube (MGIT) constitutes a rapid alternative with comparable results, but manual MGIT (mMGIT) is favorable in low-resources areas because its lower cost. This study evaluates the concordance for DST of LJ and manual MGIT in a country with one of the highest prevalence of tuberculosis in the Americas. Sputum samples collected from respiratory TB suspects, enrolled in diagnostic trials during 2007-2011 in Lima (Capital of Peru), underwent LJ and MGIT. Only samples with positive Capilia test for Mycobacterium tuberculosis (MTB) were included in the DST analysis. Resistance to Isoniazid (INH), Rifampin (RIF), Streptomycin (SM) and Ethambutol (EMB), as well as resistance to INH plus RIF (MDR) were evaluated by Proportion Method (PM) in LJ medium and SIRE system in MGIT. Comparison between performance of MTB detection and susceptibility patterns were assessed by Kappa indices. DST in both solid and liquid mediums was performed in 319 samples. PM-LJ and SIRE-MGIT detected resistance to INH: 21.6% (69/319) and 20.7% (66/319) (Kappa=0.92, p<0.001); RIF: 12.0% (38/319) and 11.0% (35/319) (Kappa = 0.94, p<0.001); SM: 26.3% (84/319) and 25.1 (80/319) (Kappa = 0.82, p<0.001); and EMB: 11.9% (38/319) and 9.4% (30/319) (Kappa = 0.77, p<0.001). Furthermore, PM-LJ and SIRE-MGIT found 10.0% (32/319) and 8.8% (28/319) as MDR cases, respectively (Kappa = 0.89, p<0.001). Manual MGIT emerges as a faster DST alternative to LJ in low resources settings like Peru, with optimal concordance between LJ and mMGIT DST.

1777

COMPARATIVE PERFORMANCE OF WEEKLY TELEPHONE CALLS VERSUS WEEKLY HOME VISITS TO IDENTIFY CASES OF INFLUENZA-LIKE ILLNESS AMONG A COHORT OF PREGNANT WOMEN - GUATEMALA, 2013

Chris Bernart[1], Maria R. Lopez[1], Marvin Xuya[1], Juan C. Moir[2], Saad B. Omer[3], Jorge Jara[1], Eduardo Azziz-Baumgartner[4], Joe P. Bryan[4], John P. McCracken[1]

[1]Universidad del Valle, Guatemala, Guatemala, [2]Guatemalan Ministry of Health, Guatemala, Guatemala, [3]Emory University, Schools of Public Health and Medicine and Emory Vaccine Center, Atlanta, GA, United States, [4]Global Disease Detection Branch, Division of Global Health Protection, Atlanta, GA, United States

Influenza-like illness (ILI) is a leading cause of illness globally. A substantial proportion of ILI is not detected by health services-based surveillance because not all persons with ILI seek healthcare; this may be exacerbated in settings where healthcare access or utilization is limited. Active surveillance through household visits can detect most ILI cases, but is costly and labor-intensive. We followed a cohort of pregnant women in rural Quetzaltenango, Guatemala for ILI (fever and cough or sore throat) by using either weekly phone calls or home visits. Women with < 20 weeks gestation were randomized 1:1 to either a weekly phone call or home visit. Staff attempted up to 3 contacts per week to administer a study questionnaire for ILI symptoms. Probable ILI cases identified by the call or visit were then evaluated within 24 hours by a nurse during a home visit. Participants with ILI had a nasopharyngeal swab collected which was tested by polymerase chain reaction for respiratory syncytial virus (RSV), human metapneumovirus, influenza A/B, parainfluenza virus 1/2/3, and adenovirus. During May-November 2013, 167 women were enrolled, of whom 85 (51%) were randomized to weekly phone calls and 82 (49%) to home visits. Surveillance was completed for 864 (63%) of the 1,364 expected person-weeks of follow-up by phone calls versus 1,010 (73%) of the 1,381 expected person-weeks by home visits (p=0.01). Weekly follow-up identified 9 ILI cases in the phone call group versus 13 ILI cases in the home visit group (p=0.5 for ILI). We detected 3 infections in the phone call group (adenovirus, parainfluenza-2, RSV) versus 6 infections in the home visit group (RSV, flu-B, parainfluenza types 2 and 3) (p=0.8). Although more costly and time consuming, home visits were more likely to have successfully completed questionnaires than phone calls. The sample size was inadequate to determine the difference in detection of lab-confirmed ILI cases between visits and calls. The choice between phone calls and home visits to identify case-patients will depend on the surveillance objectives and available resources.

1778

ATTEMPTED ALTERNATIVE METHODS FOR THE DIAGNOSIS OF *PNEUMOCYSTIS JIROVECI* PNEUMONIA (PCP) IN HIV/AIDS PATIENTS IN RESOURCE-LIMITED SETTINGS

Melissa Reimer-McAtee[1], Meredith Lora[2], Carolina Mejia[3], Monica Pajuelo[4], Faustino Torrico[3], Daniel Lozano Beltran[3], Robert Gilman[5]

[1]Tulane University, New Orleans, LA, United States, [2]University of San Francisco California, San Francisco, CA, United States, [3]Universidad San Simon, Cochabamba, Plurinational State of Bolivia, [4]Universidad Peruana Cayetano Heredia, Lima, Peru, [5]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Despite the increased use of prophylactic therapy and improved access to antiretroviral therapy, *Pneumocystis jiroveci* pneumonia (PCP) remains one of the most common life-threatening opportunistic infections in HIV-infected patients worldwide. Difficulty obtaining an adequate sputum sample is often a barrier to the diagnosis of PCP in sputum-scarce patients where broncheoalveolar lavage is not available. This study examined the rates of PCP in HIV patients with respiratory symptoms in Cochabamba, Bolivia, and evaluated detection of PCP in samples other than sputum in an attempt to overcome the challenge of sample collection for the diagnosis of PCP in resource-limited settings. Fifty-one HIV patients admitted to the hospital for respiratory symptoms in Cochabamba, Bolivia in 2010 were enrolled in the study. In addition to induced sputum, an oral rinse, stool, and gastric secretion sample obtained by the string test were collected from each subject. Presence of PCP was evaluated by real-time quantitative PCR in a laboratory in Lima, Peru after the completion of sample collection. Of the 51 induced sputum samples collected, seven (13.7%) were positive for PCP. The oral rinse and string test each detected PCP in one of the samples. PCP was not detected in any of the stool samples. The mortality rate of those diagnosed with PCP was 42.9% (3/7). Although other studies have reported the ability to diagnose PCP by the use of PCR on oral rinse samples, our study was not able to repeat this. Additionally, we were not able to detect PCP in either stool or gastric secretion samples obtained by the string test (as has been used for the detection of Mycobacterium tuberculosis in other studies). Our study does show that PCP is a cause of substantial morbidity in the HIV population in Bolivia, and it is possible that there were additional cases of PCP that went undiagnosed in our study because of we were limited to induced sputum. Thus, improved diagnostic modalities are needed for the detection of PCP in resource-limited settings (delete all in italics). Although the rates of PCP in our study, and in most resource-scarce settings, are significantly less than tuberculosis, our study shows that PCP is still a cause of substantial morbidity and mortality in the HIV population in Bolivia. We were not able to detect PCP in stool samples, and our yield was very low in oral rinse and gastric secretion samples obtained by the string test. It is possible that there were additional cases of PCP undiagnosed in our study because we were limited to samples of induced sputum. Our study highlights the fact that improved diagnostic modalities are needed for the detection of PCP in resource-limited settings.

1779

HAMSTER WEIGHT PATTERNS PREDICT THE INTENSITY AND COURSE OF *SCHISTOSOMA HAEMATOBIUM* INFECTION

Thien-Linh Le

Stanford School of Medicine, Stanford, CA, United States

Although Syrian golden hamsters are a widely used host for experimental infection by *Schistosoma haematobium*, surprisingly little is known about the associated intensity and course of infection, making the use of these animals potentially unreliable. As such, we sought to define inexpensive, simple, noninvasive, and accurate methods for assessing and predicting the severity of disease in *S. haematobium* infected hamsters in order to prevent premature hamster sacrifice and unexpected morbidity and mortality. Through monitoring the weight and behavior of infected hamsters, we determined that the weight loss patterns of infected hamsters are highly correlated with commonly used measures of the severity of infection (i.e. numbers of eggs passed in the stool and worm burdens). In contrast, we found no significant correlation between hamster weight loss patterns and egg yields from liver and intestinal tissues. Our findings suggest that a more complex relationship exists among worm burden, fecundity, and egg passage in the feces than previously appreciated. Regardless, our data may be useful for workers seeking to optimize harvests of *S. haematobium* eggs and worm pairs from infected hamsters for downstream applications.

1780

LONG DELAY IN DIAGNOSIS AND HIGH LITHIASIS PROPORTION IN SURGERY SUGGEST UNDERESTIMATION OF HUMAN FASCIOLIASIS IN ARGENTINA

Veronica H. Agramunt[1], Roberto l. Mera Y. Sierra[2], Laura Sidoti[2], Maria Dolores Bargues[1], Santiago Mas-Coma[1]

[1]Departamento de Parasitologia, Facultad de Farmacia, Universitat de Valencia, Burjassot-Valencia, Spain, [2]Catedra de Parasitologia y Enfermedades Parasitarias, Facultad de Ciencias Veteriarias y Ambientales, Universidad J.A. Maza, S. Jose, Guaymallen, Mendoza, Argentina

A retrospective overview of human fascioliasis in Argentina highlighted the long delay with which many patients were diagnosed. Calculated delay average of the time elapsed between the appearance of symptoms and confirmation of infection by appropriate diagnosis is very high, of 1262 days, nearly 3.5 years, and there are references about patients having suffered from symptoms for ten or more years without diagnosis. This suggests either infected subjects not looking for professional diagnosis due to mild symptoms of low fluke burdens and/or misdiagnosis of patients due to the non-pathognomonic clinical picture, easily confused with other diseases when the patient attends a health centre not used to dealing with fascioliasis. Moreover, the number of cases in which a surgical procedure contributed to the diagnosis when *Fasciola hepatica* specimens were unexpectedly found upon liver exploration appeared to be surprisingly high. In the majority, surgery was indicated due to abdominal pain and biliary obstruction suggestive of lithiasis. The importance of intraoperative cholangiography was highlighted in cases in which, even though gallstones were removed, evidence of obstruction observed during the cholangiography led to the finding of flukes. In most of these surgical cases with lithiasis suspicion, the patient inhabited a large city (Buenos Aires, Córdoba, Mendoza, Tucuman) as opposed to a rural area where attending a health care centre is less usual due to economic reasons or at least complicated due to the long journey thas has to be made. This additionally suggests a far greater underestimation of the problem in rural areas. There were patients in whom fluke infection was detected only after a second surgical intervention. Both long delay in diagnosis and high lithiasis proportion suggest that many patients are frequently overlooked and pose a question mark about fascioliasis detection in the country.

1781

RNAI OPTIMIZATION IN *FASCIOLA HEPATICA* NEWLY EXCYSTED JUVENILES: LONG DSRNA INDUCE MORE PERSISTENT SILENCING THAN SIRNA

Nicolas DellOca[1], Tatiana Basika[1], Gabriel Rinaldi[2], Ileana Corvo[1], Estela Castillo[3], Paul J. Brindley[2], Jose F. Tort[1]

[1]Departamento de Genética, Facultad de Medina, Universidad de la Republica, Montevideo, Uruguay, [2]Department of Microbiology, Immunology and Tropical Medicine, and Research Center for Neglected Diseases of Poverty, School of Medicine and Health Sciences, The George Washington University, Washington, DC, United States, [3]Sección Bioquímica, Facultad de Ciencias, Universidad de la Republica, Montevideo, Uruguay

The exponential growth of the genomic and transcriptomics knowledge of parasitic flatworms allows the identification of several novel putative genes of unknown function. In trematodes RNA interference emerges as almost the only available tool to analyze gene function since classical genetics or other reverse genetics approaches still remain unavailable. Whereas this approach has been tested in several parasites of this group it has been optimized only in schistosomes likely reflecting the difficulties in the establishment of the technology as a routine tool. In this report we present progress in the optimization of this technology in the liver fluke *Fasciola hepatica*, causative agent of fasciolosis. This disease is one of the most problematic infections affecting livestock worldwide, and the increasing appearance of human cases had lead the WHO to recognized this disease as a reemeging zoonosis. Using a single copy gene encoding leucine aminopeptidase (LAP) as the target, we refined delivery conditions, identifying electro-soaking (electroporation and subsequent incubation) as the most efficient method to introduce small RNAs into the fluke. We observed consistent knock down of LAP with low (2 µg/ml) dsRNA concentrations. While this low concentration may reduce or obviate off-target effects, it also compromise the tracking of the RNAi incorporation by fluorescent labeling. We also tested the effects of long and short interfering RNAs. While both long dsRNA and short interfering RNA (siRNA) are equally effective at inducing a short-term knock down, dsRNA induced more persistent silencing up to 21 days after treatment, suggesting that mechanisms of amplification of the interfering signal can be present in this parasite. Persistent silencing from invasive stage for up to 3 weeks (close to what it takes for the parasite to reach the liver) opens the possibility of using RNAi for the validation of new putative therapeutic targets

1782

PREVALENCE OF *HAPLORCHIS TAICHUI* AMONG HUMANS AND FISH IN LUANG PRABANG PROVINCE, LAO PDR

Jong-Yil Chai[1], Woon-Mok Sohn[2], Tai-Soon Yong[3], Keeseon S. Eom[4], Duk-Young Min[5], Dongmin Lee[4], Bong-Kwang Jung[1], Virasack Banouvong[6], Bounnaloth Insisiengmay[7], Bounlay Phommasack[7], Han-Jong Rim[8]

[1]Seoul National University College of Medicine, Seoul, Republic of Korea, [2]Gyeongsang National University School of Medicine, Jinju, Republic of Korea, [3]Yonsei University College of Medicine, Seoul, Republic of Korea, [4]Chungbuk National University School of Medicine, Cheongju, Republic of Korea, [5]Eulji University School of Medicine, Daejeon, Republic of Korea, [6]Malaria Center, Luang Prabang, Lao People's Democratic Republic, [7]Ministry of Public Health, Vientiane, Lao People's Democratic Republic, [8]Korea University College of Medicine, Seoul, Republic of Korea

This study confirmed the prevalence of the intestinal fluke *Haplorchis taichui* (Trematoda: Heterophyidae) among people and fish in Luang Prabang Province, Lao PDR. Fecal specimens were collected from 559 riparian people (229 males and 330 females), residing in 4 Districts (Luang Prabang, Xieng Ngeun, Pak Ou, and Nam Bak) and were examined by the Kato-Katz fecal smear technique. The overall helminth egg positive rate was 64.9%. The positive rate for small trematode eggs (STE), which may include *H. taichui* and other heterophyids, Opisthorchis viverrini, and lecithodendriids, was 15.2%. For recovery of adult helmniths, 10 STE-positive people were treated with 40 mg/kg praziquantel and 15 mg/kg pyrantel pamoate, and then purged. Mixed infections with 3 *Haplorchis* species (*H. taichui*, *H. pumilio*, and H. *yokogawai*), a species of cestode (*Taenia saginata*), and several species of nematodes including *Enterobius vermicularis* and hookworms were found. The worm load for trematodes was exclusively high for *H. taichui* with an average of 7691 specimens per infected person, followed by *H. yokogawai* (8.3 specimens) and *H. pumilio* (4.1 specimens). Out of 207 freshwater fish (17 species) purchased in a market in Luang Prabang District, 138 (67%) harboured *H. taichui* metacercariae (metacercarial burden per fish; 520). Lower prevalence of fish and lower metacercarial density were observed for *H. yokogawai* (52% and 50 per fish, respectively) and *H. pumilio* (18% and 3 per fish, respectively). STE found in the surveyed population of Luang Prabang Province were verified to be those of intestinal fukes, particularly *H. taichui*.

1783

MOLECULAR MODELING OF ADENYLATE KINASE 1 AND 8 KDA CALCIUM-BINDING PROTEIN OF *CLONORCHIS SINENSIS*

Tai-Soon Yong[1], Tae Yun Kim[1], Eun Joo Chung[2]

[1]Yonsei University College of Medicine, Seoul, Republic of Korea, [2]Korea National Institute of Health, Seoul, Republic of Korea

Allosteric proteins involved in signal transduction transforms their molecular structure by binding co-factors, and thus they have a potential

for new drug development. In this study, two allosteric proteins, adenylate kinase 1 (ADK1) and 8 kDa calcium-binding protein (CaBP) of the Chinese liver fluke *Clonorchis sinensis* were cloned and modeled. *C. sinensis* EST clone Cs63 and Cs296 were cloned and sequenced. To compare them with other proteins of parasites, multiple sequence alignment and phylogenetic analysis were performed. For molecular modeling, both sequences were subjected to SWISS-MODEL. Recombinant proteins generated bacterially were used for their functional analysis. By BLAST search, Cs63 and Cs296 were confirmed as ADK1 and 8kDa calmodulin-like CaBP, respectively, and thus they were named as CsADK1 and CsCa8, respectively. Sequence and hydrophobicity of them were similar to those identified from parasitic helminthes. Molecular model of CsADK1 contained CORE, LID and NMP domains and expected to transform its structure by binding co-factor-like AP5. CsCa8 was predicted to have two distinctive EF-handed calcium-binding sites by molecular modeling. Calcium ion could bind to each of EF-hands of CsCa8 model. Both recombinant proteins were functionally active in biochemical assay. Results obtained from the study provide structural basis of *C. sinensis* ADKs and CaBPs for the development of new anthelminthic drugs.

1784

AN OPEN-LABEL, RANDOMIZED, MULTICENTRIC STUDY OF TRICLABENDAZOLE FOR FASCIOLIASIS IN CHILDREN FROM PERU

Vicente Maco[1], Luis Marcos[1], Jaime Delgado[2], Julio Herrera[1], José Nestares[1], Angelica Terashima[1], Frine Samalvides[1], Eduardo Gotuzzo[1]

[1]Institute of Tropical Medicine Alexander von Humboldt, Lima, Peru, [2]Health Care Center of Progreso, Asillo, Azangaro, Puno, Peru

Human fascioliasis is an important public health problem in Latin America mainly affecting school-aged children in poor areas of the Andean Region (Peru and Bolivia). The optimal therapeutic scheme of triclabendazole (TCBZ), the recommended anthelmintic against the trematode *Fasciola hepatica*, has not been well defined (with a 10 mg/kg single dose being the most common regimen recommended), and clinical trials that assess effectiveness and tolerability in children are scarce. We aimed to evaluate the efficacy and tolerability of 2 therapeutic schemes of TCBZ in different areas of Peru. A total of 84 individuals (mean age ± SD: 9.27± 2.48 years) with *F. hepatica* eggs in their stools (chronic infection) were enrolled in an open-label, phase II clinical trial from areas located along the Peruvian Andes. Individuals were randomly allocated into 2 groups: 44 received 2 dosages of TCBZ at 7.5 mg/kg each, with a 12 h interval post-prandially (group I-tested group), and 40 received a single dose of 10 mg/kg, post-prandially (group II-standard group). The efficacy (parasitological cure) was evaluated by the presence of eggs in stools at regular intervals and up to 60 days post-treatment. Tolerability was evaluated by the presence of clinical symptoms during the first week after TCBZ administration.A parasitological cure was obtained in 100% of individuals from the tested-group, and 95.0% in the standard-group (p>0.05). The most common adverse event was biliary colic, documented in 25.0% in group II (95% CI= [Ed.1] 11.9-38.9) on day 2, and in 20.5% in group I (95% CI= 7.8-33.7) on day 4, possibly related to the expelling of the adult worms through the biliary tract.In conclusion, the tested scheme was highly efficacious (100% cure rate) and tolerable, and it may be an optimal therapeutic scheme for the treatment of fascioliasis in children in Peru. This represents the largest series of children treated with TCBZ in a non-hospital setting and the largest in Peru.

1785

IDENTIFICATION, CHARACTERIZATION AND EVALUATION OF RECOMBINANT ANTIGENS FOR THE DIAGNOSIS HUMAN PARAGONIMIASIS

Peter U. Fischer, Kurt C. Curtis, Kerstin Fischer, Samantha N. McNulty, Makedonka Mitreva, R. Reid Townsend, Gary J. Weil

Washington University School of Medicine, St. Louis, MO, United States

Paragonimiasis is a foodborne trematode infection that affects 23 million people mainly in Asia. The parasite causes chronic cough with fever and hemoptysis, and lung fluke infection is often confused with tuberculosis. We used a systems biology approach to identify antigens that might lead to improved diagnostic tests for this infection. Antibodies from patients with *Paragonimus kellicotti* were used to isolate antigens for proteomic analysis, and RNAseq data from adult worms were used for protein identification. Among the 22 most abundant identified proteins were a number of orthologues to known diagnostic antigen as well as novel candidates. Sequences for these proteins have 80-90% identity with amino acid sequences for orthologues in P. westermani. We expressed five *P. kellicotti* proteins as his-fusion proteins in E. coli, and these were used to raise antibodies in mice. Immunohistology performed with sections of adult worms showed that four of them were localized to the tegument, at the parasite host interface. In contrast, a known egg antigen was absent from the tegument but present in developing and mature eggs. We evaluated the diagnostic potential of these antigens by Western blot with sera from patients with paragonimiasis (from Missouri and the Philippines), fascioliasis, and schisosomiasis and with sera from healthy North American controls. Two recombinant proteins showed high sensitivity and specificity as diagnostic antigens. Antibodies to the egg protein seemed to be specific marker for patients with mature adult worm infections (with Paragonimus ova in stool or sputum). In conclusion, this study has identified and characterized promising antigens for diagnosis of human paragonimiasis.

1786

IMPACT OF *SCHISTOSOMA MANSONI* IN SCHOOL-AGED CHILDREN LIVING IN KASANSA, DEMOCRATIC REPUBLIC OF THE CONGO

Mbuyi M. Kabongo[1], Sylvie Linsuke[2], Sylvain Baloji[3], Wilfried Mukunda[4], Raquel Inocêncio da Luz[3], Christine Stauber[1], Richard Rothenberg[1], Jean Pierre Van geertruyden[3], Katja Polman[5], Marleen Boelart[3], Pascal Lutumba[2]

[1]Georgia State University, Atlanta, GA, United States, [2]University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [3]University of Antwerp, Antwerp, Belgium, [4]Programme National de Lutte contre la Bilharziose et Parasitoses Intestinales, Kinshasa, Democratic Republic of the Congo, [5]Institute of Tropical Medicine, Antwerp, Belgium

Schistosomiasis (SCH) is an important public health problem in developing countries and school aged children are the most affected. The aim of this study was to evaluate the impact of SCH on the population of school-aged children living in the high endemic area of Kasansa HZ in terms of malnutrition, anemia and low school performance. The overall health status of the children was poor with very high prevalence of *S. mansoni* infection (89.3%), malaria infection (65.1%), anemia (61.4%) and stunting (61.0%). School performance was also negatively affected with 54.6% of the children having failed at least one class. Regular contact with river water was the most significant risk factor related to SCH infection. Anemia was influenced by SCH infection (p=0.003) and weak egg load was associated with stunting (p=0.04). However, due to poverty the causality between chronic malnutrition and anemia can be in either direction, potentially aggravated by SCH. Low school performance was mainly influenced by low income (<1 USD). Poverty exacerbated both health and school performance. Control measures are urgently needed to improve the health status of these children with in depth studies to demonstrate the causalities for each of the diseases in this population.

1787

FIELD EVALUATION OF MINI-FLOTAC®, SPONTANEOUS SEDIMENTATION TECHNIQUE IN TUBE OF TELLO, RAPID SEDIMENTATION TECHNIQUE BY LUMBRERAS AND KATO-KATZ FOR THE DIAGNOSIS OF ENTERIC PARASITES IN THE HIGHLANDS OF PUNO, PERU

Vicente Maco[1], Catherine de Beaumont[2], Davide Ianniello[3], Giuseppe Cringoli[3], Quentin Bickle[4], Angelica Terashima[1], Eduardo Gotuzzo[1], Luis Marcos[1]

[1]Institute of Tropical Medicine Alexander von Humboldt, Lima, Peru, [2]Division of Infectious Diseases, Albert Einstein College of Medicine, New York, NY, United States, [3]Department of Pathology and Animal Health, University of Naples "Federico II", Naples, Italy, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom

Enteric parasites show a peculiar distribution along the geography of Peru. Although a low-cost, reliable, local coproparasitological technique such as the Spontaneous Sedimentation Technique in Tube of Tello (SSTT) has been used over decades this has not been compared to standardized methods. Here, we compare the performance of SSTT with the mini-FLOTAC®, a coproparasitological technique under validation, in an area where intestinal multiparasitism and fascioliasis are common. Rapid Sedimentation Technique (RST) by Lumbreras, and Kato-Katz (KK) were included. We conducted a stool survey among school-aged children in Naupa Pampa, Calapampa, and Progreso, located in Azángaro (3859 m) (Puno, Peru). Four techniques, mini-FLOTAC®, SSTT, RST, and KK were performed. The sensitivity and negative predictive value (NPV) of each technique were compared using the combined results of all positive techniques as the "gold standard". The inter-technique agreement (κ) was also evaluated. A p value 10%): *Entamoeba coli* (77.2%), *Blastocystis hominis* (66.5%), *Endolimax nana* (50.3%), *Iodamoeba buetschlii* (15.0%), *Entamoeba histolytica/dispar* (12.0%), *Giardia lamblia* (11.4%), *Fasciola hepatica* (10.8%), and *Chilomastix mesnili* (10.2%). The area of study is hyperendemic for human fascioliasis with high prevalence in Ñaupa Pampa (25%). Mini-FLOTAC® showed a higher performance than SSTT and RST for *H. nana* (13 vs. 11 vs. 8) and *F. hepatica* (15 vs. 10 vs. 8), and higher sensitivities than SSTT, RST and KK for both helminths ($p<0.05$). The use of multiple techniques is an appropriate approach for highly endemic areas where intestinal multiparasitism is common. SSTT is highly sensitive for enteric protozoa, including when compared to standardized techniques such as the mini-FLOTAC®. Mini-FLOTAC® holds promise for the diagnoses of H. nana and *F. hepatica* in endemic areas. Notably, *F. hepatica* infection continues to be highly prevalent among children in Azangaro, thus prompt and realistic field interventions are needed.

1788

GRANULOMATOUS INFLAMMATION OF THE BLADDER COMPROMISES THE OVERLYING UROTHELIAL BARRIER

Deborah Boyett

Stanford University, Stanford, CA, United States

Bladder granulomas can form as a result of retained suture material from surgery, BCG treatment, urinary tuberculosis, and urogenital schistosomiasis. We previously demonstrated in a mouse model of urogenital schistosomiasis that multiple urothelial barrier function genes (i.e., uroplakins) were downregulated on a whole bladder level after bladder wall injection with *Schistosoma haematobium* eggs. Given that egg-injected bladders exhibit hyperplasia of the urothelium overlying resulting egg granulomata, we hypothesized that this hyperplastic response was a response to egg inflammation-induced, regional compromise of the molecular urothelial barrier. Anesthetized mice underwent laparotomies and bladder exposure. Mice then underwent either bladder wall injection with *S. haematobium* eggs or a vehicle control. Five days later, mice were sacrificed and their bladders harvested and fixed. Frozen sections of each bladder were stained with Cresyl violet. Laser microdissection was used to harvest RNA from three regions of each bladder: 1) the "proximal" urothelium (urothelium overlying the egg granuloma site); 2) the "distal" urothelium (urothelium from the opposite side of the bladder relative to the granuloma site); and 3) granuloma tissue (subepithelial). Equal areas were harvested for each of the three tissue sites (478,000-1,000,000 µm2/site). RNA was isolated, reverse transcribed to cDNA, pre-amplified using the NuGen PicoSL WTA System, and then subjected to qPCR for uroplakin and housekeeping genes. Control vehicle-injected bladders exhibited subepithelial edema, normal urothelium, and some inflammation. Egg-injected bladders, in contradistinction, featured markedly thickened, hyperplastic urothelium overlying areas of significant egg-associated inflammation. The proximal urothelium in egg-injected bladders featured lower expression levels of uroplakin genes relative to the distal urothelium. Bladder granulomas, such as those induced by urogenital schistosomiasis, may locally suppress the overlying urothelial barrier. This suppression may be a mechanism by which chronic bladder inflammation results in urothelial-related bladder dysfunction. Future work will further define these mechanisms and their physiologic significance.

1789

INFLUENCE OF HISTONE MODIFYING ENZYMES AND MITOGEN-ACTIVATED PROTEIN KINASE SIGNALING PATHWAY ON *SCHISTOSOMA MANSONI* SURVIVAL AND REPRODUCTIVE DEVELOPMENT

Marina M. Mourão[1], Luiza F. Andrade[1], Livia G. Avelar[1], Juliana A. Geraldo[1], Fernanda S. Coelho[1], Laila A. Nahum[2], Raymond J. Pierce[3], Guilherme Oliveira[1]

[1]Genomics and Computational Biology Group, Instituto Nacional de Ciência e Tecnologia em Doenças Tropicais, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz – FIOCRUZ, Brazil, Center for Excellence in Bioinformatics, Belo Horizonte, Brazil, [2]Fundação Oswaldo Cruz, Faculdade Infórium de Tecnologia, Belo Horizonte, Brazil, [3]CIIL, Inserm U1019, CNRS UMR8204, University Lille Nord de France, Institut Pasteur de Lille, Lille, France

Praziquantel is an efficacious drug against schistosomiasis, however, there is the risk of drug resistance development and therefore new drugs are necessary. Although, the roles of Histone Modifying Enzymes (HMEs) and Mitogen-activated protein kinases (MAPKs) are unclear in schistosomes, they are increasingly approved as targets for drug development with a rising number of inhibitors under development. In other organisms, HMEs and MAPKs influence a number of tissue-specific biological activities such as cell survival, differentiation and proliferation. Here, we employed RNA interference (RNAi) to elucidate the functional roles of 16 HMEs and 6 genes involved in MAPK signaling pathway in *S. mansoni*. First, the HMEs and ePKs were identified in the predicted proteomes of *Schistosoma mansoni*, *S. japonicum*, and *S. haematobium* by HMM searches. Genes were annotated and selected regarding their putative function in the parasite. One histone deacetylases (HDAC8), 10 methyltranferases (HMTs), 5 demethylases (HDM), SmRas, SmERK1, SmERK2, SmJNK, SmCaMK2, and Smp38 were chosen for experimental validation. RNAi and pharmacological inhibition were used to elucidate the functional role of HMEs and MAPK signaling pathway proteins in *S. mansoni*. Mice were injected with schistosomula subsequent to RNAi and the development of adult worms observed. The data demonstrate that SmHDAC8 and SmJNK contributes to the parasite transformation and survival, whereas HDAC8, PRMT3, KDM1/KDM2, SmERK, and Smp38 seems to be involved in egg production as infected mice had significantly lower egg burdens and female worms presented underdeveloped ovaries. Additionally, SmJNK and Smp38 dsRNA treated worms exhibited tegumental damage. We also observed that Smp38 is involved in the activation of detoxification enzymes. Our results help characterize the importance of HMEs and MAPK pathway in the normal development and survival of the schistosome parasite and suggest some of these enzymes as useful drug targets to prevent schistosomiasis progression.

1790

PROTEOMIC ANALYSIS OF *BIOMPHALARIA GLABRATA* HEMOCYTES DURING ENCAPSULATION OF *SCHISTOSOMA MANSONI* SPOROCYSTS *IN VITRO*

Marília Gabriela dos Santos G. Cavalcanti[1], Grzegorz Sabat[2], Xiao-Jun Wu[2], Utibe Bickham[2], Timothy P. Yoshino[2]

[1]Federal University of Paraíba, João Pessoa, Brazil, [2]University of Wisconsin, Madison, WI, United States

In order to better understand the effector mechanisms associated with parasitic encapsulation reactions, whole hemolymph containing hemocytes was extracted from susceptible (NMRI) and resistant (BS-90) *Biomphalaria glabrata* strains and incubated in the presence or absence of newly-transformed *Schistosoma mansoni* sporocysts (Sp). After 18 h of incubation at 26 C, hemocyte capsules were isolated, frozen *en bloc*, cryo-sectioned and subjected to laser capture microdissection to yield samples enriched for cells involved in hemocyte/Sp reactions for comparison to hemocyte capsules without Sp. Isolated cryosections were analyzed by nanoLC-ESI-MS/MS for peptide isolation and sequencing. Putative protein identifications were made by BLAST analyses vs. the non-redundant NCBI protein database (db) and a 6-frame translated *B. glabrata* protein db in VectorBase. Preliminary analyses revealed at total of 358 putatively identified proteins of which 71 were from *Schistosoma* spp. (mainly *S. mansoni*) and 287 were non-*Schistosoma* sequences. Significantly more larval sequences were identified in NMRI/Sp capsules compared to BS-90/Sp samples, consistent with greater killing and larval rejection typically seen in R hemocyte reactions *in vitro*. After normalizing the dataset across samples using total unique peptide counts for actin and tubulin, other notable immune-related observations were made: (a) A greater reduction of HSP70 peptides in BS-90/Sp capsules compared to NMRI/Sp (67% BS-90 vs. 37% NMRI); (b) Frep2 was the only Frep identified in all samples, and only in the BS-90/Sp sample, implying an upregulation of Frep2 during parasite encounters; (c) An upregulation of extracellular matrix/adhesion proteins (dermatopontin2, HMG1, matrilin2 and α-integrin) only in BS-90/Sp or BS-90 control samples suggesting possible roles in immune-related cell-parasite or cell-cell adhesion reactions; and (d) Enrichment of MnSOD, a potential effector molecule, in the BS-90 hemocyte capsules compared to those of NMRI snails. It is anticipated that continued mining of this rich dataset will yield valuable insights into hemocyte immune function.

1791

COMPARATIVE ANALYSIS OF GENE EXPRESSION IN *SCHISTOSOMA MANSONI*-EXPOSED *BIOMPHALARIA GLABRATA* (BS90 STOCK) SNAILS MAINTAINED AT PERMISSIVE AND NON-PERMISSIVE TEMPERATURES

Matty Knight[1], Ramzi Temanni[2], Wannaporn Ittiprasert[3], Andre Miller[3], Yuan Li[2], Najib M. El-Sayed[2]

[1]George Washington University, Washington, DC, United States, [2]University of Maryland, College Park, MD, United States, [3]Biomedical Research Institute, Rockville, MD, United States

We have shown previously that the refractory phenotype in the *Biomphalaria glabrata* BS-90 stock is a temperature-dependent trait. Thus, using mild non-lethal heat (32°C) to induce stress genes such as Hsp70 and Hsp90 prior to *S. mansoni* infection rendered these normally resistant snails susceptible. In order to determine differences between transcription profiles of these snails responding to early parasite infection when they are ether resistant or susceptible, RNA samples from *Schistosoma mansoni* exposed juvenile BS-90 snails, maintained through several generations either at the permissive (32°C), or non -permissive temperature (25°C) were sequenced. Bioinformatic analyses of RNAseq datasets revealed a preponderance of stress related transcripts in parasite-exposed BS-90 snails maintained at the permissive temperature compared to similarly exposed reference stock maintained at room temperature. For example, at 2 hours post - exposure, a 77- fold induction of Hsp70 transcript was observed in susceptible BS-90 snails maintained at 32°C, corroborating earlier results that showed that this transcript was induced differentially between juvenile resistant and susceptible parasite-exposed snails. Differential expression of other stress genes, Hsp 90 (12-fold induction), Hsp 83 (40-fold induction) and Hsp 68 (5-fold induction) was also detected in these BS-90 snails responding to *S. mansoni* at the permissive temperature. These data, taken together with previous results provides further evidence for the role of stress in the snail- host and schistosome interaction.

1792

IMMUNOMODULATORY PROTEINS OF *SCHISTOSOMA HAEMATOBIUM*

Luke F. Pennington[1], Debalina Ray[2], Chi-Ling Fu[1], Yi-Ju Hsieh[1], Michael H. Hsieh[1]

[1]Stanford University, Palo Alto, CA, United States, [2]University of California San Francisco, San Francisco, CA, United States

Approximately 120 million individuals are infected with *Schistosoma haematobium* in sub-Saharan African alone. *S. haematobium* adult worms lay eggs throughout the urogenital tract that induce a pronounced inflammatory response. To date very little is known about how *S. haematobium* is able to produce such a robust immune response while evading immune clearance and immunity in many individuals. One plausible explanation lies in parasite secreted immunomodulatory proteins, however, due to the historical lack of a robust *S. haematobium* animal model, very little is known about the role of these proteins in urogenital pathology. We have recently cloned a homolog of the IL-4-inducing principle of *S. mansoni* eggs (IPSE), a protein originally identified in *S. mansoni,* the sister species of *S. haematobium* responsible for hepatic and enteric schistosomiasis. *S. mansoni* IPSE has been shown to bind IgE on the surface of basophils in an antigen independent manner, and drive basophil degranulation. To date it is not clear why it is advantageous for the parasite to secrete a protein capable of activating effector cells associated with anti-parasite responses. Intriguingly the *S. haematobium* IPSE homolog shares only 63% identity with its *S. mansoni* counterpart, suggesting that the protein may have evolved to suit each species' infectious niche. Furthermore studies with recombinant *S. haematobium* IPSE and *S. mansoni* IPSE suggest that the immunoglobulin isotype binding profiles of IPSE differ across species. Despite IPSE's sequence divergence and differences in immunoglobulin binding, several important protein features appear to be conserved across species, including a nuclear localization sequence. Using a novel model of *S. haematobium* egg-induced pathology we have shown that immunization with IPSE prior to *S. haematobium* egg injection alters urothelial inflammation. Together, these results suggest that *S. haematobium* IPSE also functions as an immunomodulatory protein, and plays an important and distinct role in regulating tissue pathology in urogenital schistosomiasis.

1793

GENDER DEPENDENCE OF P53-RELATED ABNORMALITIES IN MOUSE BLADDER UROTHELIUM DUE TO *SCHISTOSOMA HAEMATOBIUM* EGG-INDUCED INFLAMMATION

Jared Honeycutt[1], Olfat Hammam[2], Michael H. Hsieh[1]

[1]Stanford University School of Medicine, Stanford, CA, United States, [2]Theodor Bilharz Research Institute, Cairo, Egypt

The bladder urothelium is dramatically altered during *Schistosoma haematobium* infection (urogenital schistosomiasis). These alterations include hyperplasia, ulceration, dysplasia, squamous metaplasia and frank carcinogenesis. Defining the pathways that drive these urothelial changes will contribute to a deeper understanding of how *S. haematobium* egg-induced expulsion, hematuria, and bladder cancer develop in humans. Defects in the function of the tumor suppressor gene p53 are evident in many cancers, including bladder cancer generally and schistosomal bladder cancer specifically. To identify any role p53 might play in urothelial alterations due to urogenital schistosomiasis, we employed transgenic mice with tamoxifen-inducible cre recombinase activity in cells expressing

uroplakin-3a, a urothelial-specific gene (Upk3a-GCE mice). We confirmed specificity of cre expression in Upk3a-GCE mice by crossing them with TdTomato-floxed-EGFP reporter mice and administering tamoxifen to their progeny. As expected, these progeny switched from TdTomato to EGFP expression in their bladder urothelium. We then crossed Upk3a-GCE mice to p53-floxed mice. The resulting progeny (Upk3a-GCE+/wt;p53fl/wt) were given tamoxifen or vehicle control to render them urothelial p53-haploinsufficient or -intact, respectively. We then injected *S. haematobium* eggs or control vehicle into the bladder walls of these mice. Three months later, mice were sacrificed and their bladders subjected to histological analysis (H&E staining). Male p53-intact, egg-injected mice exhibited similar histological changes as their p53-haploinsufficient counterparts, including urothelial hyperplasia and ulceration. In contrast, female p53-intact, egg-injected mice featured no urothelial ulceration, whereas their p53-haploinsufficient counterparts often had significant ulceration. Additionally, some egg-injected p53-haploinsufficient females exhibited regions of squamous metaplasia. Thus, intact p53 activity seems to be required, in a gender-specific manner, for urothelial homeostasis during *S. haematobium* infection in this model. Ongoing work includes (1) examining histological changes in Upk3a-GCE+/wt;p53fl/wt mice beyond 3 months after egg-injection, and (2) measuring alterations in the cell cycle status of the urothelium as a consequence of schistosomiasis.

1794

CD4+ T-CELL COUNTS IN WOMEN WITH UROGENITAL SCHISTOSOMIASIS

Elisabeth Kleppa[1], Kari F. Klinge[1], Kristine Lillebø[1], Hashini N. Galaphaththi-Arachchige[1], Sigve D. Holmen[1], Siphosenkosi G. Zulu[2], Birgitte J. Vennervald[3], Pavitra Pillay[2], Mathias Onsrud[1], Svein Gunnar Gundersen[4], Myra Taylor[2], Eyrun F. Kjetland[1]

[1]Oslo University Hospital, Oslo, Norway, [2]University of KwaZulu-Natal, Durban, South Africa, [3]University of Copenhagen, Copenhagen, Denmark, [4]Sorlandet Hospital, Kristiansand, Norway

Schistosoma haematobium causes urogenital schistosomiasis and has been shown to be associated with HIV in cross-sectional studies. Schistosomiasis infection has been hypothesized to affect the HIV viral load and HIV disease progression. Schistosomiasis treatment has been shown to increase CD4 counts in HIV negative individuals. Between May and October 2013, a CD4 count was done in 797 young women who were invited for a gynecological examination. One urine sample was collected from all, and microscopy for schistosome ova was done. HIV testing was done in 769 women, of which 123 were HIV positive (16.0%). The mean CD4 count was 864 x10^6 cells / L. It was lower in the HIV negative group than in the HIV positive group (931 vs. 511 $p < 0.001$). Urinary schistosomiasis was not associated with a lower CD4 count (862 in urine negative vs. 871 in urine positive women, p=0.75). Likewise, there was no significant association between CD4 counts between women with moderate or severe degree genital schistosomiasis and women without genital lesions (846 vs. 829, respectively, p= 0.70). Further there was no significant difference between schistosomiasis positive and negative women were found after stratifying for HIV (data not shown). This cross-sectional study did not show any significant difference in CD4 T-cell counts when comparing women with and without urogenital schistosomiasis.

1795

PATTERNS OF REACTIVITY TO *SCHISTOSOMA MANSONI* EGG GLYCAN ANTIGENS IN A POPULATION OF TREATMENT-NAÏVE KENYAN SCHOOL CHILDREN

Nina S. Prasanphanich[1], Jasmine A. Landry[2], Ryan E. Wiegand[3], Eric M. Ndombi[4], Bernard Abudho[4], Diana K. Riner[5], Megan L. Mickum[1], Diana M. Karanja[4], Daniel G. Colley[5], Richard D. Cummings[1], W. Evan Secor[3]

[1]Emory University, Atlanta, GA, United States, [2]Macalester College, St. Paul, MN, United States, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Kenya Medical Research Institute, Kisumu, Kenya, [5]University of Georgia, Athens, GA, United States

Children in schistosomiasis-endemic areas develop partial resistance to infection as they age. This resistance is associated with immune responses, including IgE and IgG, to parasite antigens. Anti-glycan antibodies can kill parasite larvae *in vitro* and mediate resistance in some animal models of helminth infection, but their significance in human *Schistosoma mansoni* infection is still unclear. Plasma from *S. mansoni*-infected children demonstrate specific reactivity with several epitopes on schistosome glycan microarrays. To explore the relationship of such antibodies with naturally-acquired partial immunity, we measured IgG and IgM to mock- and periodate-treated *S. mansoni* soluble egg antigen (SEA), and two parasite cross-reactive glycoproteins, keyhole limpet hemocyanin (KLH) and horseradish peroxidase (HRP), in plasma from a population of treatment-naïve Kenyan school children. The ratio of antibody reactivity with periodate-resistant (primarily non-glycan) versus total epitopes in SEA increased, and antibodies to KLH and HRP glycans decreased slightly as children aged. These trends were especially pronounced throughout adolescence. The anti-glycan antibodies detected included a variety of IgG subtypes. Our results suggest that immune recognition of the glycan epitopes examined in this study are negatively associated with age, but some may warrant further investigation as diagnostics or indicators of the length of exposure to schistosomes. Future studies on anti-glycan antibodies to other epitopes or of other isotypes/subtypes in naturally-acquired immunity, and on whether anti-glycan antibodies may be involved in resistance to reinfection after praziquantel treatment could be informative for vaccine development.

1796

CHILDREN WITH CEREBRAL MALARIA LACK SERORECOGNITION OF A DISTINCT PFEMP1 SUBSET

Mark A. Travassos[1], Amadou Niangaly[2], Jason A. Bailey[1], Amed Ouattara[1], Drissa Coulibaly[2], Kirsten Lyke[3], Matthew B. Laurens[1], Jozelyn Pablo[4], Algis Jasinskas[4], Rie Nakajima-Sasaki[4], Andrea A. Berry[1], Matthew Adams[1], Shannon Takala-Harrison[3], Bourema Kouriba[2], Abdoulaye K. Kone[2], J. Alexandra Rowe[5], JoAnn Moulds[6], Ogobara K. Doumbo[2], Mahamadou A. Thera[2], Philip L. Felgner[4], Christopher V. Plowe[1]

[1]Howard Hughes Medical Institute/Center for Vaccine Development, Baltimore, MD, United States, [2]Malaria Research and Training Center, Bamako, Mali, [3]Center for Vaccine Development, Baltimore, MD, United States, [4]University of California Irvine, Irvine, CA, United States, [5]University of Edinburgh, Edinburgh, United Kingdom, [6]LifeShare Blood Centers, Shreveport, LA, United States

Plasmodium falciparum erythrocyte membrane protein-1 (PfEMP1) antigens play an important role in parasite sequestration and host immune system evasion. Acquired antimalarial immunity is at least partially due to antibodies directed against highly variable antigens like PfEMP1 that are present on the red blood cell surface. However, the PfEMP1 antigenic domains that drive this immune-mediated protection have not been identified. We have previously shown that infected erythrocytes from persons with cerebral malaria express a distinct "stealth" PfEMP1 group that do not bind the endothelial receptor CD36. We hypothesized that children with cerebral malaria lack serorecognition to a subset of PfEMP1s

that is subsequently recognized in convalescence. A protein microarray was printed with 171 fragments of PfEMP1s based on the 3D7 reference genome. For comparison, 268 diverse apical membrane 1 (AMA1) fragments, 20 merozoite surface protein 1 (MSP1) fragments, and 30 Rh5 fragments were also included on the array, based on sequences derived from field samples. Reactivity was measured in 195 serum samples from Malian children, including 43 cases of cerebral malaria and age-matched controls who were healthy or had uncomplicated malaria. Children with cerebral malaria had lower seroreactivity to stealth and non-stealth PfEMP1 antigen variants than both healthy controls and uncomplicated malaria controls. Seroreactivity to AMA1, Rh5, and MSP1 variants tested did not increase from acute cerebral malaria illness to convalescence, but seroreactivity to four stealth PfEMP1 fragments increased, suggesting that a lack of immunity to a subset of PfEMP1s may be associated with vulnerability to cerebral malaria.

1797

CYTOKINE RESPONSES TO THE VAR2CSA VACCINE CANDIDATE IN CORD BLOOD FROM BENINESE NEWBORNS

Nadine Fievet[1], Komi Gbedandé[2], Gilles Cottrell[1], Bertin Vianou[2], Carine Agbowaï[2], Samad Ibitokou[2], Marita Troye-Blomberg[3], Thor Theander[4], Ali Salanti[4], Achille Massougbodji[2], Nicaise Tuikue Ndam[1], Philippe Deloron[1], Adrian Luty[1]

[1]IRD UMR216 "Mère et enfant face aux infections tropicales", Paris, France, [2]Centre d'Etude et de Recherche sur le Paludisme Associé à la Grossesse et à l'Enfance, Faculté des Sciences de la Santé, Cotonou, Benin, [3]Department of Immunology, Wenner-Gren Institute, Stockholm University, Stockholm, Sweden, [4]Department of International Health, Institute of International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark

The STOPPAM consortium conducted 2 longitudinal cohort studies in pregnant women in Benin and Tanzania in order to evaluate the immunopathological consequences of infections with *Plasmodium falciparum* during pregnancy for the newborns. In order to study the exposure of the foetal immune system to parasite-derived antigens *in utero*, we evaluated both, cytokine (IL10, IL12, IL13, IL17, IFN-γ, TNF-α responses and T cell IFN-γ specific responses to the vaccine candidate antigen DBL5 domain of VAR2CSA as a function of placental infection with *P. falciparum*. In Come, southwestern Benin, we conducted a longitudinal prospective study of ~1000 pregnant women. Women at ≤24 weeks of pregnancy were enrolled and followed at each antenatal visit until delivery. For the immunological sub-study of the cord blood mononuclear cellular (CBMC) responses to VAR2CSA-DBL5 *in vitro*, a group of 200 pregnant women was selected at delivery on the basis of their history of infection with *P. falciparum* (uninfected during pregnancy/infected during pregnancy but uninfected at delivery/infected at delivery). Those harbouring *P. falciparum* infections at delivery were matched by gravidity and gestational age with mothers with no infection and those with no history of infection earlier in the pregnancy. The amounts of IL10, IL12, IL13, IL17, IFN-γ and TNF-α produced in response to mitogen (PHA) and to VAR2CSA-DBL5 were quantified in supernatants of stimulated CBMC. The *ex vivo* frequencies of IFN-γ secreting CD4 and CD8 T cells in response to PHA and VAR2CSA domains were also evaluated. At the time of writing, all data have been collected, cytokine concentrations have been evaluated and multivariate analyses are under way. Results will be discussed in the context of cytokine profiles that reflect the *in utero* acquisition of a specific cellular memory response to the vaccine candidate.

1798

RNA-SEQ ANALYSIS OF WHOLE BLOOD FROM MALARIA-SUSCEPTIBLE AND IMMUNE CHILDREN REVEALS AN EARLY PRO-INFLAMMATORY RESPONSE TO *PLASMODIUM FALCIPARUM* INFECTION THAT CORRELATES WITH CONTROL OF PARASITE GROWTH: A PROSPECTIVE STUDY IN MALI

Tuan M. Tran[1], Aissata Ongoiba[2], Marcus Jones[3], Jeff Skinner[1], Shanping Li[1], Safiatou Doumbo[2], Didier Doumtabe[2], Younoussou Kone[2], Aboudramane Bathily[2], Seydou Dia[2], Moussa Niangaly[2], Abdrahamane Traore[2], Charles Dara[2], Jules Sangala[2], Ogobara K. Doumbo[2], Pratap Venepally[3], Kassoum Kayentao[2], Boubacar Traore[2], Ewen F. Kirkness[3], Peter D. Crompton[1]

[1]Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]Mali International Center of Excellence in Research, University of Sciences, Technique and Technology of Bamako, Bamako, Mali, [3]Genomic Medicine Group, J. Craig Venter Institute, Rockville, MD, United States

Non-sterile, antibody-mediated immunity that reliably protects from febrile malaria is acquired gradually through repeated *Plasmodium falciparum* infections; however, the nature of cellular immune responses at the onset of clinically apparent versus clinically silent blood-stage infections in children is unclear. In a prospective study in Mali, we collected whole blood RNA, PBMCs and plasma from healthy, uninfected children aged 6-11 years (n=79) before the 6-month malaria season and from the same children during their first *P. falciparum* infection of the ensuing season—detected retrospectively through bi-weekly active surveillance by PCR. We used RNA-seq to compare whole-blood transcriptomes of children whose clinically silent infections never progressed to fever (immune, n=21), children whose infections progressed to fever within 2-14 days (late fever, n=32) and children who were febrile at the time of infection (early fever, n=26). We found that baseline transcription profiles before the malaria season distinguished children whose future *P. falciparum* infections either progressed to fever or not, including upregulation of B-cell-receptor signaling pathways in immune children. Transcription profiles induced by the first detected *P. falciparum* infection of the season revealed upregulation of pro-inflammatory genes in immune versus late fever children, despite both groups having similar levels of parasitemia and the clinical absence of fever initially. In addition, this early upregulation of pro-inflammatory genes was associated with slower subsequent parasite growth rates *in vivo*. In ongoing work, we are testing hypotheses generated by this study at the protein level and in functional assays using contemporaneous PBMCs and plasma samples from the same children. Molecular and cellular signatures that correlate with protection from malaria are yielding novel insights into the mechanisms underlying naturally acquired immunity to malaria. The resulting datasets may inform the development of interventions that prevent or mitigate malaria disease.

1799

IMMUNE CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* PARASITES WITH A SHARED GENETIC SIGNATURE: VARIANT SURFACE ANTIGENS AND *VAR* REPERTOIRES

Amy K. Bei[1], Ababacar Diouf[2], Kazutoyo Miura[2], Daniel B. Larremore[3], Ulf Ribacke[1], Gregory Tullo[2], Eli L. Moss[4], Daniel E. Neafsey[4], Rachel F. Daniels[1], Amir E. Zeituni[2], Iguosadolo Nosamiefan[1], Sarah K. Volkman[1], Ambroise D. Ahouidi[5], Daouda Ndiaye[6], Tandakha Dieye[5], Souleymane Mboup[5], Caroline O. Buckee[3], Carole A. Long[2], Dyann F. Wirth[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [3]Harvard School of Public Health, Center for Communicable Disease Dynamics, Boston, MA, United States, [4]Broad Institute of Massachusetts Institute of Technology and Harvard, Boston, MA, United States, [5]Laboratory of Bacteriology and Virology, Hopital Aristide Le Dantec, Dakar, Senegal, [6]Laboratory of Parasitology and Mycology, Dakar, Senegal

As malaria transmission intensity has declined in some regions, *Plasmodium falciparum* parasite populations are displaying decreased clonal diversity resulting from the emergence of many parasites with identical genetic signatures. We have monitored genetically identical parasite clusters from 2006-2013 in Thiès, Senegal, and we have characterized the immune response against these parasites. We focus on one cluster of identical parasites that was present in 24% of clinical isolates in 2008 and declined to 3.4% of clinical isolates in 2009. We studied the susceptibility of 2 representative common genetic signature (CGS) parasites and 1 representative non-CGS parasite and measured the infected RBC IgG reactivity for 109 individual plasmas distributed between both years by variant surface antigen (VSA) flow cytometry. By VSA flow, the non-CGS parasites are similary recognized by plasma IgG from 2008 and 2009, but reactivity is increased in 2009 compared to 2008 for the CGS parasites. We characterized the *var* genes expressed by CGS parasites by *var* Ups qRT-PCR and by sequencing using degenerate DBL1alpha domain primers. We observed that the CGS parasites expressed the same *var* Ups classes, and the same dominant *var* repertoires as identified by both DBL1alpha sequence analysis as well as RNAseq. Additionally, we used network analysis to compare the diversity of the *var* repertoires with that of globally diverse parasites. We generated a *var* sequence network that shows that the *var* repertoires of CGS-1 and CGS-2 overlap substantially, while the repertoire of the non-CGS parasite is unique at the level of globally diverse parasites. Taken together, our work indicates that these CGS parasites express similar *var* genes, more than would be expected by chance in the population, and there is year-to-year variation in immune recognition of these CGS parasites at the level of surface expression of VSAs. We are currently expanding these findings to other large clusters such as one that emerged in 2009 and persisted at a high frequency (26-30%) for multiple years before disappearing from the population in 2013.

1800

SINGLE-CELL TRANSCRIPTIONAL ANALYSIS OF MALARIA-SPECIFIC CD4+ T LYMPHOCYTES FOLLOWING PFSPZ VACCINATION AND PROTECTION IN HUMANS

Kailan Sierra-Davidson[1], Adam Wheatley[1], Patricia Darrah[1], Greg Finak[2], Madhu Prabhakaran[1], Pratip Chattopadhyay[1], Mario Roederer[1], Helen McShane[3], Kim Lee Sim[4], Stephen Hoffman[4], Raphael Gottardo[2], Adrian McDermott[1], Robert Seder[1]

[1]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [2]Vaccine and Infectious Disease Division, Seattle, WA, United States, [3]Jenner Institute, Oxford, United Kingdom, [4]Sanaria, Rockville, MD, United States

Intravenous immunization with highly purified, radiation-attenuated parasites (PfSPZ Vaccine) is safe, immunogenic and confers high-level protection against controlled malaria infection in humans. Protection was associated with a dose-dependent increase in PfSPZ-specific antibodies, $CD4^+$ and $CD8^+$ T cell responses. Heretofore, multi-parameter flow cytometry has been used to characterize the magnitude and quality of PfSPZ-specific T cell responses following vaccination or infection. To substantially expand the analysis of such responses, we performed high-resolution, quantitative transcriptome analysis of PfSPZ-specific $CD4^+$ T cells. Accordingly, PfSPZ-specific $CD4^+$ T cells expressing the costimulatory marker CD154 (CD40L) were sorted following *in vitro* activation with sporozoites. We first demonstrate that ~30 percent of PfSPZ-specific $CD154^+$ $CD4^+$ T cells do not produce IFN-γ, IL-2 or TNFα, the most common cytokines used to assess T cell responses. This finding highlights the increased sensitivity of the CD154 capture assay for broader assessment of antigen-specific responses. Furthermore, isolation of live malaria-specific $CD4^+$ T cells permits downstream mRNA analysis using valved microfluidic chips from Fluidigm. Quantitative expression of ~100 genes can be rapidly analyzed from isolated samples of single antigen-specific T cells. Initial transcriptome analysis of protected subjects revealed that malaria-specific $CD4^+$ T cells express a unique gene expression signature that is distinct from influenza-specific $CD4^+$ T cells in the same individual. These data will serve as an internal control to compare virus- and parasite-specific responses. We are currently analyzing the gene signature of PfSPZ-specific $CD4^+$ T cells from vaccinated and protected subjects prior to challenge vs. nonvaccinated controls during infection. Overall, this analysis should advance our understanding of the heterogeneity of parasite-specific $CD4^+$ T cell responses at the single-cell level and provide insights into how $CD4^+$ T cells may influence protection against human malaria infection.

1801

IMMUNOLOGICAL PROFILING AFTER SPOROZOITE IMMUNIZATION UNDER CHEMOPROPHYLAXIS IN THE CONTROLED HUMAN MALARIA INFECTION MODEL

Robert W. Sauerwein

Radboud University Medical Center, Nijmegen, Netherlands

A unique tool to study malaria immunology and efficacy of immunisation strategies form Controlled Human Malaria Infections (CHMI) and has proved to be a reproducible, predictable and safe method of inducing *Plasmodium falciparum* (Pf) malaria. An efficient method for induction of complete protection in humans was achieved by exposing human subjects to Pf-infected mosquitoes while taking blood-stage suppressive chloroquine prophylaxis. When tested in clinical trials, this protocol induced > 95% clinically and parasitologically sterile protection against a standard challenge infection. Longlasting CPS-induced protection was primarily mediated by immunity to sporozoite and liver stages rather than to asexual blood-stages. This opens opportunities to explore mechanisms of protective immunity, allowing the search for immune correlates/signatures of protection and clinical development of a whole sporozoite based vaccine. Humoral and cellular immune responses associated with protection to *Plasmodium falciparum* parasites will be presented.

1802

GENE PROFILING IN NAÏVE AND SEMI-IMMUNE COLOMBIAN INDIVIDUALS SUBJECTED TO EXPERIMENTAL CHALLENGE WITH *PLASMODIUM VIVAX* SPOROZOITES

Myriam Arévalo-Herrera[1], Monica Rojas-Peña[2], Andres F. Vallejo[1], Nora Martinez[1], Greg Gibson[2], Sócrates Herrera[1]

[1]Caucaseco Scientific Research Center, Cali, Colombia, [2]School of Biology, Georgia Institute of Technology, Atlanta, GA, United States

In the context of a *Plasmodium vivax* malaria vaccine program, a total of 16 Colombian malaria naïve (n=7 from Cali) and semi-immune (n=9 from Buenaventura) volunteers were subjected to an experimental P.vivax sporozoite infectious challenge using direct infected *Anopheles* mosquito bites (2-4) and followed up to determine the prepatent period, immune response and clinical outcome of the infection. Volunteers were closely monitored and treated as soon as malaria infection was detected by microscopy. The study offered a unique opportunity to assess the gene expression profile induced by *P. vivax* malaria infection in naïve and previously infected human volunteers Blood samples were used for immunological analyses and RNA preserved in Tempus tubes was isolated for transcriptomic analyses. We used a Fluidigm nanofluidic qRT-PCR array to profile the expression of 92 genes in the 16 individuals across 6 time-points following infection. The genes were chosen to represent 10 axes of variation that describe major components of transcriptional variation in peripheral blood. Strong covariance of transcript abundance was observed for 8 of these axes, 2 of which correspond to the first two overall principal components of variation. The results show that there is strong up-regulation of an interferon-response axis at the peak of parasitemia, but a down-regulation of the inflammatory response at the same time. Another set of transcripts was observed to be significantly up-regulated both in the naïve samples and at the peak of parasitemia, but across the entire experiment the difference between the naïve and pre-immune samples was minor relative to among individual variation. Nevertheless, these results strongly suggest that whole transcriptome profiling will uncover a set of genes that respond differently to infection as a function of the degree of prior exposure to malaria..

1803

GAMETOCYTE CLEARANCE IN MELANESIAN CHILDREN TREATED FOR *PLASMODIUM FALCIPARUM* AND *P. VIVAX* MALARIA WITH ARTEMISININ COMBINATION THERAPY

Stephan Karl[1], Moses Laman[2], Tamarah Koleala[3], Anna Rosannas-Urgell[4], Brioni Moore[2], Peter Siba[3], Timothy G. St.Pierre[2], Ivo Mueller[1], Timothy Davis[2]

[1]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [2]The University of Western Australia, Perth, Australia, [3]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [4]Antwerp Institute of Medical Research, Antwerp, Belgium

We present a detailed analysis of gametocyte clearance in Melanesian children infected with *Plasmodium falciparum* or *P. vivax* and treated with either Artemether-Lumefantrine (AL) or Artemisinin-Naphthoquine (AN). In addition, a detailed comparison of three methods, namely standard light microscopy (LM), magnetic fractionation (MF) and reverse transcriptase polymerase chain reaction (RTPCR) for detection of *Plasmodium falciparum* and *Plasmodium vivax* gametocytes is presented. Children (0.5-5 years) from the north coast of Papua New Guinea were randomly assigned to either AL or AN treatment upon presentation at the health centre with either *P. falciparum* or *P. vivax* malaria. LM was conducted by 2 trained microscopists with discordant reads judged by an expert microscopist. MF was conducted as previously described on the same day using 200 µL of blood. Samples for RTPCR were placed directly into RNA-later and stored at -80°C until analysis. MF and RTPCR were similarly sensitive and specific, and clearly superior to LM detection of gametocytes. *P. falciparum* gametocyte clearance characteristics were found to be different between AL and AN, mostly due to a longer predicted gametocyte sequestration time in the AN group. However AN treatment provided longer protection from gametocytaemic relapse and/or reinfection. *P. vivax* gametocytes were found to be cleared very rapidly and along with the asexual blood stages upon treatment with AL or AN, highlighting the fundamental differences between the *P. falciparum* and the *P. vivax* parasite species. This study represents the first direct comparison of LM, MF and RTPCR on a large number of field isolates. It provides clear evidence that magnetic fractionation is superior to light microscopy and can be used to detect gametocytaemic patients under field conditions with similar sensitivity and specificity as RTPCR. Furthermore this study illustrates fundamental differences between ACT mediated clearing of *P. falciparum* and P.vivax gametocytes and describes differences in the effect of AL and AN on *P. falciparum* gametocytes.

1804

DEVELOPMENT AND EVALUATION OF A SIMPLIFIED MOLECULAR DIAGNOSTIC PLATFORM FOR MALARIA: THE DIRECT ON BLOOD PCR-NALFIA SYSTEM

Petra Mens, Johanna Roth, Laura de Bes, Henk Schallig

Royal Tropical Institute, Amsterdam, Netherlands

Molecular tools allow for specific and sensitive malaria diagnosis, but current formats, like PCR with gel-electrophoresis, are difficult to implement in resource poor settings. Therefore, a simple, fast, sensitive and specific molecular diagnostic platform, direct on blood (db)PCR combined with nucleic acid lateral flow immunoassay (NALFIA) to detect amplified PCR products of *Plasmodium*, including species differentiation, and human GAPDH (internal amplification control) was developed. This platform does not require DNA extraction and circumvents complex read-out system. The platforms was evaluated under laboratory conditions, a multi country ring trial and in two malaria endemic countries (Burkina Faso and Thailand). Analytical sensitivity and specificity of the dbPCR-NALFIA in a single laboratory evaluation was >95% and the test was able to detect less than 1 parasite/µl blood. All four laboratories in the ring trial reported ease of use of the system and could successfully perform the protocol. Overall laboratory inter-variability was low and the agreement of reported results was high. Overall k-value was 0.89 (95% CI: 0.83 - 0.94; $p<0.001$). Overall test sensitivity and specificity was >95% with very small confidence intervals. Field evaluations by local staff without prior training in performing the dbPCR-NALFIA in malaria endemic countries, Thailand and Burkina Faso, were performed. In Burkina Faso (*P. falciparum* environment) the relative sensitivity was 94,8% and relative specificity 82,4% compared to microscopy and 93,3% and 91.4% compared to RDT. In Thailand (*P. vivax* environment) the relative sensitivity and relative specificity was 93,4% and 90,9 respectively compared to microscopy and 95,6% and 87.1 % compared to RDT. These numbers are an underestimation of test performance as the results are not PCR corrected. The prototype dbPCR-NALFIA test will now be moved forward in diagnostic test development (supported by EU funding: www.diagmal.eu) to provide a molecular diagnostic test to detect malaria in for example near elimination settings. The final format will include a closed transfer unit to reduce possible workspace contamination with amplicons. Funding: EU FP7 grant 601714: Translation of the direct-on-blood PCR-NALFIA system into an innovative near point-of-care diagnostic for malaria

1805

DEVELOPMENT OF A SINGLE NUCLEOTIDE POLYMORPHISM BASED BARCODE FOR THE IDENTIFICATION AND TRACKING OF *PLASMODIUM VIVAX*

Mary Lynn Baniecki[1], Aubrey Faust[2], Rachel Daniels[3], Kevin Galinsky[4], Marcelo U. Ferreira[5], Nadira Karunaweera[6], Elizabeth Winzeler[7], David Serre[8], Peter Zimmerman[9], Tom Wellems[10], Lise Musset[11], Stéphane Pelleau[11], Alexandre Melnikov[1], Daniel Neafsey[1], Sarah Volkman[4], Daniel L. Hartl[2], Dyann Wirth[4], Pardis Sabeti[2]

[1]The Broad Institute, Cambridge, MA, United States, [2]Harvard University, Cambridge, MA, United States, [3]Harvard School of Public Health, Cambridge, MA, United States, [4]Harvard School of Public Health, Boston, MA, United States, [5]University of São Paulo, São Paulo, Brazil, [6]University of Colombo, Colombo, Sri Lanka, [7]The University of California San Diego, San Diego, CA, United States, [8]Cleveland Clinic, Cleveland, OH, United States, [9]Case Western Reserve University, Cleveland, OH, United States, [10]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [11]Institut Pasteur de la Guyane, Cayenne, French Guiana

For global eradication of malaria to occur an enhanced understanding of the population structure of *Plasmodium vivax* is needed. An inability to maintain *P. vivax* continuously in culture has caused research on this parasite to lag behind that of the other *Plasmodium* species. Recent advances in molecular methods have enabled the *P. vivax* genome to be assayed directly from infected humans, providing the tools needed to develop genotyping methods. Single nucleotide polymorphism (SNP) genotyping provides a robust, inexpensive, field-deployable technology that allows malaria parasites to be tracked and identified by creating a unique genetic signature or barcode for each parasite. Using our experience in developing a SNP genotyping tool with quantitative PCR and High Resolution Melting (qPCR-HRM) for *P. falciparum*, we have developed a SNP barcode for *P. vivax.* The candidate SNPs were selected with a high minor allele frequency (MAF) from available *P. vivax* genome sequence data and were located at sites including intergenic, intragenic, or were 4-fold degenerate coding sites. Here, we report a pilot screen of a 95 SNPs by genotyping a set of 89 *P. vivax* containing clinical samples from geographically distinct parasite populations from the Americas (Brazil, French Guiana), Africa (Ethiopia) and Asia (Sri Lanka). Candidate SNPs were winnowed to a 41-SNP barcode based on robustness and reproducibility of the genotyping calls, and the ability to accurately detect polygenomic infections. The assays are robust with a detection range from 10 ng to 0.001 ng with an average assay efficiency of 90% among clinical samples tested. All 41 assays had an average minor allele frequency (MAF) > 0.1. Based on principle component analysis the clinical samples form distinct clusters that correspond to their geographic origin. Interesting, these analysis revealed a high level of polygenomic samples among all populations with Brazil (84%), Sri Lanka (95%), Ethiopia (78%), and French Guiana (62%).

1806

THE APICOPLAST OF *PLASMODIUM FALCIPARUM* PROVIDES A NOVEL TARGET FOR MOLECULAR DIAGNOSIS OF MALARIA USING POLYMERASE CHAIN REACTION AND LOOP MEDIATED ISOTHERMAL AMPLIFICATION ASSAYS

Eniyou C. Oriero[1], Jan Jacobs[2], Jean-Pierre Vangeertruyden[3], Umberto D'Alessandro[1], Davis Nwakanma[1]

[1]Medical Research Council Unit, The Gambia, Fajara, Gambia, [2]Institute of Tropical Medicine, Antwerpen, Belgium, [3]University of Antwerp, Antwerpen, Belgium

Despite a recent increase in procured rapid diagnostic tests (RDTs), and rate of diagnostic testing in the public sector of the African region, malaria still remains a global health burden with an estimated 627,000 deaths worldwide in 2012. WHO recommends all suspected malaria cases to be confirmed by microscopy or RDT prior to treatment however, millions of people with suspected malaria still do not receive diagnostic tests. As these methods do not rapidly and accurately detect sub-microscopic infections which can also contribute to transmission, high throughput molecular assays such as polymerase chain reaction (PCR) are used to detect asymptomatic and/or low-grade infections. Isothermal amplification methods were recently developed to address some of the major shortcomings of PCR. The most common target amplified in these molecular assays is the conserved small subunit ribosomal RNA 18S locus, which in the *Plasmodium falciparum* chromosomal genome exists in five to eight copies, depending on the strain. In this study, we report the development and optimization of the apicoplast of *P. falciparum* as a target for molecular diagnosis of malaria using a single step PCR (ssPCR), nested PCR (nPCR) and loop-mediated isothermal amplification (LAMP) assay. *P. falciparum* sequences from 15 Gambian isolates and 8 laboratory clones were aligned against the PlasmoDB reference sequence (ID: emb|X95275.2|) and primers were designed from a highly conserved region of the consensus sequence, approx. 1.5kb segment of the gene coding for a ribosomal RNA protein (AP|0010:rRNA). The primers were validated in silico and mapped unto the consensus sequence with a web based tool. The assays were optimized for temperature and concentration of primers, deoxyribonucleotides (dNTPs) and magnesium chloride (MgCl2). 272 archived DNA samples from across West Africa and S.E Asia were analyzed against a reference PCR method targeting the 18SrRNA gene. Preliminary results show perfect agreement for ssPCR and nPCR compared with the reference PCR method, while Sensitivity of 100 % (95% CI: 94 % to 100 %), Specificity of 84 % (95% CI: 68 % to 94 %), Positive Predictive Value (PPV) of 91 % (95% CI: 81 % to 97 %), Negative Predictive Value (NPV) of 100 % (95% CI: 89 % to 100 %) and Kappa index of 0.86 (95% CI: 0.76 to 0.97) were obtained for LAMP. Based on the results, the apicoplast genome appears to be a suitable target for sensitive detection of P.falciparum.

1807

GEOGRAPHICAL DISPERSION AND GENETIC CHARACTERIZATION OF PFHRP2 NEGATIVE *PLASMODIUM FALCIPARUM* PARASITES IN THE PERUVIAN AMAZON: IMPLICATIONS FOR RAPID DIAGNOSTIC TESTS (RDTS) BASED ON DETECTION OF HRP2

Dionicia Gamboa[1], Jorge Bendezu[1], Katherine Torres[1], Sheila Akinyi[2], Curtis S. Huber[2], Elizabeth Villasis[1], Sandra Incardona[3], David Bell[3], Joseph Vinetz[4], John W. Barnwell[2], Venkatachalam Udhayakumar[2]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Foundation for Innovative New Diagnostics, Geneva, Switzerland, [4]University of California San Diego, San Diego, CA, United States

There is prior evidence of *Plasmodium falciparum* parasites from clinical cases lacking pfhrp2 and pfhrp3 genes in the Peruvian Amazon Region. As various countries in South America move to introduce malaria rapid diagnostic tests (RDTs) as an alternative for diagnosis, the geographical distribution and genotypic characterization of parasites lacking pfhrp2 and pfhrp3 genes will have major implications for procurement choices for RDTs in the region. Ninety-three *P. falciparum* samples, collected in different communities from the Peruvian Amazon Region between 2009 and 2010, were used in this study. Genomic DNA was used to amplify 18SrRNA and pfmsp2 to confirm the diagnosis and DNA quality, respectively; pfhrp2, pfhrp3, and their flanking genes in order to assess the frequency of their deletions. Microsatellite analysis was performed using seven neutral microsatellites (MS) and seven novel MS loci flanking the pfhrp2 gene on chromosome 8 (-41kb, -10kb, -4kb, 1.4kb, 2.5kb, 5.2kb and 15kb). The data showed deletion of the pfhrp3 gene in 53.76% (50/93) and pfhrp2 gene deletion in 33.33% (31/93) of the samples. The proportion of the parasite populations that lacked these genes was quite variable from community to community. Among the flanking genes, PF3D7_0831900 (Mal7P1.230) showed the highest deletion frequency,

78.49% (73/93). Neutral MS marker analysis revealed the widespread distribution of *P. falciparum* hybrid lineages with a hybrid of the A clonal lineage (named AV1) being the most prevalent among parasites lacking pfhrp2 and pfhrp3 genes. MS data from loci flanking the pfhrp2 gene showed that the haplotypes α and Δ were the most abundant among the isolates analyzed. This study confirms that field isolates lacking either pfhrp2, pfhrp3 or their respective flanking genes were still present in the area in 2010. In addition, we identified five *P. falciparum* hybrid lineages circulating in this region. It is possible that certain parasite genetic backgrounds (haplotypes) could favor the maintenance and expansion of pfhrp2 and pfhrp3 gene deletions in the Peruvian Amazon, however further studies will be required to prove this possibility and also to elucidate the genetic basis for the pfhrp2 gene deletion in wild *P. falciparum* parasites.

1808

AN EXTERNAL QUALITY ASSURANCE PROTOCOL FOR PLACENTAL MALARIA HISTOPATHOLOGY STUDIES

Atis Muehlenbachs[1], Miriam K. Laufer[2], Tamiwe Tamoka[3], Veronica Ades[4], Grant Dorsey[5], Julie Gutman[6]

[1]Infectious Diseases Pathology Branch, Division of High Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Department of Pediatrics, University of Maryland, Baltimore, MD, United States, [3]University of Malawi College of Medicine, Blantyre, Malawi, [4]Department of Obstetrics and Gynecology, New York University Langone Medical Center, New York, NY, United States, [5]Department of Medicine, University of California San Francisco, San Francisco, CA, United States, [6]Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States

Placental histology can contribute to studies on the diagnosis, prevention and treatment of malaria in pregnancy (MIP). Infections prior to delivery can be identified by detecting hemozoin deposition, and parasitized erythrocytes can be seen in cases where peripheral blood is negative. However histology is prone to artifacts, and requires considerable training in slide reading. There is a general need for external quality control and quality assurance for MIP histopathology studies, where poor preparation can lead to the appearance of a hemozoin-like material which may be mistaken for evidence of prior malaria infection and debris can be mistaken for parasites, leading to false positive reads. In addition, very low level infections may be present in the placentas of semi-immune women, leading to false negatives if insufficient fields are examined or if the slide reader is inexperienced. We developed a protocol to formally provide confidential, blinded, retrospective review of slides with feedback in order to ensure high quality study data and to work towards building local expertise. A random subset of 10% of negative and 25% of positive slides are recommended for review by a single, blinded expert reader. Slides are formally scored on both the presence of parasites and hemozoin in fibrin in addition to general quality. Following unblinding, discrepant slides are re-examined to determine source of discrepancy, and consensus opinion with the submitter is attempted by using high quality photomicrographs. All slides are returned to the submitter so that they can be used for further education. Error rates less than 20% are considered standard for passing a laboratory proficiency test, and further rounds of QC can be performed as needed. For histology studies of malaria in pregnancy, discordance over 10% should trigger consideration of additional training and targeted re-review of study slides. In pilot studies with experienced readers, there was concordance in 85/94 (90.5%) of submitted cases; discrepancies primarily included false positives for hemozoin due to artifact, and false negatives due to low levels of hemozoin. We anticipate that a standardized histopathology QC/QA protocol will be of value to pregnancy malaria research community, and have potential to strengthen local histopathology expertise.

1809

IMPACT OF MALARIA RAPID DIAGNOSTIC TESTS ON CARE OF FEBRILE PATIENTS: CROSS-PROJECT RESULTS FROM THE ACT CONSORTIUM

Baptiste Leurent, Katia Bruxvoort, Eleanor Grieve, Eleanor Hutchinson, Joanna Reynolds, Toby Leslie, ACT Consortium

London School of Hygiene & Tropical Medicine, London, United Kingdom

The introduction of malaria rapid diagnostic tests (RDTs) is aimed to increase the proportion of febrile patients tested for malaria, but there are gaps in knowledge of what happens to patients who receive an RDT compared to those who do not. Multi-project data from the ACT Consortium provide an opportunity to examine the impact of RDTs on patient care outcomes under different contexts and settings. Twelve projects from seven countries contributed data, including projects in public health facilities, private retailers, and community health worker settings. Each compared different sets of diagnostic methods, resulting in 30 arms for analysis, which were grouped into four diagnostic categories: presumptive, microscopy, RDT, or enhanced RDT (in which RDTs were provided alongside supportive interventions). Patient care outcomes such as RDT use, antimalarial prescription, antibiotic prescription, referrals, patient satisfaction, and consultation out-of pocket costs were summarised for each arm and compared between categories. Preliminary results indicate a lower prescription of ACTs and a generally higher prescription of antibiotics where RDTs were used, compared to arms where only presumptive diagnosis was available. This difference was more marked in enhanced RDT arms. Referral to higher level care was also more frequent among RDT arms. However, the impact of RDTs was not always consistent and may depend on study setting and design, type of sector, characteristics of patients and providers, and measures of RDT implementation and support. Exploration of these contextual factors is underway, with complete results expected to be available in August 2014. While many studies have reported a positive impact of RDTs on patient outcomes, the effect is complex and likely to vary by setting and context. Further scrutiny is needed to better understand the impact of RDTs on patient care beyond their role as an important diagnostic tool.

1810

SCHISTOSOME POPULATION GENOMICS USING SINGLE ARCHIVED MIRACIDA

Frédéric Chevalier[1], Winka Le Clec'h[1], Philip LoVerde[2], Rafael Ramiro de Assis[3], Guillerme Corrêa-Oliveira[3], Safari Kinunghi[4], Anouk Gouvras[5], Bonnie Webster[5], Joanne P. Webster[6], Aidan Emery[5], David Rollinson[5], Tim Anderson[1]

[1]Texas Biomedical Research Institute, San Antonio, TX, United States, [2]Departments of Biochemistry and Pathology, University of Texas Health Science Center, San Antonio, TX, United States, [3]Centro de Pesquisas René Rachou-Fiocruz, Belo Horizonte, Brazil, [4]National Institute for Medical Research, Mwanza Centre, Mwanza, United Republic of Tanzania, [5]Wolfson Wellcome Biomedical Laboratories, Department of Life Sciences, Natural History Museum, London, United Kingdom, [6]Department of Infectious Disease Epidemiology, School of Public Health, Imperial College Faculty of Medicine, London, United Kingdom

Population genomic analyses of schistosomes have not previously been possible due to the difficulty of sampling adult worms and the high repeat content and the large size (360-400Mb) of their genomes. We have developed a robust, inexpensive approach for capture and sequencing of the ~15Mb *Schistosoma mansoni* exome that can be used for single larval miracidia. The approach uses whole genome amplification of miracidia preserved on FTA cards, solution-based capture of exome sequences using 120bp RNA baits, and Illumina sequencing, and can be extensively multiplexed to reduce costs and simplify sequence library preparation. To demonstrate the utility of these methods we sequenced exomes from 45 single miracidia collected from a Brazilian location in three lanes of an Illumina HiSeq. We captured >99% of the exome sequences targeted,

obtained between 30-80x read depth per miracidia, and robustly called >70,000 SNPs. The method also efficiently captures exomes from the related parasite, S. rodhaini, providing outgroup sequences and opening up the possibility of detailed dissection of interspecific hybridization within schistosome populations. We are currently using these methods to characterize African *S. mansoni* populations from the SCAN collection at the British Natural History Museum. The exome data will be used to characterize SNP variation at candidate vaccine and drug resistance loci, to examine geographic differentiation in allele frequencies, and to identify genome regions under strong directional and balancing selection. We believe that this approach will have multiple uses for schistosome epidemiology, population biology and evolutionary genomics.

1811

POPULATION AND COMPARATIVE GENOMICS OF AFRICAN *SCHISTOSOMA MANSONI*

Thomas Crellen[1], James Cotton[2], Sophia David[2], Caroline Durrant[2], Thomas Huckvale[2], Nancy Holroyd[2], Fiona Allen[3], David Rollinson[3], Joanne Webster[1], Matthew Berriman[2]

[1]Imperial College, London, United Kingdom, [2]Wellcome Trust Sanger Institute, Cambridge, United Kingdom, [3]Natural History Museum, London, United Kingdom

Schistosomiasis is among the most important parasitic diseases, with over 200 million people infected and 300,000 deaths annually across Africa, Asia, South America and the Caribbean caused mainly by three closely related species. Around 90% of cases are in sub-Saharan Africa, where *Schistosoma mansoni* is one of the two most clinically important species, and the principal cause of intestinal schistosomiasis. A draft reference genome is available for *S. mansoni* and is being actively curated and improved, based on an isolate from Puerto Rico that has been maintained in research labs for many years. Here, we present genome sequence data and assemblies from seven adult male *S. mansoni* that were recently collected from the field with minimal lab passage, including six diverse African isolates - the first genomic data from the region of greatest public health interest. We confirm that the *S. mansoni* reference sequence is a suitable substrate for genomic analysis of African populations. We use this genomic diversity data to investigate signatures of natural selection on the *S. mansoni* genome, and apply two coalescent-based models to infer the population history of *S. mansoni* on two continents. Our results show that the New World strains have smaller past effective population sizes (Ne) than African strains, suggesting the possible occurrence of a past population bottleneck. We estimate the divergence time between the African and New World populations, finding support for the hypothesis that *S. mansoni* colonised the New World via the 16-19th century West African slave trade. In the light of this potential population bottleneck, we investigate systematic differences between South American populations and African populations in both genome structure (copy number variants) and single nucleotide polymorphisms (SNPs).

1812

USING MICROSATELLITE MARKERS TO DETERMINE *SCHISTOSOMA MANSONI* GENETIC DIVERSITY UNDER CONTRASTING CHEMOTHERAPY CONTROL STRATEGIES IN LAKE VICTORIA, TANZANIA

Anouk N. Gouvras[1], Bonnie L. Webster[2], Fiona Allan[1], Muriel Rabone[1], Safari Kinunghi[3], Aidan Emery[1], Joanne P. Webster[2], David Rollinson[1]

[1]Natural History Museum, London, United Kingdom, [2]Imperial College, School of Public Health, London, United Kingdom, [3]National Institute for Medical Research, Mwanza, United Republic of Tanzania

National schistosomiasis control programs rely on mass drug administration using the drug praziquantel (PZQ). The widespread use of a single drug raises concerns of drug tolerance spreading in the parasite population. In the absence of specific markers for PZQ tolerance, neutral genetic markers such as microsatellite loci, and analysis of population genetics can be used to monitor changes in parasite populations and the effect of PZQ-reliant schistosomiasis control on the parasite's adaptation and evolution. In the Lake Victoria region of Tanzania (Mwanza), as part of a larger treatment study on intestinal schistosomiasis caused by *Schistosoma mansoni*, we are collecting parasite genetic material from school children from 16 villages with contrasting PZQ treatment pressures: annual Community-Wide Treatment (CWT-highest treatment pressure) and biennial School-Based Treatment (SBT-lowest treatment pressure). The infection prevalence at the start of the study was over 25%. In each village larval miracidia samples were collected prior to treatment from infected school children (n=30). For baseline (2012) 18,649 *S. mansoni* miracidia were collected from 263 children in 16 villages. In Year 2 (2013), 4,724 *S. mansoni* miracidia were collected from 95 children in the 8 annual CWT villages. Future follow-up collections are planned for May 2014 (16 villages). Samples were collected on to FTA cards and stored in SCAN (http://scan.myspecies.info/). Samples are being analysed using a new set of multiplex panels developed from 20 previously published microsatellites for *S. mansoni*. Comparison of genetic diversity indices such as number of alleles per locus, allelic richness, observed and expected heterozygosity will be made between baseline and post treatment samples and between annual CWT and biennial SBT control intervention strategies. Initial analysis of baseline-collected material shows little difference in the genetic diversity indices between the two different treatment arms. Year 2 miracidia samples are currently being analysed to determine if there has been a change in genetic diversity compared to baseline in the 8 CWT villages. This study utilizes field-collected material and microsatellite multiplex panels to determine the differential impact of annual CWT vs. biennial SBT chemotherapy strategies on parasite clearance and population genetic outcomes.

1813

REPEATED TREATMENTS ARE REQUIRED TO AFFECT SCHISTOSOME POPULATION STRUCTURE

Lucio M. Barbosa[1], Luciano K. Silva[1], Eliana A. Reis[1], Theomira M. Carmo[1], Claudio R. dos Santos[1], Jackson M. Costa[1], Peace T. Aminu[2], Thassila N. Pitanga[1], Walter A. Blank[2], Mitermayer G. Reis[1], Ronald E. Blanton[2]

[1]Oswaldo Cruz Foundation, Salvador, Brazil, [2]Case Western Reserve University, Cleveland, OH, United States

Repeated rounds of praziquantel treatment are able to reduce prevalence and morbidity of schistosomiasis, but parasite populations recover within a few years. To understand the dynamics of this recovery, two rural Brazilian communities were surveyed and treated in 3 different years: 2009, 2012 and 2013. On average, 96% of the residents participated in each year, and those who were positive on at least one of 3 stools were treated. *Schistosoma mansoni* eggs were collected from stool and genotyped using 11 microsatellite markers. Parasite differentiation was evaluated at the level of infrapopulations and component populations. Component populations were defined by host characteristics, village of residence or year of study. During this time, human population demographics changed little. New arrivals were 16% and 5% of the populations in 2012 and 2013, respectively. In both years ~12% moved elsewhere. After 2 rounds of treatment, prevalence decreased by 64% and intensity by 57%. Children 15-20 years old showed the greatest decline, while adults between 51-60 showed the least. Reinfection was 34% in 2012 and 18% in 2013, while incidence was 22% and 15%, respectively. The decreasing rates suggest that these treatments have an effect on transmission. Individual infrapopulations were moderately differentiated (D=0.055-0.077) on reinfection, indicating the pre-treatment multilocus genotypes were not fully reacquired. Differentiation between the 2 villages decreased from 0.046 to 0.031, consistent with an increase in gene flow between them. Parasites from new immigrants were little differentiated from natives (D = 0.012). Between consecutive years, there was little differentiation (D = 0.008), but comparing 2009 to 2013, differentiation increased notably (D = 0.014). Population structure began to change only after 2 rounds of treatment when total parasite burden decreased by >10 fold. This seems

to be the tipping point for producing a genetic bottleneck or reducing effective population size. Intensive therapy is required to significantly impact the parasite's genetic potential.

1814

EPIGENETIC CONTROL OF ENDOGENOUS AND EXOGENOUS (RETRO)TRANSPOSABLE ELEMENTS IN *SCHISTOSOMA MANSONI*

Hong-Bin Yan, Paul J. Brindley, **Gabriel Rinaldi**

George Washington University, Washington, DC, United States

The epigenetic landscape includes modifications on the chromatin template that establish and propagate patterns of gene expression and silencing, not based on differences in the DNA sequence. Four major epigenetic phenomena are involved: 1) DNA methylation; 2) histone modifications; 3) nuclear gene repositioning; and 4) regulatory non-coding RNAs. Whereas early reports suggested absence of DNA methylation of the schistosome genome, it has now been shown that cytosine methylation regulates schistosome oviposition and embryo development. In addition, post-translational core histone modifications in epigenetic control of transcription of schistosome genes have been described, as have a set of non-coding RNAs -- integral components of the epigenetic machinery, including epi-miRNAs, i.e. miRNAs that regulate expression of enzymes involved in chromatin remodeling and DNA methylation. To evaluate the effect of the DNA methyltransferase inhibitor 5′-azacytidine (5′-AzaC) on the expression of both long terminal repeat (LTR) and non-LTR retrotransposons, schistosomules of *Schistosoma mansoni* were cultured in 100 µM and 500 µM of 5′-AzaC. The parasites were harvested 2 or 7 days after treatment, RNA was isolated and the expression of the *Boudicca* (LTR-retrotransposon) and *SR2* (non-LTR-retrotransposon) multi-copy endogenous mobile genetic elements of schistosomes analyzed by qRT-PCR. The expression level of these retrotransposons was upregulated 10 to 20 times in the presence of 5′-AzaC. In addition, expression of reporter transgenes increased in schistosomules that had been transformed with virions of pseudotyped murine leukemia virus following culture in media supplemented with 5′-AzaC. These findings demonstrated that endogenous mobile elements, which comprise ~45% of the genome of this schistosome are controlled by epigenetic marks. In addition, they indicated a central influence of methylation status on transgene activity, suggesting one avenue forward for enhancing transgenesis of this tropical neglected tropical disease pathogen.

1815

SCHISTOSOMA MANSONI: ROLE OF BIOGENIC AMINES IN NEURONAL CONTROL OF MOTOR FUNCTION

Nelly El-Sakkary, Paula Ribeiro

McGill University, Ste. Anne-de-Bellevue, QC, Canada

Schistosoma mansoni is the main causative agent of schistosomiasis, a disease which infects over 200 million people worldwide. Treatment of the disease is primarily with praziquantel. With the lack of an available alternative, and the widespread use of the drug, there is a fear of the development of resistance. Biogenic amines (BAs) are the largest family of classical neurotransmitters in the schistosome nervous system. They are typically involved in motor control and are important to host infection and worm survival. The goal of this study is to determine the role of BA neurotransmitters in schistosomes, focusing on tyrosine derivatives, which include phenolamines and catecholamines. Using confocal immunolocalization, receptors responsive to the catecholamine, dopamine (DA), SmGPR3 and SmD2, were shown to localize to the main nerve cords of the central nervous system (CNS), and the peripheral nervous system (PNS), respectively. Both receptors localized to neurons innervating worm musculature, indicating a possible role in motility for the receptors. Octopamine (OA), a phenolamine, was also immunolabeled in the adult parasite, and showed widespread labeling in the main neurons of the worm CNS, the first indication that OA is present in schistosomes. In other studies we tested the role of several BAs on schistosomes motility. Treatment with both OA and DA caused marked changes in worm motility as compared to the control. Next, we performed RNAi targeting proteins predicted to be involved in DA and OA signaling in larvae and adult schistosomes, and effects of downregulation were assessed. Several of the RNAi-targeted animals showed strong changes in frequency of body movements and in worm morphology as compared to the control. Together these studies highlight the importance of tyrosine derived BAs in the control of motor activity in schistosomes.

1816

MICRORNAS IN THE EXCYSTATION OF *FASCIOLA HEPATICA* METACERCARIAE

Jose F. Tort[1], Santiago Fontenla[1], Nicolas Delloca[1], Pablo Smircich[1], Maria del Mar Siles-Lucas[2]

[1]Universidad de la Republica, Montevideo, Uruguay, [2]Instituto de Recursos Naturales y Agrobiología, Salamanca, Spain

Host invasion by the trematode parasite *Fasciola hepatica* is initiated by the activation of the metacercariae usually in the host stomach. The released juvenile forms actively transverse the gut wall towards the abdominal cavity and follow their journey to the biliary ducts of the liver. The activation is a rapid switch finely tuned by signals from the environment, that can be easily reproduced *in vitro*. miRNAs have emerged as relevant modulators of gene expression at the post-transcriptional level (either by tranlation blocking or mRNA degradation), playing essential roles in development. We aimed to study the miRNA expressed in the liver fluke metacercarial activation, and for that purpose we purified and sequenced the small RNA populations expressed at this developmental transition. After filtering the reads with homology to mRNA, repetitive sequences and other non coding RNAs, we ended up with several thousand reads that were compared to miRBase, Rfam and all the miRNA previoulsy identified in other flatworms. Within the known miRNAs found, those common to all metazoans and protostomes were the most abundant. Some miRNA so far only detected in other flatworms were also found, highlighting the existance of flatworm specific miRNA families. Furthermore within sequences with no homology, novel *F. hepatica*-specific miRNAs were predicted. We observed subtle differences between dormant and activated metacercariae and further differences to newly excysted juveniles. While sequence conservation in mature miRNA is high across the metazoan tree, we observed that in general flatworm miRNA are more divergent than in other lineages, with strict conservation restricted to seed region. Whether this variability leads to changes in the regulated target genes associated to the parasitic way of life deserves further investigation.

1817

IDENTIFICATION OF SYMBIONTS IN THE REPRODUCTIVE TRACT MICROBIOME OF NATURAL *ANOPHELES GAMBIAE* POPULATIONS AND IMPLICATIONS FOR NOVEL VECTOR CONTROL METHODS

Francesco Baldini[1], Nicola Segata[2], Julien Pompon[3], Perrine Marcenac[4], W. Robert Shaw[5], Roch K. Dabiré[6], Abdoulaye Diabaté[6], Elena A. Levashina[7], Flaminia Catteruccia[4]

[1]University of Glasgow, Glasgow, United Kingdom, [2]University of Trento, Trento, Italy, [3]University of Strasbourg, Strasbourg, France, [4]Harvard School of Public Health, Boston, MA, United States, [5]Imperial College London, London, United Kingdom, [6]Institut de Recherche en Sciences de la Santé/ Centre Muraz, Bobo-Dioulasso, Burkina Faso, [7]Max-Planck Institute for Infection Biology, Berlin, Germany

Identification of symbionts in the reproductive tract microbiome of natural *Anopheles gambiae* populations and implications for novel vector control methods Recent findings show that mosquito-microbiota interactions are crucial determinants of mosquito fitness and vectorial capacity opening novel and promising areas of research in the utilization of symbiotic bacteria for the control of vector-borne diseases. Furthermore, recent

works propose the use of paratransgenesis as a novel vector control tool, which exploits genetically engineered symbionts to deliver anti-parasite molecules in the vector. In order to identify potential determinants of mosquito biology as well as putative candidates for paratransgenesis, we have characterized the reproductive microbiome of two major African malaria vectors *Anopheles gambiae* and *An. coluzzii* from field population in Burkina Faso. Specifically, we performed high throughput sequencing of the bacterial 16S gene in the male and female reproductive tracts of mosquitoes. We identified two bacteria genera that are present in all the analysed specimens, representing the core taxa of mosquito reproductive organs with possible symbiotic interactions with the vector. Nevertheless, although a general core microbiome was identified, we observed a general high diversity among different specimens that might indicate that the reproductive microbiome is highly dynamic and might be influenced by external factors. Indeed, we identified some taxa whose abundance was significantly associated with the environment where the mosquitoes lived. Finally, we identified intracellular bacteria that were previously believed not to colonize natural populations of *Anopheles*. Remarkably, these bacteria are capable of spreading into insect populations and negatively impact mosquito vectorial capacity by reducing their lifespan and boosting the immune response against parasites. To our knowledge this was the first identification of these intracellular bacteria in malaria mosquitoes, which opens new promising opportunities to exploit these organisms as vector control agents against malaria vectors. These results started to elucidate the composition of malaria mosquito reproductive tract microbiome offering novel opportunities to exploit symbiotic bacteria in the fight against malaria.

1818

PERIOSTIAL HEMOCYTE AGGREGATION IN *ANOPHELES GAMBIAE* OCCURS FOLLOWING DIVERSE IMMUNE STIMULI AND IS ACCOMPANIED BY CHANGES IN MOSQUITO HEART PHYSIOLOGY

Julian F. Hillyer, Leah T. Sigle, Tania Y. Estevez-Lao

Vanderbilt University, Nashville, TN, United States

Malaria parasites and other mosquito-borne pathogens must traverse the insect hemocoel prior to being transmitted. This process is affected by hemolymph circulation, as flow influences both pathogen movement and the movement of mosquito-produced immune factors. Using the African malaria mosquito, *Anopheles gambiae*, as our study system, we recently identified a novel immune tissue, called periostial hemocytes, that exemplifies the co-adaptation of the insect immune and circulatory systems. Specifically, in response to infection, circulating hemocytes (immune cells) migrate to the valves of the mosquito heart, where they sequester and kill pathogens. We have previously reported the aggregation of hemocytes on the surface of the heart following *Plasmodium* and *Escherichia coli* infection, however, little is known about the breadth of this immune response or about how heart physiology changes following infection. In the present study we tested whether periostial hemocyte aggregation occurs following diverse immune stimuli, whether this response is uniform across the length of the heart, and whether infection affects mosquito heart physiology. We found that periostial hemocyte aggregation occurs following all types of infections tested, confirming the fundamental role of this immune response. Moreover, periostial hemocyte aggregation is not uniform along the length of the heart, as larger hemocyte aggregates consistently form in abdominal segments 4, 5, and 6. Finally, periostial hemocyte aggregation is accompanied by a decrease in heart contraction rates. In summary, these data further describe a recently discovered immune tissue in mosquitoes, and demonstrate how the immune and circulatory systems have co-adapted to fight infection.

1819

BREAKING THE LAW OF EFFECTIVE TEMPERATURE: ECOLOGICAL CONTEXT MATTERS FOR DEVELOPMENT RATE VARIATION MOSQUITO VECTORS

Jannelle Couret[1], Ellen Dotson[2], Mark Q. Benedict[3]

[1]Emory University, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]University of Perugia, Perugia, Italy

The resurgence and spread of arboviruses in recent years underscores the continued need to understand the biology of its mosquito vectors including, *Aedes aegypti* and *Culex pipiens* complex. Despite advances in the development of vaccines, control of the mosquito vector populations remains the most effective control measure. In order to understand how the environment impacts disease transmission, a thorough understanding of the impact of environmental variables on mosquito biology is needed. Historically, the emphasis has been on temperature and the linear association with insect development rate. This association is widely observed across ectotherms, and is often referred to as the law of effective temperature. We tested the hypothesis that the law of effective temperature is contingent on the ecological context of the larval environment in mosquito development. Through a combination of statistical modeling of published rearing experimental research and a larval rearing experiment under gradients of conditions in environmental chambers, we find that intraspecific density and dietary resources mediate the importance of temperature in explaining variation of mosquito development rate. Our results support the hypothesis of environmentally contingent impacts of temperature on mosquito development. These findings have broad implications for the modeling of mosquito population dynamics, climate change and vectorborne disease transmission, and our understanding of nature of mosquito life history evolution.

1820

PARASITE CO-INFECTION AND STRAIN DIFFERENCES AS DRIVERS OF PATHOGENIC VARIATION IN THE CHAGAS DISEASE PARASITE *TRYPANOSOMA CRUZI* WHEN INFECTING ITS INSECT VECTOR, *RHODNIUS PROLIXUS*

Jennifer K. Peterson[1], Andrea L. Graham[1], Andrew P. Dobson[1], Omar Triana[2]

[1]Princeton University, Princeton, NJ, United States, [2]University of Antioquia, Medellín, Colombia

Avoiding the over-exploitation of resources in a food patch while still obtaining all the resources needed to live and reproduce is a common challenge among all creatures. For many parasites that live inside another organism this is especially tricky because they do not have the option of moving to a new patch should they be too pathogenic and overexploit their current one. Classical theory suggests that the avoidance of host death should favor intermediate levels of parasite pathogenicity (i.e., negative effects) to the host, however that this is not the case: parasites actually exhibit a wide range of pathogenic effects on their hosts. We investigated parasite co-infection and strain differences as drivers of pathogenic variation in the Chagas disease parasite *Trypanosoma cruzi* and its sister species, *Trypanosoma rangeli* when infecting their insect vector, *Rhodnius prolixus.* Using insect survival, reproduction, and parasite load (qPCR amplification of parasite DNA extracted from each insect) as proxies for parasite pathogenicity, we found that *T. cruzi-T. rangeli* co-infection significantly reduces the survival of *R. prolixus* up to 30 days post-infection, but increases reproduction. We also found that *T. cruzi* pathogenicity in *R. prolixus* is highly variable, with *R. prolixus* death at 90 days ranged from 5-80% depending on *T. cruzi* strain, and at times was far more pathogenic than *T. rangeli*, a parasite believed to be highly pathogenic to triatomines. Furthermore, insects with higher parasite loads tended to have higher fecundity, presenting evidence for terminal investment in infected bugs.

Our results suggest that the pathogenic variation often found in *T. cruzi* infection of vertebrate hosts extends to its invertebrate hosts as well, with strain variability and co-infection with *T. rangeli* as two of the main drivers.

1821

THE EFFECTS OF FORCED-EGG RETENTION ON THE BLOOD-FEEDING BEHAVIOR AND REPRODUCTIVE POTENTIAL OF *CULEX PIPIENS* (DIPTERA: CULICIDAE)

Brian J. Johnson[1], Dina M. Fonseca[2]

[1]Graduate Program in Ecology and Evolution, Rutgers University, New Brunswick, NJ, United States, [2]Center for Vector Biology, Department of Entomology, Rutgers University, New Brunswick, NJ, United States

High rates of West Nile virus (WNV) transmission to humans are associated with exceptionally hot and dry summers. This is paradoxical since the eggs of *Culex* vectors of WNV depend on the persistence of containers with water, which decline during droughts. We examined the effects of forced-egg retention on the reproductive success of female *Culex pipiens* as well as behavioral responses, such as likelihood of secondary blood meals. As controls we examined the effects of female age and delayed mating. We found that early mating is essential to achieve reproductive success and, consistent with an "all-or-none" ovipositing strategy, *Cx. pipiens* females are able to retain considerable reproductive potential while searching for oviposition sites. Specifically, although forced-egg retention resulted in significant decreases in fitness, the decline was moderate for 5 weeks and most can be accounted for by increases in female age. Consequently, no females took blood more than once per gonotrophic cycle, which eliminates the possibility that heightened vectorial capacity due to multiple blood-feedings increases WNV transmission during periods of drought. Instead, our findings suggest that during droughts populations of *Cx. pipiens* have time to locate the remaining water holes, which are associated with human populations and WNV-competent bird species.

1822

BEHAVIORAL CHANGE IN *ANOPHELES FUNESTUS*: AN OBSTACLE TO MALARIA ELIMINATION

Sougoufara Seynabou[1], Diedhiou Mocote Seynabou[1], Doucoure Souleymane[1], Trape Jean François[1], Sokhna Cheikh[1], Sembene Mbacke[2], Harry Myriam[3], Ndiath Mamadou Ousmane[4]

[1]IRD, Dakar, Senegal, [2]University, Dakar, Senegal, [3]LEGS-CNRS, Paris, France, [4]Institute Pasteur, Bangui, Central African Republic

In the long road that leads towards malaria elimination, vector control was undoubtedly an essential component of success. Two major strategies have marked the vector control: the use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). However, the effectiveness of these tools is being challenged by the emergence of insecticide resistance and behavioural resistance which thwarting the goal of decreasing malaria transmission. In this study, we focus on behavioural changes of malaria vectors that can hamper the efficacy of vector control interventions in Dielmo, a Senegalese rural village where a longitudinal study of malaria has been conducted. In this village, universal coverage with LLINs was done in July 2008, and in July 2011 all these LLINs were renewed. Adult mosquitoes were collected by human landing catches (HLC) from July 2011 to April 2013 and hourly from 19:00 and 07:00. Collecting mosquitoes was also done by pyrethrum spray catch (PSC) during this period. From January to April 2013, mosquito catches were continued until 11:00 and the entomological different parameters were investigated. This study shows that *Anopheles funestus* which have disappeared after first introduction of LLINs (July 2008) comes back in malaria transmission in Dielmo. *An. funestus* remains anthropophilic and endophilic but adopt a behavioural change in biting activity after introduction of LLINs. The human biting rate of mosquitoes collected from 07:00 to 11:00 was eight times higher than the one from 19:00 to 07:00. So the alarming phenomenon is the positive mosquitoes found in the day capture (mean CSP rate of 1.28%) while since distribution of LLINs in this village, no *An. funestus* has been found positive to CSP. These disturbing observations show the capacity of *Anopheles* to adapt and circumvent strategies aimed at reducing malaria transmission. In an arms race between malaria control programs and the vector populations, the behaviour change in *Anopheles* threatens to thwart the goal of decreasing malaria transmission.

1823

INFECTION OF LABORATORY-COLONIZED *ANOPHELES DARLINGI* MOSQUITOES BY *PLASMODIUM VIVAX* AND TEMPORAL CHANGE IN GENETIC VARIATION IN *AN. DARLINGI*

Marta Moreno Leirana[1], Carlos Tong[2], William Lainhart[3], Mitchel Guzman[2], Raul Chuquiyauri[2], Alejandro Llanos-Cuentas[4], Hugo Rodriguez[5], Dionicia Gamboa[4], Jan E. Conn[6], Joseph M. Vinetz[1], Joseph M. Vinetz[2], Joseph M. Vinetz[4]

[1]University of California San Diego, La Jolla, CA, United States, [2]Laboratorio ICEMR-Amazonia, Laboratorios de Investigacion y Desarrollo, Facultad de Ciencias y Filosofia, Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Department of Biomedical Sciences, School of Public Health, State University of New York-Albany, Albany, NY, United States, [4]Instituto de Medicina Tropical "Alexander von Humboldt", Universidad Peruana Cayetano Heredia, Lima, Peru, [5]Loreto Regional Directorate of Health, Iquitos, Peru, [6]Wadsworth Center, New York State Department of Health, Albany, NY, United States

Anopheles darlingi Root is the most important malaria vector in the Amazonia region of South America. However, continuous propagation of *An. darlingi* in the laboratory has been elusive, limiting entomological, genetic/genomic, and vector–pathogen interaction studies of this mosquito species. We report the establishment and maintenance of an *An. darlingi* colony (since July 2013) derived from wild-caught mosquitoes obtained in the northeastern Peruvian Amazon region of Iquitos in Loreto Department. We demonstrate that the numbers of eggs, larvae, pupae, and adults continue to rise at least to the F9 generation. In addition, comparison of feeding *Plasmodium vivax* by artificial membrane feeding of F4-F9 to F1 generation mosquitoes showed the comparable presence of oocysts and sporozoites, with numbers that corresponded to blood-stage asexual parasitemia and gametocytemia, confirming *P. vivax* susceptibility in the colonized mosquitoes. Additionally, analyses of fourteen microsatellites markers were performed on a subsample of *An. darlingi* offspring to detect genetic variation and expected reduction in heterogeneity through generations. These results provide new avenues for research on *An. darlingi* biology and study of mosquito–*Plasmodium* interactions and malaria transmission in the Neotropics, including new genomic analysis and assessment of transmission biology of malaria parasites.

1824

RATIONAL DESIGN OF OXAMNIQUINE DERIVATIVES THAT KILL SCHISTOSOMES

Stacey Stahl[1], Alexander B. Taylor[1], Xiaohang Cao[1], Stanton McHardy[2], P. John Hart[1], Timothy J. Anderson[3], **Philip T. LoVerde**[1]

[1]University of Texas Health Science Center, San Antonio, TX, United States, [2]University of Texas at San Antonio, San Antonio, TX, United States, [3]Texas Biomedical Research Institute, San Antonio, TX, United States

Schistosomiasis, a major cause of morbidity, infects >200 million people worldwide. Schistosomiasis control is based on a monotherapy consisting of repeated doses of praziquantel (PZQ). Drug resistance is a concern, especially as it is expected to increase in treatment coverage in sub-Sahara Africa (250 million doses per year for each of the next 5 years). New anti-schistosomal drugs are needed to reduce reliance on a single drug. A new drug could be used in combination with PZQ to minimize the probability of resistance arising to either drug. The goal of this research is to modify an existing anti-schistosomal drug oxamniquine (OXA) to make it more efficacious. We recently identified the gene encoding the Sm-sulfotransferase (SmSULT) responsible for drug activation and determined

the structure of the SmSULT•cofactor•OXA ternary complex at 1.75 Å resolution. These analyses provide detailed information of the mechanism of action of OXA against Sm, while structural analyses of drug•protein interactions direct redesign of OXA. We designed and synthesized 12 OXA derivatives based on four key design aspects; 1) the structural requirements of OXA and its derivatives based on available space in the substrate binding cavity in SmSULT and the key residue interactions from crystallographic studies, 2) the required ortho-electron withdrawing moiety necessary for the sulfonation process, 3) the design of analogs that fall within favorable "drug-like" physical chemical property ranges and 4) the development of efficient and convergent syntheses, allowing for the greatest amount of structural diversity and chemical space to establish structure-activity relationships (SAR). *In vitro* worm killing assays indicated that three of these analogs were as good as or better than OXA itself. These new compounds with antischistosomal activity have been soaked into the SmSULT•PAP crystals and their mode of binding elucidated. This information will be used to synthesize the next generation of OXA derivatives.

1825

ROLES OF ATP BINDING CASSETTE (ABC) MULTIDRUG TRANSPORTERS IN SCHISTOSOME PHYSIOLOGY, DRUG SUSCEPTIBILITY AND PARASITE-HOST INTERACTIONS

Ravi S. Kasinathan, Yue Ning, **Robert M. Greenberg**

University of Pennsylvania, Philadelphia, PA, United States

Although its value in the treatment and control of schistosomiasis is well established, praziquantel (PZQ) has significant limitations. Most notably, it is largely ineffective against immature schistosomes. It is also essentially the only drug available for a disease afflicting hundreds of millions. New therapeutics or adjuncts to enhance PZQ activity and overcome possible drug resistance are urgently needed. We hypothesize that ATP binding cassette (ABC) multidrug transporters offer attractive candidate targets for new or repurposed drugs that either act as anthelmintics on their own, or that enhance parasite susceptibility to existing anthelmintics. ABC transporters such as P-glycoprotein (Pgp) mediate efflux of metabolic toxins, xenobiotics, and signaling molecules, and are associated with drug resistance in many organisms, including parasitic helminths. They exhibit broad substrate specificity and are inhibited by several drugs currently in clinical use. ABC transporters are also implicated in a variety of normal physiological activities such as excretion, maintenance of permeability barrier function, and modulation of immune responses. They transport many potent signaling molecules with high affinity, including several with immunomodulatory activity. Schistosomes exposed to PZQ increase expression of ABC transporters such as Pgp (SMDR2) and multidrug resistance associated protein (SmMRP1), and worms with reduced PZQ sensitivity show higher basal expression of these transporters. PZQ is also both an inhibitor and likely substrate of schistosome Pgp. Disruption of transporter expression (by RNAi) or function (by inhibition) enhances the activity of PZQ against adult parasites, and renders PZQ-refractory juvenile worms susceptible to the drug. Schistosome ABC transporters also appear to be important for normal schistosome egg production. We are currently exploiting molecular and pharmacological tools to understand the mechanism by which schistosome ABC transporters alter PZQ susceptibility and to assess the role of these transporters in the parasite's modulation of host immune responses. These experiments could lend important insights into schistosome physiology and possibly provide targets for novel antischistosomals.

1826

SHORT-TERM ANTIBIOTIC TREATMENT INTERRUPTS THE EXCHANGE OF POLYMORPHIC VESICLES BETWEEN *WOLBACHIA* AND THEIR FILARIAL HOST

Kerstin Fischer, Peter U. Fischer

Washington University School of Medicine, St. Louis, MO, United States

Wolbachia endosymbionts are crucial for growth, reproduction, and survival of many medically important filarial parasites. *Wolbachia* have a tightly regulated lifecycle within filarial nematodes. While *Wolbachia* density is low in microfilariae and vector stage larvae, much higher numbers of the endobacteria are observed in developing larvae and young adult worms recovered from mammalian hosts. We have recently reported that *Wolbachia* release polymorphic outer membrane vesicles (OMV) that may be essential for their mutualistic relationship with filarial worms. OMV may transport bacterial products that are required for parasite growth and development. Tetracyclines (TET, antibiotics that inhibit bacterial protein synthesis) clear *Wolbachia* from filarial worms over a period of weeks. This treatment first sterilizes the parasites and eventually kills them. The present study was performed to elucidate the early effects of TET treatment on the morphology of *Wolbachia* and filarial worms. Gerbils with i.p. *Brugia malayi* infections were treated with TET on days 19 and 20 post-infection (i.p. injection, 5 mg/kg). Immature female worms recovered on day 21 were studied by transmission electron microscopy using high pressure freezing/freeze substitution fixation. OMV were largely absent near *Wolbachia* in TET-treated worms, while about half of the *Wolbachia* in untreated control worms were associated with OMV. *Wolbachia* in treated worms were often surrounded by membranes that appeared to come from the endoplasmic reticulum. Lateral chords in treated worms were heavily vacuolated with increased glycogen granules compared to untreated worms. Thus TET treatment appears to block OMV production by *Wolbachia* and promote encapsulation of the bacteria by internal host cell membranes.

1827

POPULATION GENOMICS OF *WUCHERERIA BANCROFTI* ELIMINATION FROM PAPUA NEW GUINEA

Scott T. Small[1], David Serre[2], James F. Hester[2], Lisa J. Reimer[3], Daniel J. Tisch[1], Peter A. Zimmerman[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Cleveland Clinic, Cleveland, OH, United States, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Lymphatic filariasis (LF) is a major threat to human health in the tropics, leading to the disfiguring and debilitating conditions hydroceles and elephantiasis. There are approximately 1.2 billion people living in LF endemic countries where 120 million people currently suffer from the disease. The parasites that cause LF are separated into two genera, *Brugia* and *Wuchereria*, with *W. bancrofti* (Wb) responsible for ~90% of LF cases. Until recently, Wb has been neglected in genomic studies due to complications in sample collection and preparation, such as lack of adult stage samples. Here, we report 13 new Wb genomes from the Dreikikir region of Papua New Guinea (PNG). We utilized multiple displacement amplification to amplify and sequence 13 juvenile stage (L3) Wb worms from four patient infections. We report the discovery of 60,000 novel single nucleotide polymorphism (SNPs) and 200 polymorphic microsatellite loci from the genomes. Within patient infections we find that genetic diversity is high, yet concentrated in specific regions of the genome, with large tracts of intervening homozygous sequence. We also identify candidate regions that harbor genes with extended haplotypes and shifted frequency spectrums, signals of either ongoing or recent positive selection. We discuss our results in the context of the recent mass drug administration, and identify SNP loci ideal for future monitoring of elimination success.

1828

NOVEL TEGUMENT EXPRESSED KUNITZ TYPE PROTEASE INHIBITOR FROM *SCHISTOSOMA MANSONI*

Shiwanthi L. Ranasinghe, Katja Fischer, Geoffrey Gobert, Donald P. McManus

QIMR Berghofer Medical Research Institute, Herston, Australia

Schistosomiasis is one of the most prevalent and serious parasitic diseases of tropical and subtropical regions with 779 million people being at risk and 207 million infected. Kunitz type proteins belong to the I2 family of protease inhibitors and are involved in diverse biological processes in invertebrates. One secretory type sequence (SmKI) having similarity to Kunitz type protease inhibitors was identified from recent mining of the genome of *Schistosoma mansoni*. Recombinant SmKI was expressed in *E. coli,* purified and an antiserum against rSmKI was produced in mice and subsequent immunolocalization and western blotting carried out. Gene expression levels were determined within key lifecycle stages of *S. mansoni* using real-time PCR. Serine protease inhibitory assays were also used to determine the inhibitory effect of the rSmKI on trypsin, chymotrypsin, neutrophil elastase (NE), pancreatic elastase and Cathepsin G. Real time PCR indicated SmKI is highly expressed in adult worms which reside in the mesenteric venules of the definitive host. Immunolocalization showed the Kunitz protein is present in the tubercles of the male tegument and along the tegument of the female worm. Notably, western blots showed the level of SmKI was higher in the excretory secretory products of adult worm pairs than in soluble worm antigens. Further, rSmKI inhibited trypsin, chymotrypsin and NE, with the highest inhibitory activity recorded against trypsin. Initial screens indicated that rSmKI interfere with both intrinsic and extrinsic blood coagulation pathways as well, indicating another important function. Thus, the SmKI protein may play an important role in schistosome survival in blood by inhibiting NE as well as playing a key role in evading host immune responses. As SmKI is secreted and exposed to the host immune system, we consider that rSmKI may be a useful candidate as novel vaccine target to control schistosomiasis. Assays are underway to further understand the function of SmKI and vaccine/challenge experiments will be undertaken to evaluate its protective efficacy.

1829

PLASMODIUM VIVAX LIVER STAGE DEVELOPMENT AND HYPNOZOITE FORMATION IN THE FRG HUHEP MOUSE MODEL

Sebastian A. Mikolajczak[1], Niwat Kangwanrangsan[2], Ashley M. Vaughan[1], Wanlapa Roobsoong[2], Rachaneeporn Jenwithisuk[2], Narathatai Yimamnuaychok[2], Matt Fishbauger[1], Viswanathan Lakshmanan[1], Alexis Kaushansky[1], Scott E. Lindner[3], John H. Adams[4], Jetsumon Sattabongkot[2], Stefan H. Kappe[1]

[1]Seattle BioMed, Seattle, WA, United States, [2]Mahidol University, Bangkok, Thailand, [3]Pennsylvania State University, University Park, PA, United States, [4]University of South Florida, Tampa, FL, United States

The ability of *Plasmodium vivax* to form dormant liver stages (hypnozoites) that can be activated weeks or months after initial infection to cause relapse of malaria is of crucial importance for the unprecedented epidemiological success of the parasite. Yet, little progress has been made to understand the unique biology of hypnozoite formation and activation. Due to the parasite's strong preference for nonhuman primate and human tissue, the availability of models to study *P. vivax* liver stages is extremely limited. Here we report that the FRG KO mouse model transplanted with human primary hepatocytes (huHep) efficiently supports the development of *P. vivax* liver stages as well as the formation of hypnozoites for Thai isolates of *P. vivax*. Using a series of *P. vivax* specific polyclonal and monoclonal antibodies, we were able to evaluate the liver stage progression and maturation in the infected liver. The ability of the exo-erythrocytic merozoites to establish blood stage infection upon transfusion with human reticulocytes is being currently evaluated. Furthermore, *P. vivax* infections in the FRG huHep mice carried beyond the time of the liver stage maturation showed that persistence and activation of *P. vivax* hypnozoites can be further investigated in the model to determine the biological basis for liver stage dormancy. Successful evaluation of the antimalarial drugs with known activities on *P. vivax* liver stage infection (Primaquine and Atovaquone) confirmed that the FRG huHep/*P. vivax* infection model could be used as an efficient platform for testing new antimalarial drugs *in vivo* in quest to accelerate the development of interventions for the radical cure of *P. vivax* infection.

1830

CHARACTERIZATION OF COENZYME A BIOSYNTHESIS PATHWAY REVEAL ESSENTIAL DISTINCTIVE FUNCTIONS DURING *PLASMODIUM* DEVELOPMENT IN BLOOD AND MOSQUITO

Robert J. Hart, Catherine Nation, Panpim Thongsripong, **Ahmed S. I. Aly**

Tulane University, New Orleans, LA, United States

Coenzyme A (CoA) is an essential universal cofactor and carrier of acyl groups for all prokaryotic and eukaryotic cells. In nearly all non-photosynthetic cells, CoA biosynthesis depends on the uptake and phosphorylation of vitamin B5 (pantothenic acid or pantothenate). Earlier Studies showed the importance of pantothenate acquisition and phosphorylation for *Plasmodium* survival within erythrocytes. Recently, pantothenate plasma membrane transporter (PAT) was functionally characterized in *Plasmodium falciparum*. PAT was shown to be refractory to deletion and was localized to the parasite plasma membrane. However, very little is known about the *in vivo* cellular functions of CoA biosynthesis pathway in malaria parasite life cycle stages. We have targeted all enzymes of this pathway for deletion in the mouse malaria model *P. yoelii.* We show that first enzymes of this pathway are dispensable for asexual and sexual blood stage (BS) development but they are essential for mosquito stages development and sporozoite production. However, the last enzymes of this pathway are essential for both BS and mosquito stages development. These results indicate that the first substrates and intermediate products of this pathway can be supplemented by alternative novel pathways inside the blood but not inside the mosquito midgut. Collectively, our data show that CoA de novo biosynthesis is essential for both BS and mosquito stages. This is the first *in vivo* functional characterization of CoA biosynthesis pathway in any protozoan parasite.

1831

A NOVEL RNA APTAMER SYSTEM FOR FUNCTIONAL GENETICS IN *PLASMODIUM FALCIPARUM*

Suresh Maddur Ganesan, Jacquin C. Niles

Massachusetts Institute of Technology, Cambridge, MA, United States

Malaria is a parasitic disease that is widespread in tropical and subtropical regions, and a major cause of human morbidity and mortality. The most severe form of malaria is caused by the parasite, *Plasmodium falciparum*. A limited set of antimalarial drugs is used to treat the disease, but drug resistance is an increasing problem. Hence, identification of novel anti-malarial drugs is a high priority. While our understanding of *Plasmodium* biology has increased in the post-genomic era, tools for doing functional genetics remain quite limited. This impedes progress in identifying key parasite genes and processes that can be prioritized for drug development efforts. To address this need, our laboratory previously developed a novel small molecule-regulated protein-RNA interaction (TetR-aptamer system) that facilitates robust and inducible regulation of target gene translation in eukaryotic organisms including *Plasmodium*. Here, we present the application of protein engineering approaches to integrate our synthetic control system with native *Plasmodium* translational regulatory mechanisms. In so doing, we achieve substantially increased regulatory dynamic ranges (up to 200-fold) compared to a 5-10 fold range of the original system. With a view to identifying new potential drug targets, we are using this system to study several parasite genes. We envision that

this enhancement in regulatory dynamic range will facilitate functional interrogation of larger numbers of parasite genes with greater confidence that associated biological outcomes can be readily identified.

1832

EXPLORING COMPLEX MALARIA INFECTIONS WITH SINGLE GENOME SEQUENCING

Shalini Nair[1], Standwell Nkhoma[2], Karla Gorena[3], Benjamin J. Daniel[3], Francois Nosten[4], Tim J. Anderson[1], **Ian H. Cheeseman**[1]

[1]Texas Biomedical Research Institute, San Antonio, TX, United States, [2]Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi, [3]University of Texas Health Science Center San Antonio, San Antonio, TX, United States, [4]Shoklo Malaria Research Unit, Mae Sot, Thailand

We have recently developed a single cell genomics platform capable of generating whole genome sequence from a individual parasitized red blood cells. This has been extensively validated and can generate SNP calls genome-wide with high (>99%) accuracy, even in species such as *P. vivax* for which long term culture is not possible. This method provides a powerful approach for empirically determining the composition of multiple genotype parasite infections, and provides information that is not accessible using standard illumina sequencing of parasite infections. We have applied this approach to complex malaria infections by sequencing 17 single genomes from multiple-clone infections of *Plasmodium falciparum* (n=1) or *P. vivax* (n=2). After stringent quality control we scored an average of 62,720 *P. vivax* SNPs and 61,080 *P. falciparum* SNPs from each single cell sequence allowing us to map within host divergence between single parasite genomes at exceptional resolution. We use this data to highlight how single cell sequencing can be used to reconstruct genome-wide drug resistance haplotypes from individual infections. Such "phasing" data is expected to be of critical importance for determining the outcome of drug treatment, but cannot currently be determined from bulk sequencing of infections. Second, we have examined the size of blocks of haplotype sharing between genomes within infections and compared these with population data from single clone infections. We observed that parasite genotypes within infections tend to be closely related. Application of single cell genomics and can reveal patterns of relatedness at a fine scale, both within and between malaria infections.

1833

USING GENOMICS TO TRACK PROGRESS TOWARDS MALARIA ELIMINATION

Rachel Daniels[1], Stephen F. Schaffner[2], Noah M. Daniels[3], Wesley Wong[4], Nicholas Baro[4], Hsiao-Han Chang[4], Daouda Ndiaye[5], Daniel E. Neafsey[2], Dyann F. Wirth[4], Sarah K. Volkman[6], Daniel L. Hartl[1]

[1]Harvard University, Cambridge, MA, United States, [2]Broad Institute, Cambridge, MA, United States, [3]Massachusetts Institute of Technology, Cambridge, MA, United States, [4]Harvard School of Public Health, Boston, MA, United States, [5]Cheikh Anta Diop University, Dakar, Senegal, [6]Simmons College, Boston, MA, United States

WHO recommendations for regions of low and moderate endemicity call for program re-orientation at milestones marked by changes in disease prevalence. However, genetic changes in parasite populations may occur before changes in prevalence are measurable. To identify these genomic changes, we examined *Plasmodium falciparum* samples collected from patients in Thiès, Senegal from 2006 to 2013. We genotyped 24 independent SNPs from across the parasite genome - the molecular barcode - to separate annual collections into monogenomic (single parasite genome) and polygenomic (multiple parasite genome) infections. We also performed whole genome sequencing on 190 parasites from monogenomic infections. Following increased control efforts beginning in 2008, we observed large changes in population allele frequencies each season, suggesting enhanced random genetic drift expected from a reduced effective population size. We developed tools to visualize parasite inter-relatedness by molecular barcode and sequencing and identified increasing levels of identity by descent in both. SNP genotyping of monogenomic samples showed clusters with identical molecular barcodes, including several collections where 25-30% of samples shared the same barcode. Whole genome sequence analysis revealed that approximately half of the independent isolates shared between 10 and 98% of their genomes with other sequenced samples, including one obviously hybrid parasite. To our knowledge, this is the first observation of increasing identity by descent in an African population. We show evidence of significant parasite population changes undetectable by standard epidemiological methods. Early identification of population genomic changes associated with changes in transmission offers refined criteria for milestones tracking progress towards elimination. The decreasing costs of genomic analysis make this a feasible option for surveillance of malaria control efforts.

1834

DENGUE VIRUS NONSTRUCTURAL PROTEIN 1 CONTRIBUTES TO VASCULAR LEAK *IN VITRO* AND *IN VIVO*, WHICH CAN BE BLOCKED BY ANTI-NS1 ANTIBODY

P. Robert Beatty, Sarah Killingbeck, Henry Puerta Guardo, Eva Harris

Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States

Dengue virus (DENV) is a mosquito-borne flavivirus consisting of 4 serotypes that causes ~100 million cases of dengue annually. DENV nonstructural protein 1 (NS1) is secreted by infected cells and is found at high levels in patient serum during acute infection. We examined the protective efficacy of NS1 immunization against lethal DENV2 infection in a mouse model of vascular leak. Interferon α/β receptor-deficient C57BL/6 (*Ifnar*$^{-/-}$) mice were injected intraperitoneally 3X over 6 weeks with 20 µg of DENV2 recombinant NS1 (rNS1) combined with different adjuvants, including alum, Sigma adjuvant system (SAS), CpG DNA, Addavax and/or monophosphoryl lipid A (MPLA). Two weeks after the third immunization, mice vaccinated with DENV2 rNS1 with either SAS + CpG or Addavax + MPLA were fully protected against lethal peripheral challenge with DENV2, whereas mice vaccinated with DENV2 rNS1 with alum or CpG DNA alone were not protected. In addition, heterologous cross-protection was observed, as 75% of mice vaccinated with DENV1 NS1 survived lethal DENV-2 challenge. Because NS1 vaccination blocked DENV pathogenesis, we hypothesized that NS1 itself may have direct pathogenic effects. We found that *Ifnar*$^{-/-}$ mice inoculated intravenously with 10 mg/kg of DENV2 NS1 combined with a sublethal dose of DENV2 succumbed 3-4 days post-infection equivalently to mice receiving a lethal dose of DENV2. Mice inoculated with 10 mg/kg DENV2 NS1 alone exhibited morbidity but 100% survived, as did control mice receiving a sublethal dose of DENV2. We then tested the direct toxicity of NS1 on endothelial integrity in a trans-endothelial electrical resistance (TEER) *in vitro* assay. When rNS1 was added to cultured human pulmonary microvascular endothelial cells (HPMEC) in a transwell system, the relative TEER value decreased compared to untreated or OVA-treated HPMEC cells. We next investigated if the *in vivo* lethality and *in vitro* disruption of endothelial integrity caused by rNS1 could be inhibited by NS1-immune serum. *Ifnar*$^{-/-}$ mice passively administered anti-NS1 serum after receiving sublethal DENV2 + NS1 protein were completely protected against death while those receiving control serum were not. In the HPMEC assay, the disruptive effects of rNS1 on TEER were prevented by NS1-immune serum but not serum from OVA-immunized or control mice. Thus, DENV NS1 appears to directly contribute to increased vascular permeability, which can be blocked by anti-NS1 antibody.

1835

DIFFERENCES IN TYPE I INTERFERON SIGNALING ANTAGONISM BY DENGUE VIRUSES IN HUMAN AND NON-HUMAN PRIMATE CELL LINES

Freddy A. Medina[1], Giselle Torres[2], Amanda J. Chase[3], Gilberto A. Santiago[1], Juan F. Medina[1], Luis M. Santiago[1], Jorge L. Muñoz-Jordán[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]University of Puerto Rico Medical Science Campus, San Juan, PR, United States, [3]Mercer University School of Medicine, Macon, GA, United States

The type I interferon (IFN-α/β) response has been shown to be one of the most regulated systems during dengue virus infections. Moreover, attenuation of IFN stimulated genes has been associated with severe disease. In this study we wanted to address conflicting reports of inhibition of IFN signaling by dengue viruses and if there were variations in viruses that differed in their pathogenicity. Using a method that combines flow cytometry and a four-parameter logistic regression model we compared the relative inhibition of IFN-α/β signaling between viruses. Our results showed that all dengue virus serotypes were capable of inhibiting IFN signaling in human cells. A more refined analysis of well-characterized DENV-2 clinical isolates from the five DENV-2 genotypes demonstrated that all viruses inhibited IFN signaling in human cells, but sylvatic viruses displayed a superior ability to inhibit STAT1 phosphorylation. We analyzed inhibition of STAT1 phosphorylation by sylvatic strains in non-human primate cell lines and to our surprise there was no blockage. To determine if these observations were specific to sylvatic strains we performed our IFN inhibition assay with a prototypical DENV2 Asian strain and confirmed that inhibition of STAT1 phosphorylation by dengue viruses does not occur in non-human primate cell lines. However, dengue virus was capable of inhibiting IFN signaling in both human and *Rhesus macaque* primary dendritic cells. IFN-α production was detected in supernatants of dengue virus infected *Rhesus macaque* dendritic cells and contrast to published studies that have suggested that dengue virus can inhibit IFN-α production in human cells. The observed differences in inhibition of the IFN-α/β pathway in human and non-human primate cells may be cell type specific or could result from the transformation process. Nevertheless, these studies provide awareness of differences in the manipulation of the IFN system by dengue virus in human and non-human primate cells.

1836

DENGUE VIRUS NON-STRUCTURAL PROTEIN-1 (NS1) INCREASES HUMAN PULMONARY ENDOTHELIAL CELL PERMEABILITY *IN VITRO*

Henry Puerta Guardo, Dustin Glasner, Eva Harris

Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States

Dengue is the most prevalent arboviral disease in humans and a major public health problem worldwide. Systemic plasma leakage leading to profound shock and potentially fatal complications is a critical determinant of dengue severity. Increased vascular permeability without morphological damage to the capillary endothelium seen in severe dengue suggests the shock syndrome may be due to endothelial dysfunction. In the endothelium, dynamic structures including intercellular junctional proteins and the endothelial glycocalyx control the barrier function critical for vascular homeostasis. In certain diseases, e.g., dengue, functional and structural alterations modify the normal architecture of the vessel wall, increasing plasma extravasation. Dengue pathogenesis involves a complex interaction of the virus and host immune response, including cross-reactive antibodies and T cells, complement activation, and elevated levels of cytokines and other soluble mediators that correlate with severe disease. However, the mechanism of vascular dysfunction in dengue disease is still unclear. Secreted and cell-surface-associated dengue virus nonstructural protein 1 (NS1) and anti-NS1 antibodies are implicated in contradictory roles of protection and pathogenesis, and how NS1 contributes to dengue pathogenesis remains uncertain. Here we evaluated the role of soluble NS1 (sNS1) in inducing endothelial dysfunction. Cultures of human pulmonary microvascular endothelial cells grown on a transwell permeable membrane system as a model of barrier function *in vitro* were exposed to sNS1 (0.2-20 µg/mL), and endothelial permeability was examined by continuously measuring the trans-endothelial electrical resistance (TEER). sNS1 induced a significant dose-dependent increase in endothelial permeability starting 2 hours post-treatment (hpt), at 5 and 20 µg/mL (20 and 50% decrease in TEER, respectively). This effect persisted for more than 24 h as compared to the TEER baseline values exhibited by untreated controls and treatment with unrelated protein (20 µg/mL OVA). Lower concentrations (0.2 and 1 µg/mL) showed less dramatic but still significant decreases in TEER that returned to baseline 6-12 hpt. Confocal microscopy revealed concomitant alterations in intercellular junctional proteins. Our findings suggest a new mechanism of sNS1 directly triggering endothelial vascular dysfunction that occurs in severe dengue disease.

1837

MICROVASCULAR AND ENDOTHELIAL FUNCTION IN PREDICTING CLINICAL OUTCOME OF DENGUE

Sophie Yacoub[1], Cameron Simmons[2], Heiman Wertheim[1], Nguyen Van Kinh[3], Gavin Screaton[4], Bridget Wills[2]

[1]Oxford University Clinical Research Unit, Hanoi, Vietnam, [2]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, [3]National Hospital for Tropical Diseases, Hanoi, Vietnam, [4]Imperial College London, London, United Kingdom

Dengue can present with a broad spectrum of clinical phenotypes. The hallmark of severe disease is increased vascular permeability, sometimes leading to hypovolaemic shock. However microvascular/endothelial dysfunction are difficult to assess clinically. A prospective observational study recruiting a) patients presenting to the OPD with fever for <72 hours and a clinical diagnosis of possible dengue, and b) patients hospitalized with warning signs or established severe disease, is ongoing in two hospitals in Hanoi and Ho Chi Minh City, Vietnam. Clinical, laboratory, and haemodynamic assessments are performed daily for a maximum of 6 days, and again at follow-up 2 weeks later. Microvascular imaging using Sidestream Darkfield Imaging (SDF) and endothelial function testing using peripheral artery tonometry (EndoPAT) are performed at enrollment, defervescence/hospital discharge and follow-up. To date, 167 patients have been recruited, 92 in the outpatient arm and 75 in the inpatient arm. The median age is 27 years (range 5-65 years) and 47% are male. In the outpatient arm 29/67 (43%) of the confirmed dengue cases developed warning signs and 3/67 (4%) developed shock, while 25/92 (27%) were diagnosed as having other febrile illnesses (OFI). At enrolment, the reactive hyperaemic index (RHI), a marker of endothelial function, was lowest in the patients who went on to develop severe dengue (median [range] 1. 54 [1.36-1.96]) followed by those who developed warning signs (1.78 [1.17-3.5]) and then uncomplicated dengue (2.18 [1.16-2.29]). In the OFI category the RHI was 1.63 [1.22-3.38]. Results for the inpatient arm showed a similar trend with the lowest RHI seen in severe dengue patients. The SDF images are being analysed; initial results show microvascular flow is impaired in early dengue with a lower proportion of perfused vessels, mean flow index and vessel density compared with follow-up. These preliminary results suggest microvascular and endothelial dysfunction are associated with dengue disease severity, and can be detected prior to severe clinical manifestations. These techniques may prove useful as outcome predictors and/or to monitor endothelium-directed therapies.

1838

MONOCYTE RECRUITMENT TO THE DERMIS AND DIFFERENTIATION TO DENDRITIC CELLS INCREASES THE NUMBERS OF TARGETS FOR EARLY DENGUE VIRUS REPLICATION

Michael A. Schmid, Eva Harris

Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States

The four serotypes of dengue virus (DENV1-4) cause the most prevalent arthropod-borne viral disease in humans. Although *Aedes* mosquitoes transmit DENV via the skin and studies have identified Langerhans cells as targets of DENV replication in the epidermis, no information exists about the immune response and DENV infection in the dermis. DENV suppresses the interferon response, replicates, and causes disease in humans but not in wild-type mice. Here, C57BL/6 mice lacking the interferon-α/β receptor (*Ifnar*$^{-/-}$) had normal frequencies of hematopoietic cells in the skin, were susceptible to intradermal DENV2 infection, and developed disease that displayed key features of severe dengue in humans. For the first time, we identified dermal dendritic cells (DCs), macrophages, and monocytes as targets for DENV replication in the dermis of *Ifnar*$^{-/-}$ mice. We made the following observations. (1) CD103^{+} DCs and macrophages were present in the steady-state dermis and were the first DENV-infected cells in the skin 12 hours post-inoculation (hpi); they then decreased in frequency over time and no longer contributed to DENV replication after 48 h. (2) Substantial numbers of CD11b^{+} Ly6C^{-} DCs were present and were continuously DENV-infected between 12 and 72 hpi. (3) Ly6C^{high} monocytes were actively recruited to the DENV-infected dermis as early as 12 hpi and, by 48 h, differentiated to Ly6C^{+} monocyte-derived DCs (moDCs). Ly6C^{high} monocytes and Ly6C^{+} moDCs became DENV-infected 48-72 hpi and were then the major subsets for DENV replication in the skin. Finally, adoptive transfer of Ly6C^{high} monocytes from *Ifnar*$^{-/-}$ and WT mice confirmed recruitment of circulating monocytes to the DENV-infected dermis, differentiation to Ly6C^{+} moDCs, and DENV infection of *de novo* recruited cells. Our study identifies dermal DCs and macrophages as the initial targets for DENV replication in the skin. Further, we establish a novel mechanism of how DENV exploits the immune response in the dermis by recruiting monocytes and moDCs, which then become the major targets for virus replication in the skin.

1839

DENGUE VIRUS INFECTION INHIBITS THE CGAS/STING/IRF3 PATHWAY IN INFECTED HUMAN CELLS

Sebastian Aguirre[1], Francise Lamote[1], Jenish Patel[2], Ana Maestre[1], Dabeiba Bernal-Rubio[1], Adolfo Garcia-Sastre[2], Ana Fernandez-Sesma[2]

[1]Department of Microbiology and the Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York City, NY, United States, [2]Department of Microbiology and the Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai and Icahn Graduate School of Biomedical Sciences at Mount Sinai, New York City, NY, United States

Dengue virus (DENV) has become the most relevant arthropod-borne virus that affects humans. To productively infect the host, DENV needs to inhibit the host innate immune system, particularly the type I interferon (IFN) system. Our group and others have demonstrated that DENV interferes with both, the production and signaling of type I IFN pathways through the expression of viral proteins that specifically target host proteins involved in these essential responses to pathogens. Our group showed that the DENV protease complex NS2B3 is able to interact and cleave the adaptor STING to inhibit the activation of IRF3 in infected cells. A recent report showed an anti flavivirus activity of the newly described pattern recognition receptor cGAS, which after activation generate a second messenger (cGAMP) that in turn activates the adaptor STING and the subsequent induction of type I IFN. In order to investigate the role of cGAS during DENV infection we evaluated the ability of this protein to be activated and trigger type I IFN during DENV infection. We also investigated the role of the NS2B3 protease complex in the inhibition of type I IFN production induced by cGAS. We have found a novel mechanism of type I IFN inhibition by the DENV protease through the interference of the cGAS/STING/IRF3 pathway. Also, over expression of cGAS impaired DENV replication. Alternatively, silencing of cGAS in human dendritic cells (DCs) resulted in a higher accumulation of DENV RNA after infection. These results suggest an active role of cGAS as a sensor during DENV infection and confirm the role of DENV protease as a master regulator of the type I IFN response in DENV infected cells

1840

DEVELOPMENT OF A NON-HUMAN PRIMATE MODEL FOR SECONDARY DENGUE VIRUS INFECTION USING MARMOSETS (*CALLITHRIX JACCHUS*): DETECTION OF VIRUS IMMUNE-COMPLEX USING FCΓ RECEPTOR EXPRESSING CELLS

Meng Ling Moi[1], Kenji Shirai[2], Yasushi Ami[3], Chang-kweng Lim[1], Yuriko Suzaki[3], Kazutaka Kitaura[2], Masayuki Saijo[1], Ryuji Suzuki[2], Ichiro Kurane[4], Tomohiko Takasaki[1]

[1]Department of Virology 1, National Institute of Infectious Diseases, Tokyo, Japan, [2]Department of Rheumatology and Clinical Immunology, Clinical Research Center for Allergy and Rheumatology, Sagamihara National Hospital, National Hospital Organization, Kanagawa, Japan, [3]Division of Experimental Animal Research, National Institute of Infectious Diseases, Tokyo, Japan, [4]National Institute of Infectious Diseases, Tokyo, Japan

Infection with one dengue virus (DENV) serotype does not offer protection against secondary infection against a heterologous serotype. Antibodies to dengue virus (DENV) possess two competing activities: antibody-mediated virus neutralization that leads to protection and infection-enhancement that may cause severe complications. In this study, marmosets (*Callithrix jacchus*) were infected with DENV-2 and subsequently inoculated with DENV-1, DENV-2 or DENV-3 to evaluate the model utility as an infection model for DENV infection. Viremia levels were determined by RT-PCR, BHK and FcγR-expressing cell lines. Antibody response was determined by IgM and IgG ELISA, and neutralizing antibody titers were determined by using BHK cells and FcγR-expressing BHK cells. All marmosets consistently developed viremia after secondary heterologous infection and primary infection. Viremia was absent during secondary homologous challenge. As compared to primary infection, viremia during secondary heterologous challenge persisted longer. Higher levels of viremia were detected using FcγR-expressing cells as compared to FcγR-negative cells during secondary heterologous challenge in marmosets, suggesting presence of infectious virus-immune complex during secondary infection. However, levels of viremia were similar after primary challenge using FcγR-expressing cells and FcγR-negative cells. IgM and IgG antibody response in primary and secondary inoculation were consistent to those of human DENV infection. Marmosets also exhibited thrombocytopenia, leucopenia and increase in AST, ALT and LDH levels during DENV infection. The animal model also demonstrated enlarged liver and kidney during secondary DENV infection. Neutralizing antibodies were serotype cross-reactive in FcγR-negative cells but were specific to primary serotype in FcγR-expressing cells. During secondary infection, marmosets demonstrate viremia and antibody responses consistent with those of human DENV infection. Strong antibody responses induced after secondary heterologous infection possess high neutralizing antibody titers against all four DENV serotypes. The results suggest the potential of marmosets as a useful animal model for DENV infection.

1841

MOSQUITO INFECTIVITY AND GAMETOCYTE CARRIAGE AMONG PATIENTS PRESENTING WITH UNCOMPLICATED FALCIPARUM MALARIA IN NORTHERN CAMBODIA

Jessica T. Lin[1], Ratawan Ubalee[2], Chanthap Lon[2], Michele Spring[2], Sujata Balasubramanian[1], Ponsa Chanudom[2], Namtip Trongnitpatt[2], Worachet Kuntawunginn[2], Anintita Laoboonchai[2], Christian Parobek[1], Nitima Chanarat[2], Char Meng Chuor[3], Jessica Manning[2], Steve Meshnick[1], Charlotte A. Lanteri[2], David L. Saunders[2]

[1]University of North Carolina, Chapel Hill, NC, United States, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia

Halting the spread of malaria relies on identifying the transmission reservoir and providing effective transmission blocking therapy. Membrane feeding studies have shown that a substantial proportion of *Plasmodium falciparum* infected African children are capable of infecting mosquitoes in the absence of smear-detectable gametocytes. Less is known about transmission in Southeast Asia. We sought to determine the infectivity of adults with *P. falciparum* infection in northern Cambodia relative to gametocytemia. As part of a therapeutic efficacy study of dihydroartemisinin-piperaquine, half of patients with uncomplicated *P. falciparum* were randomized to receive a 45mg single dose primaquine on the 3rd day of dosing. Patient blood was membrane-fed to *Anopheles dirus* mosquitoes prior to treatment and on days 4, 7, and 14 following treatment. At Day 9 after membrane feeding, 50 mosquitoes were dissected for oocyst detection, while another 50 were saved at Days 9 and 16 for parasite detection by real-time PCR. Among 108 patients studied, 7 (6.5%) patients carried smear-detectable gametocytes at baseline (median 66 gametocytes/μL, range 5-728), and only 2 of 7 successfully infected mosquitoes. Both transmitters had high levels of gametocytemia (705 and 728 gametocytes/μL) resulting in high oocyst prevalence (26% and 70% of mosquitoes with average 2 and 56 oocysts/midgut, respectively). Of the remaining patients without smear-detectable gametocytes, only 1 was infectious to mosquitoes, resulting in 8% oocyst prevalence with an average of 1 oocyst/midgut. These results show a 30-fold greater transmission potential in patients with microscopic *P. falciparum* gametocytemia. PCR analysis for submicroscopic gametocytemia and oocyst positivity is currently in progress. However, our findings suggest that in an area with low *P. falciparum* endemicity where gametocyte carriage is relatively rare, only a small minority of patients with symptomatic malaria contribute to the bulk of human-to-mosquito transmission.

1842

THE EFFECT OF ARTEMISININ-COMBINATION THERAPY TREATMENT OPTIONS ON *PLASMODIUM FALCIPARUM* GAMETOCYTE CARRIAGE: A POOLED ANALYSIS OF INDIVIDUAL PATIENT DATA

Teun Bousema[1], Chris Drakeley[2], Philippe Guerin[3], Georgina Humphreys[3], Kasia Stepniewska[3], Carol Sibley[3], Ric Price[3]

[1]Radboud University Medical Center, Nijmegen, Netherlands, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]WorldWide Antimalarial Resistance Network (WWARN) Gametocyte Study Group, Oxford, United Kingdom

The production of gametocytes during malaria is determined by a variety of parasite, human and environmental factors. ACTs rapidly clear the asexual parasite biomass in infected individuals, with potent gametocytocidal activity against early sexual stages of the parasites; they play a critical role in reducing the transmission of malaria and decreasing the spread of drug resistant parasites. We conducted a large pooled analysis of clinical data to examine the differential effect of ACTs on the transmission potential of *Plasmodium falciparum*. A systematic search of the literature was conducted to identify all studies published between 1960 and March 2014, in which patients were enrolled and treated with antimalarials and where gametocyte data were recorded. Individual patients data from over 100 studies (n>40,000 patients) were collated, curated and included in analysis. Data from 21 African and 6 Asian countries was analysed for gametocyte carriage following treatment with artemether-lumefantrine, amodiaquine-artesunate, dihydroartemisinin-piperaquine, and mefloquine-artesunate. An apriori data analysis plan was developed to identify factors associated with gametocyte prevalence and density prior to treatment and following treatment with an ACT. Criteria for the quality of gametocyte assessments have been ascribed to the various studies. In conclusion, the effects of asexual parasite density, age, transmission intensity and haemoglobin concentration on enrolment gametocyte prevalence and density will be presented. The differential effect of ACTs on post-treatment gametocyte carriage, density and carriage time will be examined in relation to ACT regimen, parasite clearance time, transmission intensity and human host factors. The results of this important study and their relevance for malaria elimination will be highlighted.

1843

IVERMECTIN FOR MALARIA CONTROL: INSIGHTS FROM MODELLING

Hannah C. Slater[1], Patrick G. Walker[1], Teun Bousema[2], Azra C. Ghani[1]

[1]Imperial College, London, United Kingdom, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

Ivermectin (IVM), used alongside mass treatment strategies with an artemisinin combination therapy (ACT), has been suggested as a possible tool for reducing malaria transmission. Mosquitoes feeding on humans that have recently ingested ivermectin have a reduced lifespan, meaning they have a lower probability of completing sporogony and they complete fewer gonotrophic cycles. We use human pharmacokinetic data and mortality data for mosquitoes taking bloodmeals containing IVM to quantify the mosquitocidal effect of IVM. This is incorporated into a transmission model to estimate the impact of IVM in combination with mass treatment strategies with an ACT on transmission metrics. Adding IVM increases the reductions in parasite prevalence achieved and delays the re-emergence of parasites compared to mass treatment alone. This transmission effect is obtained through its effect on vector mortality. IVM effectiveness depends on coverage with the highest impact achieved if given to the whole population rather than only those with existing detectable parasites. Our results suggest that including IVM in a mass treatment strategy can reduce the time taken to interrupt transmission as well as help to achieve transmission interruption in transmission settings in which mass treatment strategies alone would be insufficient. . We also investigate the optimal implementation of ivermectin administration in a range of intervention scenarios, for example whether it best used alongside dihydroartemsinin-piperaquine or artemether lumefantrine in a mass treatment intervention, whether there is any benefit of using primaquine alongside ivermectin, whether ivermectin could be beneficial if used as a stand-alone drug prior to the peak transmission season, and how the vector ecology and existing vector control interventions in a specific region impact the efficacy of ivermectin. Overall, we find that including IVM in mass treatment strategies could be a useful adjunct to reduce and interrupt malaria transmission.

1844

MIND THE GAP: ASSOCIATION BETWEEN HOUSE STRUCTURE AND MALARIA IN UGANDAN CHILDREN

Lucy S. Tusting[1], Humphrey Wanzirah[2], Emmanuel Arinaitwe[2], Agaba Katureebe[2], Kilama Maxwell[2], John Rek[2], Christian Bottomley[1], Sarah Staedke[1], Moses Kamya[3], Grant Dorsey[4], Steve W. Lindsay[5]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Infectious Disease Research Collaboration, Kampala, Uganda, [3]Makerere University College of Health Science, Kampala, Uganda, [4]University of California San Francisco, CA, United States, [5]Durham University, Durham, United Kingdom

Good house construction can lower malaria risk by reducing house entry by vectors. We assessed how house design may affect mosquito house entry and malaria risk in Uganda. 100 households were enrolled in each of three sub-counties: Walukuba, Jinja district; Kihihi, Kanungu district; and Nagongera, Tororo district. Light trap collections were made monthly in all homes. All children aged six months to ten years were followed prospectively to measure parasite prevalence routinely every three months and malaria incidence by passive case detection. Homes were classified as modern (cement, wood or metal walls; and tiled or metal roof; and closed eaves) or traditional (all other homes). We will present the association between house design and human biting rate, malaria infection and clinical malaria and discuss the potential of housing as an intervention against malaria, from low to very high transmission areas.

1845

MAINTAINING UNIVERSAL COVERAGE OF LONG LASTING INSECTICIDAL NETS: IMPACT OF CONTINUOUS DISTRIBUTION ON HOUSEHOLD OWNERSHIP IN EASTERN REGION, GHANA

Celine S. Zegers de Beyl[1], Richmond Ato Selby[2], Obed E. Asamoah[3], Joshua K. Ofori[4], Constance Bart-Plange[5], Hannah Koenker[6], Matthew C. Lynch[6], Kojo Lokko[6], Albert Kilian[7]

[1]Malaria Consortium, London, United Kingdom, [2]Malaria Consortium, Malaria Consortium Kampala, Uganda, [3]Malaria Consortium/NetWorks, Accra, Ghana, [4]NetWorks, Accra, Ghana, [5]National Malaria Control Programme, Accra, Ghana, [6]Center for Communication Programs, Johns Hopkins University, Baltimore, MD, United States, [7]Tropical Health LLP and Malaria Consortium, Montagut, Spain

Distribution of long lasting insecticidal nets (LLIN) is considered a key intervention for the prevention of malaria. Mass distribution is required to rapidly scale up LLIN coverage while continuous distribution systems are essential to sustain the results achieved. In the Eastern Region (ER), the National Malaria Control Programme and implementing partners supported mass LLIN distributions between December 2010 and April 2011. Continuous distribution (CD) activities were started in October 2012 and included antenatal care services), the expanded program on immunization and primary schools. The outcome was evaluated through cross sectional surveys, conducted at baseline in April 2012, 12-16 months after the campaign and at endline in December 2013, after one year of CD implementation. For each survey round, a representative sample of 900 households in ER was selected using a two-stage cluster sampling design. Household heads were interviewed using a structured questionnaire. Household ownership of at least one LLIN was 91.3% (95%CI 88.4 to 93.6) at baseline and fell to 88.4% (85.2 to 91.3) at endline 18 months later but would have been only 81.0% (76.3 to 84.9) without the LLIN from CD. Population access to an LLIN within the household decreased from 74.5% (71.1 to 77.6) at baseline to 66.5% (62.9 to 69.9) but would have been 57.4% (53.0 to 61.8) without the CD contribution. Households reached by any of the CD channels were primarily those who had not been reached by the campaign with any or sufficient ITN. In addition, the different CD channels largely complemented each other with little overlap in the first year. The continuous distribution of LLIN through primary schools and routine health services did not quite maintain the household coverage after one-year of implementation due to its late start almost two years after the campaign. Results show, however, that a CD approach is feasible.

1846

INCREASE IN INTRA-HOUSEHOLD ACCESS TO AND USE OF INSECTICIDE TREATED NETS (ITNS) IN SENEGAL

Julie I. Thwing[1], Adama Faye[2]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Université Cheikh Anta Diop, Dakar, Senegal

With the increase in funding to support malaria control, malaria endemic countries have increased the availability of insecticide treated nets (ITNs) to their populations. Senegal used primarily social marketing until 2008, when a mass distribution campaign targeting children under 5 was conducted, covering 5 of 11 regions. A nationwide campaign targeting children under 5 was conducted in 2009. A rolling universal coverage campaign started in 2010, in which every sleeping space was counted and households received sufficient ITNs to cover every sleeping space, accounting for existing nets in good condition. Between 2010 and early 2013, the universal coverage campaign was implemented in all regions of Senegal. Over this time, 690,000 ITNs were distributed in 2006; 1,065,141 in 2007; 1,586,522 in 2008; 2,532,018 in 2009; 1,258,663 in 2010; 2,465,770 in 2011; and 983,725 in 2012. We used nationally representative survey data to track the evolution of intra-household access to ITNs (a newly recommended indicator), calculated as twice the number of ITNs divided by the number of persons in the household (not to exceed 100%). Based on this indicator, only 11% and 19% of the population had access to an ITN in 2005 and 2006, respectively. In 2008, after the subnational distribution to children under 5 years, access was 36%. The post-campaign survey in 2009 indicated an increase in ITN access to 57%. Universal coverage was completed in four of 14 regions in 2010, resulting in access of 41% nationwide, and 70% in the campaign-covered regions. Access was 63% in 2012, with all but two regions covered. National-level household ownership of at least one ITN from these surveys was 20% (2005), 36% (2006), 60% (2008), 82% (2009), 63% (2010), and 72% (2012), while use by the general population was 6% (2005), 12% (2006), 23% (2008), 34% (2009), 29% (2010), and 41% (2012). Two-thirds of those with access to an ITN reported using it the previous night. Household ITN ownership is an inflated measure of ITN access. Intra-household access is a more appropriate indicator for assessing the gap between ownership and use. Access to nets closely reflects the number of nets distributed annually, and examination of access over time demonstrates the challenge of increasing and maintaining access to ITNs. Additional resources and robust routine distribution strategies are needed to maintain high access to ITNs during the interim periods between larger mass distribution campaigns.

1847

BED NET DURABILITY ASSESSMENTS: EXPLORING A COMPOSITE MEASURE OF NET DAMAGE

Jodi Vanden Eng[1], Ana Paula Abílio[2], Maria do Rosario Pondja[3], Dulcisária Marrenjo[3], Jacinta Luciano[2], Guilhermina Fernandes[3], Samira Sabindy[2], Adam Wolkon[1], Gabriel Ponce de Leon[1], Adeline Chan[1], Juliette Morgan[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Instituto Nacional da Saúde, Maputo, Mozambique, [3]Programa Nacional de Controlo da Malária, Maputo, Mozambique

The durability of Long Lasting Insecticidal Nets (LLINs) in field conditions is of great importance for malaria control programs. Although LLIN bio-efficacy has been investigated, the physical integrity of the net fabric is less well understood making it challenging to determine overall net protectiveness. The 2011 World Health Organization Pesticide Evaluation Scheme (WHOPES) guidelines provide a simple, standardized method using

a proportional hole index (PHI) for assessing net damage. We evaluated the accuracy and utility of this measure using LLINs collected over three years in Nampula Province, Mozambique following a mass distribution campaign in 2008. For each LLIN the type of damage, diameter, and distance from the bottom of the net were recorded for every hole. Holes were classified into four size categories and a PHI was calculated based on the WHOPES guidelines. The areas of WHOPES defined hole size categories were compared to circular and elliptical areas based on actual diameters of each hole; and the PHI was compared to cumulative damaged surface area of the LLIN. The damaged area of small, medium, and large holes was overestimated and the area of extra-large holes was underestimated using the WHOPES categories compared to actual measured areas (Wilcoxon signed rank test of differences p< 0.0001 for all sizes). Approximating holes as circular overestimated hole surface area by roughly 1.5 to 2 times or more. For a range of hypothetical PHI thresholds associated with a "failed LLIN" found in current literature, roughly 75 to 80% of failed LLINs can be detected by only considering large and extra-large holes (which are easier to identify and count). Future research studies may refine the PHI to better approximate overall surface area interrupted. Furthermore, research is needed to identify appropriate PHI thresholds to deem a net no longer protective. Once a cutoff is selected, logistically simpler methods of determining the effective lifespan of LLINs can help guide replacement strategies for malaria control programs.

1848

COMMUNITY ACQUIRED BACTEREMIA AMONG CHILDREN IN AREAS OF LOW AND HIGH MALARIA TRANSMISSION IN RURAL WESTERN KENYA

Frankline M. Onchiri[1], Patricia B. Pavlinac[2], Benson O. Singa[1], Elizabeth A. Odundo[3], Grace C. John-Stewart[4], Judd L. Walson[4]

[1]Kenya Medical Research Institute and University of Washington-Seattle, Nairobi, Kenya, [2]Department of Epidemiology, University of Washington, Seattle, WA, United States, [3]Walter Reed Project, United States Army Medical Research Unit, Kericho, Kenya, [4]Departments of Epidemiology, Global Health and Pediatrics, University of Washington, Seattle, WA, United States

In many African settings, malaria continues to decline as a cause of febrile illness in children, highlighting the need for improved understanding of alternative causes of fever associated with high mortality. We evaluated the prevalence, etiologies, and correlates of bacteremia in outpatient children at two rural hospitals in Western Kenya. Children aged 6 months-15 years presenting consecutively with fever to Kisii hospital; an area of low malaria endemicity (entomological infection rates [EIR]<1.5) and Homa Bay hospital; a malaria endemic area (EIR≥300), were enrolled between 2012 and 2013. Detailed socio-demographic and clinical data were collected and all children tested for malaria using smear microscopy and Paracheck Pf® rapid diagnostic tests, HIV using antibody or PCR testing, and bacteremia using BACTEC™ 9250 blood culture system. Isolates were identified and tested for antibiotic resistance using MicroScan Walkaway40®. Correlates of bacteremia were evaluated using multivariate logistic regression. Overall, 1476 children were enrolled, 742 from Homa Bay and 732 from Kisii. Children from Homa Bay were younger (mean age±SD: 33.9±18.6 vs. 36.8±24.6 months) and more likely to be malaria-infected (49.2% vs. 8.6%), HIV-infected (4.2% vs. 1.2%) or HIV-exposed (19.3% vs. 3.4%) and more severely ill based on presence of ≥1 IMCI danger signs (51.5% vs. 16.9%). Only 48 children (3.3%) had bacteremia (3.1% in Kisii and 3.4% in Homa Bay). Salmonella spp. (19 NTS and 19 typhi) were the predominant cause of bacteremia, accounting for 79.2% (38/48) of all isolates, and the distribution of pathogens did not differ between sites. Bacteremia was associated with HIV infection (aOR=4.5; 95% CI: 1.1-19.3) and lower education of caregiver (aOR=2.6; 95% CI: 1.2-5.7); and inversely associated with malaria infection (aOR=0.4; 95%CI: 0.1-0.9). Bacteremia appears to be a relatively uncommon cause of fever in outpatient children in Western Kenya. Given the infrequent availability of blood culture, targeted testing of high-risk children, including those with HIV, may be a useful strategy to reduce mortality among febrile children.

1849

SALMONELLA TYPHI-SPECIFIC EFFECTOR/MEMORY CD8+ T CELL RESPONSES ELICITED IN A WILD-TYPE *S. TYPHI* CONTROLLED HUMAN INFECTION MODEL

Stephanie Fresnay[1], Monica A. McArthur[1], Thomas C. Darton[2], Claire Jones[2], Claire S. Waddington[2], Christoph J. Blohmke[2], Gordon Dougan[3], Brian Angus[2], Myron M. Levine[1], Andrew J. Pollard[2], Marcelo B. Sztein[1]

[1]University of Maryland, Baltimore, MD, United States, [2]University of Oxford, Oxford, United Kingdom, [3]Wellcome Trust Sanger Institute, Hinxton, United Kingdom

Salmonella enterica serovar Typhi (S. Typhi) is a human restricted pathogen which causes significant morbidity and mortality, particularly in developing countries. A better understanding of the immune responses which result in protection from S. Typhi infection is imperative for the development of improved attenuated vaccines. Current knowledge is limited due to the lack of appropriate clinical and preclinical models. Recently, a controlled human infection model was re-established in which volunteers received 104 colony forming units of wild-type S. Typhi (Quailes strain) orally. Twelve volunteers were evaluated for their cell-mediated immune (CMI) responses. *ex vivo* PBMC isolated before and up to 1 year after challenge were exposed to 3 S. Typhi-infected targets, i.e., autologous B lymphoblastoid cell-lines (B-LCL), autologous blasts and HLA-E restricted AEH B-LCL cells. CMI responses were evaluated using 14-color multiparametric flow cytometry to detect simultaneously 5 intracellular cytokines/chemokines (i.e., IL-17A, IL-2, IFN-γ, TNF-a and MIP-1b) and a marker of degranulation (CD107a). Pre-challenge CD107a expression and cytokine production by S. Typhi-specific CD8+ T effector memory (TEM) following exposure to S. Typhi-infected targets were higher in most volunteers diagnosed with typhoid (TD) compared to those who were not. Direct correlations were observed between the levels of responses before challenge and time to disease onset for CD107a, IFN-g and MIP-1b following stimulation with S. Typhi-infected targets. After challenge, decreases in immune responses were observed prior to the time of disease onset, followed by a sharp increase in most TD volunteers. Multifunctional cells (i.e., concomitantly producing 3-5 cytokines/chemokine and/or expressing CD107a) were dominant at all time-points. These data suggest that S. Typhi-specific responses prior to challenge, as well as the magnitude, kinetics and quality ("multifunctionality") of these responses might play a critical role in the development of typhoid fever.

1850

ACTIVATION OF *SALMONELLA TYPHI*-SPECIFIC REGULATORY T CELLS IS ASSOCIATED WITH TYPHOID DISEASE IN A WILD-TYPE *S. TYPHI* CONTROLLED HUMAN INFECTION MODEL

Monica A. McArthur[1], Stephanie Fresnay[1], Thomas C. Darton[2], Claire Jones[2], Claire S. Waddington[2], Cristoph J. Blohmke[2], Gordon Dougan[3], Brian Angus[2], Myron M. Levine[1], Andrew J. Pollard[2], Marcelo B. Sztein[1]

[1]University of Maryland, Baltimore, MD, United States, [2]University of Oxford, Oxford, United Kingdom, [3]Wellcome Trust Sanger Institute, Hinxton, United Kingdom

Salmonella Typhi (*S.* Typhi), the causative agent of typhoid fever, causes significant morbidity and mortality throughout the world. Currently available vaccines are only moderately immunogenic. To develop improved vaccines, identification of immunological responses associated with protection or disease is necessary. This has been hindered, in part, by the lack of an animal model that faithfully recapitulates human disease. The re-establishment of a controlled human infection model with wild-type *S.* Typhi has made these critical studies possible. Peripheral blood mononuclear cells were obtained from volunteers (n=10) prior to and at multiple time-points after challenge with 10^4 colony forming units of wild-type *S.* Typhi (Quailes strain). Regulatory T cell (T_{reg}) responses were measured by flow cytometry and activation status and homing potential of

S. Typhi-specific T_{reg} were determined. We identified significantly higher gut homing (integrin-a4b7 expressing) *S.* Typhi-specific T_{reg} prior to challenge in volunteers diagnosed with typhoid (TD) than in those who were not (No TD). At early time-points following challenge, circulating integrin-a4b7 expressing *S.* Typhi-specific T_{reg} decreased in TD volunteers, indicating likely homing and a resulting accumulation in the gut. Additionally, *S.* Typhi-specific T_{reg} from TD volunteers demonstrated up-regulation of activation molecules following challenge, including expression of Human Leukocyte Antigen (HLA)-DR and Lymphocyte function-associated antigen (LFA)-1/CD11a as early as 1-4 days post-challenge compared to No TD volunteers. Furthermore, significantly higher expression of the chemokine receptor CXCR3, a molecule associated with homing to sites of active inflammation, was observed on the surface of *S.* Typhi-specific T_{reg} in TD volunteers 1-4 days post-challenge compared to No TD volunteers. Taken together these results suggest that activation of T_{reg} that home to the site of *S.* Typhi infection may play a role in disease pathogenesis, possibly through suppression of *S.* Typhi-specific effector T cell responses.

1851

CHARACTERIZATION OF *CANDIDATUS BARTONELLA ANCASHI*: A NEW AGENT ASSOCIATED WITH CARRION'S DISEASE

Kristin E. Mullins[1], Jun Hang[2], Robert Clifford[2], Fatma Onmus-Leone[2], Ju Jiang[3], Marina Leguia[4], Ciro Maguina[5], Richard G. Jarman[2], David Blazes[1], Allen L. Richards[3]

[1]Uniformed Services University, Bethesda, MD, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Naval Medical Research Center, Silver Spring, MD, United States, [4]U.S. Naval Medical Research Unit - 6, Lima, Peru, [5]Universidad Peruana Cayetano Heredia, Lima, Peru

"The genus" *Bartonella* consists of Gram negative, facultative intracellular, vector-borne bacteria, which infect a wide range of mammalian hosts. *B. bacilliformis*, *B. henselae*, and *B. quintana* have long been recognized as pathogens of human importance, while other species, such as *B. clarridgeiae* and *B. rochalimae,* are newly recognized human pathogens. During a 2003 clinical treatment trial a novel *Bartonella* species, *Candidatus* B. ancashi, was uncovered. This treatment trial was conducted, in Ancash, Peru (where *B. bacilliformis* is endemic), to test the efficacy of azithromycin as a treatment for *B. bacilliformis*. During this trial, two patients were found to be infected with a *Bartonella* species disparate from other *Bartonella* species based on *gltA* sequence typing. Subsequently, this new agent, *Candidatus* B. ancashi, was more completely characterized by 1) observations of *in vitro* microscopic, phenotypic, colonial morphology, and growth characteristics, 2) multilocus sequence typing (MLST), multispacer typing (MST), and whole genome analyses, and by 3) the development of species-specific qPCR assays to identify *Candidatus* B. ancashi's presence in possible vectors (*Lutzoma* spp). Gram-staining and transmission electron microscopy showed the isolates to be small, Gram-negative bacilli with variable expression of unipolar flagella. Biochemical testing provided a single phenotype for all the isolates, which is consistent with other *Bartonella* spp. Fully genome sequencing and subsequent genome analyses confirmed these isolates to be genetically identical to one another, yet distinct from other *Bartonella* species. Genome analyses revealed *B. bacilliformis* to be the closest relative to *Candidatus* B. ancashi, although unlike *B. bacilliformis*, the genome of *Candidatus* B. ancashi encodes virulence determinates not seen in *B. bacilliformis*. Surprisingly, whole genome mapping showed major gene rearrangements between the isolates. Additional genome analyses uncovered a possible link between the rearrangements and flagella expression. Based on the results from these studies, we believe *Candidatus* B. ancashi is a novel pathogen of human importance.

1852

INVESTIGATION OF GENOTYPE VARIATIONS IN *ORIENTIA TSUTSUGAMUSHI* OBTAINED FROM PATIENTS WITH MODERATE AND SEVERE SCRUB TYPHUS

Kristin E. Mullins[1], Daniel Paris[2], Sabine Dittrich[3], Stuart Blacksell[2], Paul Newton[3], Allen L. Richards[4]

[1]Uniformed Services University, Bethesda, MD, United States, [2]Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, [3]Wellcome Trust–Mahosot Hospital–Oxford Tropical Medicine Research Collaboration, Vientiane, Lao People's Democratic Republic, [4]Naval Medical Research Center, Silver Spring, MD, United States

Orientia tsutsugamushi is a Gram negative, obligate intracellular bacterium that is transmitted by the bite of infected chiggers (*Leptotrombidium* spp) and is the causative agent of scrub typhus. Scrub typhus presentation varies from a mild illness to severe disease, including pneumonitis, meningitis, encephalitis, disseminated intravascular coagulation, and in some cases death. Additionally, *O. tsutsugamushi* is found in Southeast Asia, the southwestern Pacific Islands, Korea, and parts of Russia, China, Japan, Australia, New Zealand, Pakistan, India, and Afghanistan, where over 1 billion persons are at risk for infection and approximately one million are infected annually. While the genus *Orientia* contains only two known species *O. tsutsugamushi* and *O. chuto*, the former is extremely genetically diverse, with >100 genotypes currently recognized. A study is currently underway to examine the relationship between disease severity and genotype. 322 clinical isolates were collected from 6,740 adult patients who presented with suspected scrub typhus in Vientiane (n=4875), Luang Namtha (n=1335), and Salavan (n=530) provinces of the Lao People's Democratic Republic (Lao PDR) from 2004 until 2012. The patients (n=322) were divided by disease severity, with 69 patients exhibiting severe disease and 253 patients exhibiting moderate disease. Severe disease included patients with reduced consciousness (Glasgow Coma Score < 15), shock (systolic pressure < 80 mmHg), jaundice (clinical observations), meningitis/encephalitis (clinical observations), and/or difficulty breathing (respiration rate > 30 breaths/minute). While moderate disease included patients with malaise, fever, headache, and/or rash, who were sick enough to seek medical attention. Additionally, 30 clinical isolates from various locations, within the endemic area for *O. tsutsugamushi,* will be used to improve *O. tsutsugmaushi* genotyping methods. Single Nucleotide Polymorphism (SNPs) analyses will be employed to look for differences between the isolates from various locations in the endemic region and between the isolates that cause severe disease and those isolates that cause a moderate illness in Lao PDR. Through this study, we hope to, identify predictors for severe disease as well as create a more accurate evolutionary phylogeny for *O. tsutsugamushi*.

1853

SPEEDIER *LEPTOSPIRA* DIAGNOSIS USING HEMOCULTURE FLUIDS

Sabine Dittrich[1], Phouthasen Hyongvongsithy[2], Anisone Chanthongthip[2], Michael Knappik[2], Weerawat Phuklia[2], David A. Dance[1], Paul N. Newton[1]

[1]University of Oxford, Vientiane, Lao People's Democratic Republic, [2]Lao-Oxford-Mahosot Hospital Wellcome Trust Research Unit, Vientiane, Lao People's Democratic Republic

Leptospirosis is a common bacterial zoonosis worldwide, with infections occurring after exposure to contaminated water. Despite this global problem, diagnosis is difficult with culture results taking up to three months and Microscopic Agglutination Test (MAT) serology being retrospective by nature. Molecular assays are ~55% sensitive and 90% specific to detect infection on admission blood samples, with low bacterial density complicating detection. Leptospira were shown to survive and multiply in blood culture media and we hypothesised that extracting DNA from incubated hemoculture fluid (HCF) from blood

culture bottles, followed by quantitative real-time PCR (qPCR) could improve the sensitivity and speed of leptospira diagnosis. We assessed this retrospectively, using pre-incubated HCF of leptospira positive (n=109) and negative (n=63) (as determined by culture, PCR directly on clinical samples and MAT on convalescent serum) febrile patients in Vientiane, Lao PDR. After optimization, receiver-operator-characteristics analysis was employed to identify the most suitable qPCR-threshold and corresponding diagnostic values. The finalized method showed promising sensitivities of 82% (95%CI: 71-90), 66% (95%CI: 55-76) and 59% (95%CI: 49-68) compared to culture, culture+PCR or culture+PCR+MAT, as the respective reference standards. The specificities were >95%, for all three comparisons. This approach may enable the diagnosis of leptospiral infection without the submission of additional samples and the incubation step may further increase the sensitivity without compromising the specificity. The optimized protocol and its usefulness in a routine laboratory setting will be further evaluated prospectively during May-October 2014 and these data will be presented.

1854

INNOVATING DIAGNOSIS OF BACTERIAL BLOODSTREAM INFECTIONS IN MALARIA-ENDEMIC SETTINGS: FROM DISEASE METABOLOMICS TO RAPID DIAGNOSTIC TESTS

Saskia Decuypere[1], Jessica Maltha[2], Issa Guiraud[3], Bérenger Kaboré[3], Marc Tahita[3], Stijn Deborggraeve[2], Malcolm McConville[4], Stephen Graham[5], Halidou Tinto[3], Jan Jacobs[2], Jonathan Carapetis[1]

[1]Telethon Kids Institute, Perth, Australia, [2]Institute of Tropical Medicine, Antwerp, Belgium, [3]Clinical Research Unit of Nanoro, Nanoro, Burkina Faso, [4]Metabolomics Australia and Bio21 Institute of Molecular Sciences and Biotechnology, University of Melbourne, Melbourne, Australia, [5]Centre for International Child Health, Melbourne, Australia

The increasing use of malaria rapid diagnostic tests has revealed that febrile illnesses are often caused by other pathogens than *Plasmodium*. Amongst them, bacterial bloodstream infections (bBSI) are increasingly recognized as an important cause associated with a high mortality, especially in the African setting. Currently, diagnosis of bBSI is clinical as microbiological culture testing is usually not available and if available, takes 2 to 3 days for a result. Missed diagnosis can result in preventable deaths, while overdiagnosis results in inappropriate or unnecessary use of antibiotics. There is an urgent need to develop rapid diagnostic tests targeting bBSI. We hypothesize that the pathophysiological processes triggered by bBSI induce characteristic changes in the > 4,000 different blood metabolites. Our objective is to harness these characteristic metabolite features for bBSI diagnosis. We are conducting a first metabolomics study to examine whether blood plasma contains metabolites that could be useful to diagnose bBSI in a malaria-endemic setting. We quantified 1600 polar and lipid metabolites in plasma from 83 children with severe febrile illness admitted to a rural district hospital in Burkina Faso using liquid-chromatography mass-spectrometry. The patients included (i) 12 bBSI cases confirmed by blood culture, (ii) 34 severe malaria cases with a positive thick blood film and (iii) 37 cases with negative blood culture and negative blood film. A distinct metabolite profile was identified in children with culture-confirmed bBSI compared to children with severe malaria. A first diagnostic model including 10 polar metabolites has a sensitivity of 80% (95% CI: 44.4-96.9%) and specificity of 76.5% (95% CI: 62.5-87.2%) to identify culture-confirmed bBSI. Mining of the lipid data is ongoing to fine-tune this diagnostic model for differential diagnosis of bBSI and severe malaria. We will present the predicted diagnosis of the 83 patients by the final metabolite diagnostic model(s), and compare to the results obtained with blood culture, PCR-based SepsiTest™, malaria thick blood smear and HRP2 rapid diagnostic test. This study demonstrates the potential of plasma metabolites to identify causality in children with severe febrile illness. We will discuss the translation to metabolite-based rapid diagnostic tests and their potential impact on clinical management of severe febrile illness in malaria-endemic settings.

1855

MYCOBACTERIUM ULCERANS DISEASE: PERFORMANCE OF DIAGNOSTIC TESTS AND CLINICAL OPINION COMPARED TO DIFFERENT REFERENCE STANDARDS IN AKONOLINGA, CAMEROON

Yolanda K. Mueller[1], Patrick Nkemenang[2], Bitoungui Mboua[3], Sara Eyangoh[4], Genevieve Ehounou[2], Clotilde Rambaud-Althaus[1], Eric Comte[2], Earnest Njih Tabah[5], Jean-Francois Etard[1], Laurence Toutous Trellu[6]

[1]Epicentre, Paris, France, [2]Doctors Without Borders, Geneva, Switzerland, [3]Central Hospital, Yaounde, Cameroon, [4]Centre Pasteur of Cameroon, Yaounde, Cameroon, [5]National Program against Buruli Ulcer, Yaounde, Cameroon, [6]Geneva University Hospitals, Geneva, Switzerland

In the absence of gold standard, the performance of laboratory test to diagnose *Mycobacterium ulcerans* disease (MU) is often overestimated. We compared the diagnostic accuracy of clinical judgment and laboratory tests to diagnose MU. Between 2011 and 2013, all individuals presenting at Akonolinga District Hospital, Cameroon, with a skin lesion suspect of new MU were enrolled after consent. Clinical data and clinicians' judgment on probability of MU (four grades) were prospectively collected, before the results of laboratory examination (ZN, PCR, culture and skin biopsy). Photographs of lesions were reviewed independently by two dermatologists, and skin biopsies by two histopathologists. We constructed a first composite reference standard combining results of laboratory tests, clinical opinion, and final diagnosis reached by expert consensus, and a second based on WHO definition of at least two positive laboratory tests. The 364 included patients had a median age of 34 years (range 0 to 87), 233 (64%) were males and 66 (19%) were HIV-positive. The 364 patients had a total of 422 lesions, of which 381 (90%) were ulcerative. Lesion severity was of category 1, 2 and 3 in 32%, 41% and 26%, respectively. According to expert consensus, MU was diagnosed in 113 (27%) lesions. Main differential diagnoses were vascular ulcers (25%), other bacterial infections (19%), post-traumatic lesions (7%) and non MU osteomyelitis (6%). Area under ROC curve (AUC) for clinical diagnosis compared to consensus reference standard was 0.84 (95CI 0.80 - 0.88), comparable to PCR (0.84, 95CI 0.80 - 0.89, p=0.98), and 0.82 (95CI 0.69 - 0.95) and 0.69 (95CI 0.65 - 0.74) for ZN performed in Akonolinga and Yaounde, respectively. When using a composite standard of two positive tests (pending final culture results for 58/422 lesions), AUC for PCR (0.94 (95CI 0.92 - 0.97) was superior (p<0.001) while clinical judgment and ZN of both sites were comparable (p=0.47). Clinical judgment is at least comparable to ZN to diagnose MU, while PCR is equivalent or superior depending on reference standard used.

1856

OVERCOMING THE CHALLENGES OF CLINICAL DATA MANAGEMENT IN LOW AND MIDDLE-INCOME COUNTRIES: A CONTEXT-ADAPTED DATA MANAGEMENT PLAN AND LIFECYCLE

Yves Claeys[1], James Smedley[2], Anish Bhattarai[3], Hercule Kalonji[4], Paritosh Malaviya[5], Robert Meester[6], David Mwakazanga[7], Martin Omello[8], Sayouba Ouedraogo[9], Sopheak Sok[10], Sun Sokleng[11], Mary Thiongo[12], Hiwot Tilahun[13], Arouna Woukeu[14], Harry van Loen[1], Brian Faragher[2], Raffaella Ravinetto[1]

[1]Institute of Tropical Medicine, Antwerp, Belgium, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]Koirala Institute of Health Sciences, Sunsari, Nepal, [4]University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [5]IMS/BHU, Varanasi, India, [6]Formerly at Amsterdam Institute for Global Health and Development, Amsterdam, Netherlands, [7]Tropical Diseases Research Center, Ndola, Zambia, [8]Interact Program, Uganda, Uganda, [9]CRUN, Nanoro, Burkina Faso, [10]Sihanouk Hospital Center of Hope, Phnom Penh, Cambodia, [11]National Center for HIV/AIDS, Dermatology and STD, Phnom Penh, Cambodia, [12]International Center for Reproductive Health, Mombasa, Kenya, [13]Armauer Hansen Research Institute, Addis Abeba, Ethiopia, [14]Formerly at London School of Tropical Medicine, London, United Kingdom

More and more non-commercial clinical research is carried out on a collaborative basis in resource-limited contexts to address priority health problems in low and middle income countries (LMICs). Despite the existence of FDA- and ICH-GCP guidelines for clinical research, there is a need for a practical approach in all processes including clinical Data Management (DM) to facilitate the collection and analysis of high-quality data, despite the contextual and financial constraints. Since 2010, the members of the Association for Data Management In the Tropics (ADMIT:https://admit.tghn.org) share knowledge and tackle common issues experienced like standardization, Electronic Data Capture and Data sharing. One of the first initiatives for ADMIT was to tackle the need for uniformity in terms of context-adapted standard operating procedures (SOPs).We defined the essential DM processes within a research project. The members of the network were assigned authorship to prepare two SOPs which were presented and peer reviewed during a workshop within the wider group. A harmonization process was undertaken to ensure uniform structure, terminology and the level of detail across the suite of SOPs. During the harmonization, the alignment of the individual processes inspired the creation of an overall lifecycle for DM. As a result, a usable Data Management Plan (DMP) is now available incorporating the suite of these SOP's.(In the oral presentation,) We will present the characteristics of this DMP and describe how it may help with collaborations on non-commercial research projects where DM processes are spread across different places. We recommend the DMP to be used to ensure a uniform approach to DM, strengthening partnerships and knowledge exchange. We hope that this may be a starting point for standardization in DM in LMICs and possibly to formulate practical recommendations for regulatory and GCP guidelines. The next challenge is to look at the definition of the roles and responsibilities needed to resource these DM activities and the development of training packages.

1857

LACTIC ACIDOSIS AND RESPIRATORY DISTRESS ARE FREQUENT IN CEREBRAL MALARIA AND SEVERE MALARIAL ANEMIA, BUT PREDICT MORTALITY ONLY IN CEREBRAL MALARIA

Nathan R. Brand[1], Robert O. Opoka[2], Karen E. Hamre[3], Chandy C. John[3]

[1]Columbia University, College of Physicians and Surgeons, New York, NY, United States, [2]Makerere University, Kampala, Uganda, [3]University of Minnesota, Minneapolis, MN, United States

We investigated the pathogenesis of lactic acidosis (LA) and deep breathing (DB) and their associated mortality in 249 children with cerebral malaria (CM) and 216 children with severe malarial anemia (SMA) in Kampala, Uganda. Platelet count and hemoglobin, lactate and histidine rich protein-2 (HRP-2) levels at admission were assessed. Children with a hemoglobin of <5 g/dL were transfused (all SMA, 59 CM). LA was more frequent in children with SMA (43.1%) than CM (32.1%, P=0.02), and DB was similar in the two groups (CM, 8.8%, SMA, 7.4%, P=0.6), but mortality was higher in children with CM (12.5%) than SMA (0.5%, P<0.001). In children with CM, mortality was increased in children with LA (odds ratio (OR), 2.2, 95% confidence interval (CI), 1.0, 4.7, P=0.04) or DB (OR 5.0, 95% CI, 1.9, 13, P=0.001), but in children with SMA neither LA nor DB was associated with mortality. Children with CM had higher HRP-2 levels and lower platelet counts than children with SMA, while children with SMA had lower hemoglobin levels than children with CM (all P<0.001). In children with CM, both LA and DB were associated with decreased platelet counts and increased HRP-2 levels (all P<0.01), while in children with SMA, LA and DB were not associated with platelet counts, and only LA was associated with increased HRP2 levels (P=0.02). Conversely, hemoglobin levels were inversely associated with lactate levels in children with SMA (P<0.001) but not CM. In children with CM and DB, each natural log increase in HRP-2 levels was associated with an 8.6 fold increased risk of mortality (95% CI, 1.1, 67.0, P=0.04). DB and LA in CM are associated with parasite sequestration with platelet adhesion, while in SMA they are associated with low hemoglobin. These differences may in part explain the high mortality associated with DB and LA in CM, and the lack of DB- or LA-associated mortality in children with SMA who receive a blood transfusion.

1858

WHAT IS AN ASYMPTOMATIC CARRIER IN AN ENDEMIC AREA?

Alejandro Llanos[1], Miguel Campos[2], Mitchel Guzman[3], Roberson Ramirez[3], Raul Chuquiyauri[1], Dionicia Gamboa[3], Hugo Rodriguez[4], Joseph Vinetz[5]

[1]Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Facultad de Ciencias y Filosofia, Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Laboratorio ICEMR-Amazonia, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru, [4]Dirección Regional de Salud de Loreto, Ministry of Health, Loreto, Peru, [5]University of California San Diego, San Diego, CA, United States

Nowadays there is a greater consensus that is crucial to identify asymptomatic carriers in order to eliminate/eradicate malaria. The methods of evaluating clinical malaria in endemic areas, which include both passive (PCD) and active case detection (ACD), are not very effective in detecting asymptomatic infections, limiting treating asymptomatic carriers. Aggressive infection detection (AID) is other strategy where malaria parasites are searched in people of endemic areas, regardless of the presence of clinical symptoms, using PCR and thick smear (TS). However, applied on a large scale AID would be expensive and impractical. In this perspective, we try to understand who is an asymptomatic carrier in an endemic area through an open population cohort study, conducted in the Peruvian Amazon, and involved 2000 people from 8 communities around

Iquitos. AID+ACD and PCD were used. All the subjects were visited once a week in their homes, and they were given a survey where they were asked systematically for 13 malaria-related symptoms, daily recall for the past week. A blood smear for TS was taken weekly and a sample for PCR monthly. It was found that the prevalence of any symptom in the group with PCR(-) and TS(-) was 21.35% (95%CI:20.67-22.43), for PCRPv(+) and TS(-) group was 29.87% (95%CI:26.17-33.40), PCRPv(+) and TS(+) group was 70.5% (95%CI:68.1-72.8) and PCRPf (+) mostly TS(-) group was 25.49% (95%CI:21.48-29.51). Fever was the symptom that best discriminated infection, but it only achieved 60% specificity and 20% sensitivity (40% for the PCRPv(+)/TS(-) group). In addition, 41.4% (95%CI: 38.9- 43.9) of the infections (asymptomatic or no) were negatives to TS. In conclusion, in the Peruvian Amazon study area, 30% of the group with sub-microscopic malaria for *P. vivax* (PCRPv+ and TS-) had at least one symptom. There are statistical differences between the symptom prevalence of PCRPv (+)/TS(-) and PCRPv (+)/TS(+). These results suggest that it is feasible to develop a clinical marker score to detect potential asymptomatic carriers that should be treated.

1859

ANEMIA AND TRANSFUSION REQUIREMENTS AMONG CHILDREN WITH SEVERE MALARIA TREATED WITH ARTESUNATE AT A RESOURCE-POOR HOSPITAL IN UGANDA

Michael Hawkes[1], Andrea Conroy[2], Robyn Elphinstone[2], Sophie Namasopo[3], Chandy John[4], Robert Opoka[5], Kevin C. Kain[2]

[1]University of Alberta, Edmonton, AB, Canada, [2]University of Toronto, Toronto, ON, Canada, [3]Jinja Regional Referral Hospital, Jinja, Uganda, [4]University of Minnesota, Minneapolis, MN, United States, [5]Makerere University, Kampala, Uganda

Treatment of non-immune travelers with malaria using parenteral artesunate is associated with late-onset hemolysis. Most cases of severe malaria occur in children in sub-Saharan Africa, where the hematologic effects of artesunate have not been well documented. We report a prospective case series of 92 children with severe malaria, all treated with parenteral artesunate, managed at a resource-poor hospital in Africa, with detailed longitudinal data on hemoglobin (Hb) levels. The median (range) age was 2 (1-8) years and 43 (47%) were female. Fifteen patients had tea-coloured urine at admission and 14 were visibly jaundiced. The median (IQR) admission Hb level was 69 (56-80) g/L and 17 patients (19%) had severe anemia (Hb<50 g/L). During hospitalization, 69 patients (76%) received one or more transfusions of packed red blood cells or whole blood, for a total of 114 transfusions. The median (IQR) total volume of blood administered was 10.4 (5.6-20) mL/kg. Patients with jaundice at presentation received significantly larger number (p=0.014) and volume (p=0.043) of transfusions. Fatal outcome in 8 patients was associated with severe anemia in 6/8 cases. Follow-up Hb measurement was performed on 35 patients (38%) at day 14 after initial hospital admission; the remaining patients had no clinical evidence of anemia (no pallor, tachycardia, hyperdynamic circulation, parental report of lethargy, or easy fatigability) at the follow-up visit. The convalescent Hb was median (range) 90 (60-138) g/L, which was significantly higher than the paired admission levels (median increase +28 g/L, p<0.001). The day 14 Hb level was higher than any level measured during hospitalization in 22 (63%) patients, but decreased or remained the same in 13 (33%). Among children with a decrease in Hb level by day 14, the magnitude of the Hb change ranged from -8 to -66 g/L, but none reached the threshold for severe anemia (lowest day 14 Hb was 60 g/L). None required transfusion after hospital discharge. In this representative cohort of young children with severe malaria in a hyper-endemic setting treated with artesunate, anemia was common at admission, required one or more transfusions in a majority of patients, and on average was improving by day 14. However, a substantial proportion of children had persistent or worsening anemia at follow-up. Further study is needed to determine whether this effect is attributable to artesunate.

1860

VALIDATION OF A TUBERCULOUS MENINGITIS CASE DEFINITION IN MBARARA REGIONAL REFERRAL HOSPITAL, UGANDA

Susan A. Adakun[1], Emily Evans[2], Bradley Weinbaum[2], Yap Boum II[3], Patrick Orikiriza[3], Mark J. Siedner[4], Tony Wilson[5], Christopher C. Moore[2]

[1]Makerere University College of Health Sciences, Kampala, Uganda, [2]University of Virginia, Charlottesville, VA, United States, [3]MSF Epicentre, Mbarara, Uganda, [4]Massachusetts General Hospital Centre for Global Health, Harvard Medical School, Boston, MA, United States, [5]Mbarara University of Science and Technology, Mbarara, Uganda

Tuberculous meningitis (TBM) is a leading cause of death in areas with a high prevalence of both tuberculosis and HIV such as sub-Saharan Africa. A major obstacle to successful treatment of TBM is the inability to obtain a rapid and accurate diagnosis. We evaluated the accuracy of a recently proposed TBM case definition (Marais, et al) which is based on clinical, CSF, and radiological findings among patients admitted with suspected meningitis to the Mbarara Regional Referral Hospital in Uganda. CSF was obtained for routine analysis, bacterial and mycobacterial culture, and PCR via GeneXpert® MTB/RIF. Blood was obtained for random blood sugar, lactate, malaria blood smear, complete blood count, blood culture, HIV serology and CD4+ count. We determined the diagnostic accuracy for the TBM clinical score by evaluating the sensitivity and specificity, as well as positive and negative predictive value of each score threshold. We used a positive mycobacterial culture of cerebrospinal fluid as a reference standard. We enrolled 141 participants and the prevalence of TBM was 6%. Patients with higher TBM scores were more likely to have a diagnosis of TBM, OR 1.44, p=0.04 CI (1.00-2.06). The ROC curve for the prediction of TBM by the TBM score was 0.75. Of the three case definition criteria (clinical, CSF, evidence of TB), only the CSF criterion was strongly associated with TBM (OR 7.73 95% CI (1.04-57.0) p=0.04). For a TBM score threshold <7, sensitivity was 100%, specificity 38.7%, PPV 7.1%, NPV 100%. The TBM score has good sensitivity but low specificity for the diagnosis of TBM. It has an excellent negative predictive value and may be used to rule out TBM in resource limited settings.

1861

MYOCARDIAL AND HAEMODYNAMIC RESPONSES TO FLUID MANAGEMENT IN SEVERELY MALNOURISHED AND WELL-NOURISHED AFRICAN CHILDREN WITH SEVERE SHOCK AND GASTROENTERITIS

Kathryn Maitland[1], Nchafatso Obonyo[1], Bernadette Brent[2], Peter Olupot Olupot[3], Julius Nteziyaremye[3], Irene Kuipers[4], Michael Boele van Hensbroek[4], Job van Woensel[4]

[1]KEMRI Wellcome Trust Programme, Kilifi, Kenya, [2]Imperial College, London, United Kingdom, [3]Mbale Regional Referral Hospital, Mbale, Uganda, [4]Emma Children's Hospital, Amsterdam, Netherlands

The FEAST (Fluid Expansion as Supportive Therapy) trial, the only randomised controlled trial of fluid resuscitation, demonstrated that fluid boluses lead to excess mortality in children receiving bolus fluids. Further analysis indicated adverse effects of fluid boluses resulted from cardiovascular events rather than fluid overload. Studies of myocardial and haemodynamic responses to boluses are thus warranted in groups not included in FEAST (gastroenteritis and malnutrition), where fluid resuscitation continue to be recommended in international guidelines. We describe in Ugandan children myocardial (echocardiographic and ECG) and haemodynamic responses to fluid bolus (i.e. 0, ½, 1, 1½, 2, 3, 4, 8, 12, 16, 20, 24, 32, 40 and 48 hours) pre- and post fluid challenges recommended by WHO guidelines. Blood and urine samples for analysis of electrolytes and other markers of myocardial dysfunction were collected at admission, 8, 24 and 48 hours. A total of 29 children with severe shock and dehydration (due to gastroenteritis) were studied: 19 had severe malnutrition (SM); 10 were well-nourished (controls). For the

SM group receiving WHO guideline resuscitation (15mls/kg over 1 hour, repeated twice if indicated) mortality was 73% (8/11 patients). Following a protocol amendment to slower rehydration (10mls/kg over 1 hour up to a maximum of 50mls/kg) 3/8 died (38%) compared to a mortality of 2/10 in controls. Echocardiographic and haemodynamic data pre-bolus showed marked evidence of underfilling. Boluses lead to early rapid shock reversal, in those treated with slower rehydration shock reversal was more protracted. In all study participants we found no evidence of that mortality was due to fluid overload. Fluid boluses administered to children with SM (per WHO guideline)-resulted in early shock reversal, but this was not associated a survival benefit. Slow rehydration strategy in cases (SM) and control patients appeared to be well-tolerated. Further research is required to optimize fluid management and other supportive strategies to inform future guidelines.

1862

SEVERE MALARIA INFECTIONS IMPAIR GERMINAL CENTRE REACTIONS AND INHIBIT EFFICIENT ANTIBODY RESPONSES TO INFECTION

Diana S. Hansen, Victoria Ryg-Cornejo, Ann Ly, Lisa J. Ioannidis, Axel Kallies

The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia

Naturally acquired immunity to malaria develops only after many years of repeated exposure to *Plasmodium* parasites. Protective immunity predominantly targets blood-stage parasites and requires antibody responses. Despite the key role that antibodies play in protection against malaria, the cellular processes leading to the slow acquisition of immunity remain unknown. Children in high transmission settings that experience frequent malaria clinical episodes are characterized by a delayed development of parasite-specific memory B cells, suggesting that the inflammatory factors contributing to disease hinder these responses. To address that hypothesis we used a severe malaria infection model to investigate the development of germinal centres (GC), memory B cells and plasma cells. C57BL/6 mice were infected with *P. berghei* ANKA, followed by treatment with anti-malarial drugs or immunized with equivalent antigenic loads of irradiated parasites. Reduced numbers of GC B cells and T follicular helper cells (Tfh) were found in mice experiencing an active infection compared to immunized control animals. Despite normal IL-21 secretion, Tfh cells from infected mice displayed an unusual phenotype characterized by low surface expression of PD-1 and CXCR5, required for their successful localization in GCs. Consistently, confocal microscopy experiments revealed that clinical malaria inhibits the establishment of GC reactions in the spleen. The frequency of memory B cells and relative antibody affinity of long-lived plasma cells emerging from GCs was also examined. Unlike immunization with irradiated parasites, active infections appeared to compromise these processes. Pro-inflammatory cytokines involved in the induction of severe malaria episodes were found be partly responsible for the inhibition of B cell responses. Thus these data indicate that clinical malaria negatively impact the development of long-term humoral immunity by disrupting critical early stages in the development of B cell responses.

1863

ACUTE MALARIA INDUCES CTLA4+PD1+ EFFECTOR T CELLS WITH REGULATORY PHENOTYPE

Maria S. Mackroth[1], Annemieke Abel[2], Christiane Steeg[2], Julian Schulze zur Wiesch[1], Thomas Jacobs[2]

[1]University Hospital Hamburg-Eppendorf, Hamburg, Germany, [2]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

Acute *Plasmodium falciparum* (Pf) malaria induces proinflammatory T cell responses which have been shown to confer protection against malaria but also contribute to the development of severe disease. A tight regulation of T effector (T_{eff}) responses is therefore crucial to protect the host. An important mechanism to fine-tune T cell responses in the periphery is the induction of co-inhibitory receptors such as CTLA4 and PD1 and their ligands but their role in the immune response in Pf malaria remains poorly understood. To test the hypothesis that co-inhibitory receptors modulate the T cell response in acute malaria, blood samples were obtained from patients with acute uncomplicated Pf malaria treated in Hamburg, Germany as well as from healthy volunteers. Flow cytometric analysis showed high expression of CTLA4 and PD1 on $CD4^+$ T cells of malaria patients and the ligands for PD1, PDL1 and PDL2, were upregulated on monocytes, B cells and T cells. We then stimulated PBMCs with Pf-infected red blood cells (iRBCs) to detect antigen-specific cytokine production and proliferation. The majority of antigen-specific T_{eff} cells were $CTLA4^+PD1^+$. IFNg was the most frequently detected cytokine and >50% of $IFNg^+$ $CTLA4^+PD1^+$ T cells simultaneously produced IL10. In some donors T cell proliferation was inhibited by PD1 and blockade of PD1-ligation enhanced antigen-specific proliferation. We further isolated $CTLA4^+PD1^+CD4^+$ T cells based on surface expression of PD1 and CTLA4 and investigated their inhibitory function in *in-vitro* proliferation assays stimulated with aCD3/28 or iRBCs. $CTLA4^+PD1^+CD4^+$ T cells suppressed aCD3/28-induced as well as plasmodial-antigen-specific T-cell proliferation in a cell-extrinsic manner. In summary, acute Pf infection leads to induction of malaria-specific $CTLA4^+PD1^+T_{eff}$ cells which coproduce IFNg and IL10 while inhibiting $CD4^+$ T cell proliferation in a cell extrinsic manner. Induction of T_{eff} cells with regulatory function might be an important mechanism to control T cell responses and prevent severe inflammation in acute malaria and potentially other acute infections.

1864

LOSS AND DYSFUNCTION OF VΔ2+ ΓΔ T CELLS IS ASSOCIATED WITH CLINICAL TOLERANCE TO MALARIA

Prasanna Jagannathan[1], Charlie C. Kim[1], Bryan Greenhouse[1], Felistas Nankya[2], Katherine Bowen[1], Ijeoma Eccles-James[1], Mary K. Muhindo[2], Emmanuel Arinaitwe[2], Jordan W. Tappero[3], Grant Dorsey[1], Moses R. Kamya[4], Margaret E. Feeney[1]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Infectious Diseases Research Collaboration, Kampala, Uganda, [3]Centers for Diseases Control and Prevention, Atlanta, GA, United States, [4]Makerere University, Kampala, Uganda

The $V\Delta2^+$ subset of γΔ T cells possess intrinsic reactivity to malaria antigens, but their role in acquired immunity to malaria is unclear. To evaluate γΔ T cell responses in children living in a highly malaria-endemic area, peripheral blood mononuclear cells (PBMCs) were obtained from 78 HIV-uninfected 4-year old children enrolled in a longitudinal cohort study in Tororo, Uganda. The incidence of symptomatic malaria in this cohort was 5.4 episodes ppy (IQR 3.2-7.0) and peaked at 25 months of age with a subsequent gradual decline in malaria episodes and a corresponding increase in asymptomatic parasitemia. PBMCs were stimulated with *Plasmodium falciparum*-infected red blood cells (iRBC) or controls and assessed by multiparameter flow cytometry and gene expression microarray. We noted a striking inverse association between frequencies of $V\Delta2^+$ cells and the prior cumulative incidence of malaria (Rho=-0.39, P=0.003). Repeated episodes of malaria were also associated with decreased cytokine production (Rho=-0.41, P=.0002) and decreased proliferation (Rho=-0.58, p=0.009) of $V\Delta2^+$ cells in response to malaria antigen stimulation, suggesting that children who have survived repeated clinical malaria episodes exhibit dysfunction as well as loss of $V\Delta2^+$ cells. Whole transcriptome analysis of sorted, unstimulated $V2^+$ cells revealed increased expression of immunoregulatory genes in children with heavy prior malaria, including genes encoding Tim-3, BATF, and CD57, suggesting that repeated infection may lead to the upregulation of immunoregulatory pathways that dampen the innate V2 inflammatory response. Finally, loss and dysfunction of pro-inflammatory $V\Delta2^+$ γΔ T cells was associated with a reduced likelihood of symptoms upon subsequent *P. falciparum* infection. Together, these results suggest that repeated malaria infection during childhood results in progressive loss and dysfunction of $V\Delta2^+$ γΔ T cells that may facilitate immunological tolerance of the parasite.

1865

LONGITUDINAL ANTIBODY RESPONSES TO ANTIGENS ON THE SURFACE OF *PLASMODIUM FALCIPARUM* GAMETOCYTE-INFECTED ERYTHROCYTES IN GHANAIAN SCHOOL CHILDREN

Bismarck Dinko[1], Teun Bousema[2], Colin J. Sutherland[2]

[1]University of Health and Allied Sciences, Ho, Ghana, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

Little is known about the immune responses directed at circulating *Plasmodium falciparum* gametocytes in humans, knowledge of which would be useful in the development of intervention strategies to reduce and block malaria transmission. Consequently, antibody responses to surface antigens of *P. falciparum* gametocyte-infected RBCs (GSA) were determined in plasma samples from malaria asymptomatic Ghanaian school children between the ages of 5-17 years. These children were screened for malaria parasites and treated with dihydro-artemisinin piperaquine and followed up weekly for one month. Gametocytes were produced from a laboratory adapted parasite line, 3D7 and a recent patient isolate from Kenya (HL1204). From a cohort of 113 children, 56% of the children exhibited marked antibody responses to GSA (immune response above the median within the cohort per sampling time) that recognized GSA on a proportion of mature gametocyte-infected RBCs of 3D7 by flow cytometry. These responsive individuals were identified by measuring both the proportion of mature gametocytes recognised by antibodies and the intensity of the antibody binding to GSA. Longitudinal data provided an additional 10% developing GSA responses during the 1 month follow-up. Children with GSA antibodies present at enrolment, were less likely to develop new gametocytaemia at subsequent visits (odds ratio = 0.29, 95% CI 0.06 - 1.05; P = 0.034). 3D7a is a laboratory adapted parasite line so a selection of positive plasma samples was tested against mature gametocyte preparations from HL1204 and strong plasma antibody binding was again shown. No binding to the surface of RBCs infected with immature gametocytes of HL1204 was detected. In conclusion, a proportion of malaria infected asymptomatic children harbour plasma antibodies which strongly recognized antigens on the surface of mature gametocyte-infected RBCs. Strong plasma antibody responses were associated with the control of gametocytaemia *in vivo*. Ghanaian GSA responses recognized antigens on both 3D7 and a Kenyan parasite line, suggesting that conserved antigenic determinants are present on the surface of gametocyte-infected erythrocytes.

1866

A SYSTEMATIC CHARACTERIZATION OF MALARIA-ASSOCIATED ATYPICAL MEMORY B CELLS

Silvia Portugal[1], Severin Zinoecker[1], Youssounou Kone[2], Christine E. Schindler[1], Safiatou Doumbo[2], Didier Doumtabe[2], Kishore Kanakabandi[3], Daniel E. Sturdevant[3], Stephen F. Porcella[3], Ogobara K. Doumbo[2], Kassoum Kayentao[2], Aissata Ongoiba[2], Susan Moir[4], Jean-Nicholas Schickel[5], Eric Meffre[5], Boubacar Traore[2], Susan K. Pierce[1], Peter D. Crompton[1]

[1]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]Mali International Center of Excellence in Research, Bamako, Mali, [3]RML, National Institutes of Health, Hamilton, MT, United States, [4]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [5]Department of Immunobiology, Yale University School of Medicine, New Haven, CT, United States

Antibodies (Ab) play a critical role in malaria immunity, but Ab-mediated protection is only acquired after years of repeated infections, leaving children in endemic areas vulnerable to repeated bouts of febrile malaria. Many *Plasmodium falciparum* antigens are diverse and clonally variant, contributing to the inefficient acquisition of protective Abs. However, mounting evidence suggests that *Plasmodium*-induced dysregulation of B cell function may also play a role. Several studies have shown that malaria exposure is associated with an expansion of atypical memory B cells (MBCs) which are distinguished from classical MBCs by the expression of inhibitory receptors. A similar subset of B cells has been described in individuals infected with HIV and HCV, yet the origin and function of this B cell subset remains unclear. We performed a comprehensive investigation of atypical B cells collected from individuals exposed to intense malaria in Mali. Sorted naïve B cells ($CD19^+$ $CD21^+$ $CD27^-$), classical MBCs ($CD19^+$ $CD21^+$ $CD27^+$), and atypical MBCs ($CD19^+$ $CD21^-$ $CD27^-$) were subjected to genome wide expression profiling, VDJ sequence analysis (Ab heavy and light chain gene usage and somatic hypermutation rate), KREC analysis (replicative history), as well as proliferative and cytokine production analysis following *in vitro* stimulation. We found that classical and atypical MBCs have distinct expression profiles, but are similar in heavy and light chain variable gene usage as well as replicative history. Atypical MBCs have, however, lower levels of somatic hypermutation in heavy and light chain sequences, indicating less antigen-dependent selection compared to classical MBCs. We further show how these B cell subsets differ in proliferative and cytokine production capacity, and how the expression of inhibitory receptors on atypical MBCs impairs their proliferation. This thorough characterization of B cell subsets in malaria-exposed individuals has generated new hypotheses on how chronic *Plasmodium* exposure leads to B cell dysregulation and the inefficient acquisition of protective antibodies.

1867

CHARACTERIZATION OF MALARIA PARASITE LINES (*PLASMODIUM FALCIPARUM*) SELECTED BY LONG-TERM CULTURE IN THE PRESENCE OF INHIBITORY ANTIBODIES TO APICAL MEMBRANE ANTIGEN-1 (AMA1)

Sheetij Dutta, Lisa Dlugosz, J. Kathleen Moch, Adrian Batchelor, John D. Haynes

Walter Reed Army Institute of Research, Silver Spring, MD, United States

Vaccination has been the most effective medical intervention (after sanitation, hygiene, and nutrition) for preventing and eliminating infectious diseases. The development of resistance to anti-malarial drugs by the parasite has spurred the search for effective vaccines. It remains unclear, however, if the antigenically highly polymorphic malaria parasite will also evolve resistance to vaccines. Antigenic escape, for example, was observed for Combination B (MSP2) and FMP2.1 (AMA1) malaria vaccines, and the parasite could also shift to alternative invasion pathways that circumvent the requirement of the vaccine antigen. Thus, it is important to study the effects of long term persistence of inhibitory levels of antibodies induced by a *Plasmodium falciparum* blood stage vaccine - using *in vitro* and if possible *in vivo* animal models of malaria. We have recently shown that AMA1 strain-specific antigenic escape could be overcome by inducing broadly inhibitory antibodies using a Quad-allelic formulation of AMA1 (QuadVax, or QV: 3D7+ FVO+HB3+W2mef allelic forms) which elicited high levels of invasion inhibitory antibodies in rabbits against not only all four vaccine strains but also against 22 antigenically diverse non-vaccine strains (Dutta et al. 2013, PLOS Pathogens). We now used anti-QV antibodies to exert immune pressure on two parasite strains (3D7 and W2mef) in long-term cultures. Parasites were maintained in culture for six months in the presence of ~50% inhibitory concentration of anti-QV rabbit serum while the control parasites were maintained in parallel in the absence of antibodies. During the cultures, parasite lines were frozen at various time-points and at the end of the 6 months of culture the parasites were cloned by limiting dilution. Selection pressure was finally removed and anti-AMA1 selected clones were compared to the parental or control selected parasites. Comparative data will be presented regarding (a) growth and invasion rates in the presence or absence of anti-AMA1 antibodies, (b) parasite DNA sequences, (c) invasion into enzyme treated red cells, and (d) quantity and location of AMA1 and its proteolytic processed products. This study informs an important decision point for future development of an AMA1 vaccine as well as malaria blood stage vaccine development in general.

1868

ENHANCED MULTIFUNCTIONAL CD4+ T CELL MEMORY RESPONSES TO MALARIA ANTIGENS IN MALIAN CHILDREN CO-INFECTED WITH *SCHISTOSOMA HAEMATOBIUM*

Kirsten E. Lyke[1], Bourema Kouriba[2], Abdoulaye Dabo[2], Charles Arama[2], Modibo Daou[1], Modibo Daou[2], Issa Diarra[2], Drissa Coulibaly[2], Christopher V. Plowe[1], Ogobara Doumbo[2], Marcelo B. Sztein[1]

[1]University of Maryland, Baltimore, MD, United States, [2]University of Sciences Techniques and Technologies, Bamako, Mali

Durable immunologic memory to malaria is limited in young children, where repetitive and ongoing exposure to malaria is required to achieve and maintain immunity. We have previously demonstrated that schistosomiasis-positive (SP) Malian children, aged 4-8 years, are protected from malaria compared to matched schistosomiasis-negative (SN) children. The effect of concomitant *S. haematobium* upon acquisition of memory to malaria antigens is unknown. We examined antigen-specific T cell frequencies in 48 Malian children aged 4-14 to malaria blood-stage antigens, Apical Membrane Antigen 1 (AMA1) and Merozoite Surface Protein 1 (MSP1) and to schistosoma antigens, Soluble Worm Antigenic Preparation (SWAP) and Schistosoma Egg Antigen (SEA) during a malaria episode and at convalescence 6 months later. CD4+ T cell memory cytokine (IFN-γ, TNFα, IL2 and/or IL17A) responses specific to schistosoma antigens was measured in 18/23 SP children at one or both time points, compared to 4/23 SN children ($p < 0.0001$). At the time of malaria infection, CD4+ T cells from 12/24 SN children and 15/23 SP children ($p=0.29$) stimulated with malaria antigens demonstrated significantly increased levels of cytokine production. In contrast, 7/23 SN children and 16/23 SP children ($p=0.009$) had responses in paired convalescent samples. 46.2% of cytokine-secreting CD4+ T cells expressed a single cytokine after stimulation with malaria antigens during the malaria episode. This fell to 40.9% at follow-up with a compensatory rise of multifunctional cytokine secretion over time (double+: 30.7 to 32.5%, triple+: 20.6 to 23.1%, and quadruple+: 2.4 to 3.8%) consistent with memory maturation. The majority (53.2%-59.5%) of cytokine responses were observed in CD45RA-CD62L- effector memory T cells with little variation depending upon the time point or the study cohort. We conclude that detectable CD4+ T cell memory response can be measured against both malaria and schistosoma antigens and that the presence of *S. haematobium* may be associated with enhanced functional T memory cell induction to malaria antigens.

1869

CAUSES OF MORTALITY IN WOMEN OF REPRODUCTIVE AGE LIVING IN AN URBAN SLUM (KIBERA) NAIROBI

Beatrice Olack[1], Leonard Otieno Cosmas[2], Daniel Ondari Mogeni[1], Joel Mark Montgomery[2]

[1]Kenya Medical Research Institute-Centre for Global Health Research and Centers for Disease for Control and Prevention, Nairobi, Kenya, [2]Global Disease Detection Branch, Division of Global Health Protection, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA USA, Nairobi, Kenya

Women of reproductive age (15-49 years) are confronted by dual burden of health concerns related to maternal conditions: infectious diseases and emerging challenges associated with non-communicable diseases. More than 30% of all deaths in resource-limited settings occur in these women, as compared to 15% in resource-rich settings. There is a paucity of mortality data and causes of death among females of reproductive age in low income countries. We present findings from verbal autopsies among women of reproductive age living in Kibera, an urban slum in Nairobi, Kenya. Verbal autopsies were conducted among women of reproductive age who were participants in a population-based surveillance system and who died between January 2009 and December 2013. Details regarding the death were obtained from close relatives and cause was assigned using the InterVA-4verbal autopsy model (version 4.02). We identified 157 deaths with an overall mortality rate of 5.4 per 1000 person-years of observation. The median age at the time of death was 31.5 years with the highest (40%) proportion of deaths occurring among women 30-39 years of age. Causes of death were identified in 51% of the individuals. Maternal deaths as defined by WHO were less frequent compared to non –maternal deaths (7% vs. 93%, respectively; $\chi^2=80.0$, $p<0.001$) in this population. Among the non-maternal deaths, 62% were due to infectious diseases, with HIV/AIDS associated illness being the leading cause (43%). Non-communicable diseases were associated with 38% of non-maternal deaths, of which cancers and cardiovascular disease were common (43% and 32%, respectively). Communicable diseases were found to be a major cause of death among women of reproductive age; however, non-communicable diseases are increasing in frequency among this population. These findings highlight the need to address and reduce the risk of deaths resulting from both communicable and non-communicable diseases, along with efforts to reduce maternal deaths among women of reproductive age.

1870

REVISITING THE BURDEN OF TYPHOID FEVER IN LOW AND MIDDLE-INCOME COUNTRIES TO INFORM POLICY DECISIONS

Vittal Mogasale, Brian Maskery, R. Leon Ochiai, Jung Seok Lee, Vijayalaxmi V. Mogasale, Enusa Ramani, Young Eun Kim, Jin Kyung Park, Thomas F. Wierzba

International Vaccine Institute, Seoul, Republic of Korea

Typhoid fever still causes significant burden in low and middle income countries where access to safe water and sanitation is compromised. There have been several efforts to quantify the global burden of typhoid fever, but the estimates have not considered the heterogeneity in risk levels within countries. Since the World Health Organization has recommended risk-based use of vaccines against typhoid, we attempted to revisit the burden on typhoid fever in low and middle income countries after adjusting to the risk levels at population. The typhoid disease burden was estimated based on community representative typhoid incidence studies applied to 2010 population after correcting for the operational issues related surveillance, limitations of diagnostic tests and risk difference due to exposure to unimproved water. Incidence estimates, correction factors and mortality estimates were derived from systematic literature review. Scenario analyses for risk factors, blood culture sensitivity and case fatality rates were conducted accounting for the uncertainty in these estimates and compared to previous disease burden estimates. Findings: The risk-adjusted estimate of typhoid fever in low and middle income countries was 11.9 million cases (CI: 9.9 - 14.7 million) and 129,000 deaths (CI: 75,000 - 208,000). In comparison, without the risk- adjustment, the burden estimate would be 20.6 million cases (CI: 17.5 - 24.2 million) with 223,000 deaths (CI: 131,000 - 344,000). Scenario analyses indicated that the risk factor adjustment and updated diagnostic test correction factor derived from systematic literature review were the drivers of difference between current estimate and past estimates. Interpretation: The risk-factor adjusted typhoid fever burden estimate is inherently more conservative than previous estimates that did not account for study site selection bias or fractions of the populations residing in urban slums or rural areas lacking access to improved water supplies. However, by distinguishing and discriminating the risk differences, it allows better estimation of the population level impact and evaluation of cost effectiveness of risk-based vaccination strategies recommended by World Health Organization.

1871

DISABILITY- AND QUALITY-ADJUSTED LIFE YEARS: MEASURING HEALTH OR···?

Thomas Fürst, Maria-Gloria Basáñez, Lesong Conteh

Imperial College London, London, United Kingdom

Disability-adjusted life years (DALYs) and quality-adjusted life years (QALYs) have risen to prominence over the past years and are frequently

used as denominators in cost-effectiveness analyses. They have become a powerful "currency" in health economics, health policy and public health decision making. By combining life years lost due to premature mortality with disability- or quality-adjusted life years reflecting morbidity and attributing these summary measures to specific health conditions and interventions, DALYs and QALYs aim at quantifying health losses and health gains respectively. An explicit assumption is that DALYs and QALYs allow for comparison of different causes of health losses and health gains and that they are therefore suitable to guide global, national and local decision making on where to invest scarce resources. However, based on a literature review, we argue that the DALYs and QALYs lack a clear definition of the concepts "health", "disability" and "health-related quality of life" and therefore also of their disability- and quality-adjustments for individuals' life years spent in less than perfect health. Mainly based on the highly topical International Classification of Functioning, Disability and Health of the World Health Organization, we developed a conceptual framework to delineate what the DALYs and QALYs do, and do not measure. Important similarities and differences between the two measures are revealed. Critical questions about the conceptualization of DALYs and QALYs are discussed and we conclude that if these questions are not addressed there is a continued risk of inefficient decision making and ill-informed advocacy.

1872

VALIDATION OF CAUSES-OF-DEATH USING VERBAL AUTOPSY DATA COLLECTED FROM NAVRONGO HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM IN GHANA: 2007-2011

Patrick O. Ansah[1], Cornelius Debpuur[1], James Akazili[1], Timothy Awine[1], John Williams[1], Nana Akosua Ansah[1], Yazoume Ye[2], Isaac Osei[1], Leopoldo Villegas[2], Osman Sankoh[3], Abraham Oduro[1]

[1]Navrongo Health Research Centre, Navrongo, Ghana, [2]ICFI, Rockville, MD, United States, [3]INDEPTH Network, Accra, Ghana

Determination of cause-specific mortality rate in developing countries is a major difficulty due to poor vital registration systems, deaths occurring outside health facilities, and deaths not being medically certified. Verbal autopsy conducted on these deaths have proven to be one of the reliable methods of compiling cause of death data. We sought to validate the causes of death assigned by physician coders and provide a cause of death structure to improve estimates on cause specific mortality in Ghana. Longitudinal VA data from the Navrongo Health and Demographic Surveillance System (NHDSS) from 2007-2011 was used. Physicians were retrained on death certification and coding using ICD 10 codes and Sample Vital Registration with Verbal Autopsy (SAVVY) methods. VA forms were recoded using the SAVVY methods. In all, 7086 VA forms were retrieved and recoded using SAVVY methods. Males constituted 56% of total deaths and 60% of deaths occurred outside health facilities. The main causes of neonatal deaths were neonatal sepsis (31.5%), birth asphyxia (18.1%) and low birth weight with prematurity (15.4%). Malaria (37.0%), diarrhea (13.1%) and acute respiratory infection (11.6%) were the leading causes of death among children aged 1-11 months. The main causes of death for 1-4 year olds were malaria (53.2%), diarrheal diseases (9.0%), and unspecified infectious diseases (4.7%). Among children 5- 15 years, the main causes of death were malaria (22.5%), accidental drowning, submersions and falls (14.6%) and meningitis (7.7%). For those above 15 years, unspecified non-communicable diseases (14.7%), malaria (6.9%) and cerebro-vascular diseases (6.5%) were the main causes of death in the districts. Variability between two coders using SAVVY method was fair (49.7%; $P<0.001$) with a higher value for neonatal deaths compared to adults. Despite the limitations of VA data the method provides an understanding of the cause of death structure at the population level in developing countries comparable with global estimates that is not possible with existing sources of data.

1873

FALSIFIED MEDICINES IN AFRICA AND PUBLIC HEALTH - 'NO ACTION-TALK ONLY'

Paul Newton[1], Patricia Tabernero[2], Prabha Dwivedi[3], Maria Culzoni[3], Maria Monge[3], Isabel Swamidoss[4], Dallas Mildenhall[5], Michael Green[4], Richard Jähnke[6], Miguel Santos de Oliveira[7], Julia Simao[7], Nicholas White[8], Facundo Fernández[3]

[1]Mahosot Hospital, Vientiane, Lao People's Democratic Republic, [2]University of Oxford, Oxford, United Kingdom, [3]Georgia Institute of Technology, Atlanta, GA, United States, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]GNS Science, Twin Hut, New Zealand, [6]Global Pharma Health Fund, Frankfurt, Germany, [7]Inspecção Geral de Saúde, Luanda, Angola, [8]Mahidol University, Bangkok, Thailand

Poor quality medicines are neglected impediments to improving global public health. In June 2012 suspected falsified medicines labelled as the antimalarial 'artemether-lumefantrine' bearing the Affordable Medicines Facility-malaria (AMFm) logo and others labelled as the antihelminthic 'mebendazole' were seized in Luanda, Angola. The tablets were analysed by an array of analytical platforms including high performance liquid chromatography, ambient ionization mass spectrometry, Raman spectroscopy, X-ray powder diffraction analysis, nuclear magnetic resonance spectroscopy, isotope-ratio mass spectrometry, botanical assays and packaging analysis, using the portable counterfeit detection device CD-3. No artemether or lumefantrine or other active pharmaceutical ingredients were detected in the 'artemether-lumefantrine' tablets. Brushite and three different yellow dyes and few pollen grains were found. No mebendazole was detected in the 'mebendazole' formulation, but calcite and levamisole (270mg/tablet) were present. Both 'products' showed marked differences in packaging characteristics from genuine products. The discovery of falsified artemether-lumefantrine, labelled as an AMFm product and without any detectable antimalarial, is of considerable concern for malaria control. Presence of levamisole in falsified 'mebendazole' is also of great concern as it has been banned for human use. This seizure illustrates many of the current problems regarding poor reporting and transparency and inaction. Enhanced collaboration between African MRAs/police and the authorities in China to stop criminal transcontinental trade in falsified essential medicines is urgently needed. Delays in reporting and action must be reduced by mandatory notification systems and independent public health risk assessments. Despite multiple reports, public health research has failed to stimulate actions required to improve the quality of global drug supply.

1874

ENHANCING PUBLIC HEALTH RESPONSE TO CLIMATE-SENSITIVE INFECTIOUS DISEASE OUTBREAKS IN FLOOD-PRONE AREAS OF BANGLADESH: ARE PRIMARY HEALTHCARE FACILITIES READY TO RESPOND?

Farhana Haque[1], Mahmudur Rahman[2], Selina Khatun[2], Mujaddeed Ahmed[2], M. Mushtuq Husain[2], Nuzhat Nasreen Banu[2], Md Saiful Islam[3], Md Kamal Hossain[3], Stephen P. Luby[4], Emily S. Gurley[3], James D. Heffelfinger[5]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh and Institute of Epidemiology, Disease Control and Research, Dhaka, Bangladesh, [2]Institute of Epidemiology, Disease Control and Research, Dhaka, Bangladesh, [3]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [4]International Centre for Diarrhoeal Disease Research, Bangladesh and Centre for Infectious Disease and Geographic Medicine, Stanford University, Stanford, CA, United States, Dhaka, Bangladesh, [5]International Centre for Diarrhoeal Disease Research, Bangladesh and Global Disease Detection Branch, Division of Global Health Protection, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States, Dhaka, Bangladesh

Global climate change is increasing Bangladesh's vulnerability to natural disasters including floods and raising the potential for infectious

outbreaks. Preparing healthcare for outbreak management is complex and requires comprehensive understanding of the systems' capacities and challenges. We evaluated preparedness of government primary care facilities in flood-prone sub-districts to respond to outbreaks. We asked 69 primary care managers to complete a self-administered survey to assess 7 core capacities critical for outbreak response. We determined percentages of facilities reporting each capacity; assessed managers' perceived preparedness level; and conducted 2 focus group discussions (FGDs) with 24 managers to explore contextual factors driving preparedness. Qualitative data were analyzed using thematic content analysis. All 69 facilities lacked preparedness plans, emergency stockpiles, funds and authority to buy drugs or supplies locally; all lacked dedicated evaluation, isolation and vehicles for infectious patients; 58% lacked sufficient clinicians and 20% lacked adequate field staff. Rapid response teams and digital communication with referral centers existed in all, limited infection control practice in 75%, and diagnostic laboratory in 80% facilities. Unlike lower stockpiling, infrastructure and human resource capacities, higher surveillance, communication, infection control and laboratory capacities were observed. Sixty-three (91%) managers felt their facilities had limited preparedness. There was moderate (71%; Kappa=0.3; P=0.001) correlation between measured and perceived preparedness. FGDs identified insufficient training of providers, low motivation and rapid turnover of clinical staff, and lack of epidemiologists posted at facilities as impediments to human resource capacity. Using a mixed methods approach, this assessment identified deficiencies in all capacities, indicated major gaps in stockpiles, infrastructure and human resource as priority areas for investing constrained resources and offered baseline data for monitoring progress in preparedness. This may be a feasible methodology to evaluate health systems in low-income countries. Development of a contingency plan that secures emergency financing and improves surge capacity by establishing a system to engage existing field workers in outbreaks can sustainably enhance primary care preparedness.

1875

A SYSTEMATIC REVIEW ON THE EFFECTIVENESS OF STRATEGIES TO IMPROVE HEALTH WORKER PERFORMANCE IN LOW- AND MIDDLE-INCOME COUNTRIES: PRELIMINARY RESULTS ON HEALTH OUTCOMES

Alexander K. Rowe[1], Samantha Y. Rowe[1], David H. Peters[2], Kathleen A. Holloway[3], John Chalker[4], Dennis Ross-Degnan[5]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Johns Hopkins University, Baltimore, MD, United States, [3]World Health Organization, New Delhi, India, [4]Management Sciences for Health, Arlington, VA, United States, [5]Harvard Medical School, Boston, MA, United States

Health workers (HWs) play essential roles in delivering health care. In low- and middle-income countries (LMICs), however, HW performance is often inadequate. To characterize the effectiveness of strategies to improve HW performance in LMICs, we conducted a systematic review of 15 electronic databases, 30 document inventories of international organizations, and bibliographies of 510 articles. We included studies meeting accepted criteria for methodological adequacy (e.g., trials with comparison groups) of any strategy on any health topic in any language, published or not. After screening, data from relevant reports were double-abstracted and entered into a database. This analysis focuses on studies that measured health outcomes (morbidity and mortality rates). Effect sizes were calculated as percent change over time in the intervention group minus percent change over time among controls. We screened >105,000 citations, 829 reports met inclusion criteria, and 60 studies measured health outcomes (28 on morbidity only, 24 on mortality only, and 8 with both). Many strategies have been tested, usually with multiple intervention components. The median effect size (MES) across all studies was an improvement of 9 percentage-points (%-points) (interquartile range [IQR]: 0, 39). Among 45 studies focused on facility-based HWs, the strategy with the greatest health impact was HW training + group problem solving (MES = 49 %-points, IQR: 24, 77). Often used strategies, such as HW training and supervision, alone or in combination, had lower effect sizes (typically ranging from no effect to +16 %-points). Among 15 studies focused only on community HWs, the strategy with the greatest health impact was consumer supports (e.g., patient education) + HW training + providing drugs or equipment (MES = 55 %-points, IQR: 15, 62). Contextual and methodological heterogeneity made comparisons difficult. Results from this review, which will be finalized by the end of 2014, should inform decision-making on how best to improve HW performance and health outcomes in LMICs.

1876

PREVALENCE OF TRACHOMA IN BRAZILIAN SCHOOLCHILDREN

Expedito J. Luna[1], Maria F. Lopes[2], Norma H. Medina[3], Maria R. Cardoso[1]

[1]Universidade de Sao Paulo, Sao Paulo, Brazil, [2]Ministerio da Saude, Brasilia, Brazil, [3]Secretaria Estadual de Saude de Sao Paulo, Sao Paulo, Brazil

The aim of the study was to estimate the prevalence and describe the distribution of trachoma among schoolchildren in Brazil. We conducted a cross-sectional study, using cluster sampling of the schoolchildren population, living in Brazilian municipalities with Human Development Index lower than the national mean. This prevalence survey was conducted by the Brazilian Ministry of Health, in the period 2002-2008. 3,144 schools, with 176,224 schoolchildren, from 1st to 4th grades, located in 1,491 municipalities, were selected. The selected schoolchildren underwent an external ocular examination, with a magnifying glass (2.5X), to detect clinical signs of trachoma according to the WHO grading criteria. The prevalence of trachoma, by state and national level, and their respective 95% confidence intervals were estimated. Chi-square and chi-square for trends tests were used to compare categorical variables. 8,526 cases of trachoma were detected, resulting in a prevalence of 5.00% (95%IC 5.05; 4.95). Most cases were mild (TF prevalence = 4.92%). Prevalence of intense inflammatory trachoma (TI) and trachomatous scarring (TS) was low: 0.03% and 0.05% respectively. There was no significant difference between the sexes. The prevalence of trachoma was 10.8% among children under 5 years of age, decreasing as age increased (chi square for trend $p < 0.00001$). There was a significant difference in prevalence between urban and rural areas, 4.3% *versus* 6.2% respectively ($p < 0.001$). Cases were detected in 1,189 municipalities (80% of the municipalities in the sample), in all 27 states of the country. In 37% of the selected municipalities, the prevalence was higher than 5%. The study has shown that trachoma is still endemic in a large proportion of the poorer Brazilian municipalities, contradicting the belief that the disease had been controlled in the country. The survey provided a baseline for evaluating planned interventions aimed at achieving the goal of certification of elimination of trachoma as a cause of blindness in Brazil by 2020.

1877

BACTERIAL LOAD AND PATHOGEN DIVERSITY IN OCULAR INFECTION WITH *CHLAMYDIA TRACHOMATIS* IN A TRACHOMA-HYPERENDEMIC ISLAND SETTING

Anna Last[1], Fengchen Liu[2], Travis Porco[2], Chrissy Roberts[1], Eunice Cassama[3], Meno Nabicassa[3], Sarah Burr[4], Taane Clark[1], Francesc Coll[1], Nick Thomson[5], Thomas Lietman[2], Martin Holland[1], Robin Bailey[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]University of California San Francisco, San Francisco, CA, United States, [3]Ministerio de Saude Publica, Bissau, Guinea-Bissau, [4]Medical Research Council The Gambia Unit, Fajara, Gambia, [5]Wellcome Trust Sanger Institute, Cambridge, United Kingdom

Trachoma is caused by ocular infection with *Chlamydia trachomatis (Ct)*. Acute conjunctival infection may recur and progress to a chronic inflammatory process causing conjunctival scarring and blindness. The

reasons why active and scarring trachoma are so prevalent on the Bijagós Archipelago of Guinea Bissau are unclear. We collected 1507 population-based conjunctival swabs with corresponding detailed clinical phenotype. We used droplet digital PCR assay to detect and quantitate *Ct* DNA on swabs. Associations between *Ct* load and clinical phenotype were examined using regression models. We used agent-based modeling to investigate the role of *Ct* load in trachoma transmission. Whole genome sequence analysis was used to identify variants in putative virulence-associated genes/loci. The geometric mean of estimated *Ct* load in clinically normal conjunctivae was 294 copies/swab (95% C.I. 165-524). In clinically active trachoma it was 8562 copies/swab (95% C.I. 5412-13546). In active trachoma *Ct* load increases with disease severity (for both follicular and inflammatory scores). The highest *Ct* loads were associated with the most severe clinical disease and the strongest associations were with increasing inflammatory grade (at maximal inflammatory score (P3) OR 30.9, 95% CI 9.39-101.5, *p<0.0001*). Genotypic differences in virulence-associated genes within this population of ocular *Ct* are suggested. The association between load and disease severity may be related to *Ct* strain diversity, where multiple strains are co-circulating. We used a novel mathematical modeling strategy to investigate the role of *Ct* load in trachoma transmission. This is the first application of these approaches in understanding the pathogenesis and transmission of *Ct* infection, which are fundamental to successful trachoma elimination and surveillance strategies.

1878

ASSESSING THE BURDEN OF PEDIATRIC ACUTE RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE --- AMERICAN SAMOA, 2011-2012

Amanda L. Beaudoin[1], Laura Edison[1], Camille E. Introcaso[2], Lucy M. Goh[3], Chris Van Beneden[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Pennsylvania Hospital, Pennsylvania Center for Dermatology, Philadelphia, PA, United States, [3]Lyndon Baines Johnson Tropical Medical Center, Pago Pago, American Samoa

In August 2013, LBJ Tropical Medical Center and the American Samoa (AS) health department notified CDC of a perceived high burden of pediatric acute rheumatic fever (ARF) and rheumatic heart disease (RHD). ARF is an immunologically mediated sequela of inadequately treated group A *Streptococcus* pharyngitis and, potentially, pyoderma. Recurrent or severe ARF can cause permanent cardiac damage and RHD. Long-term prophylactic penicillin injections post-ARF diagnosis can prevent RHD. We aimed to describe pediatric ARF and RHD and prophylaxis in AS and the pyoderma-ARF association. We used ICD-9 codes and hospital prophylaxis registries from AS's only medical system to identify all patients aged ≤18 years with a physician-recorded ARF or RHD diagnosis during 2011-2012. We recorded penicillin compliance and pre-ARF pharyngitis and pyoderma diagnoses (≤6weeks preceding) for cases. Two age- and sex-matched control subjects per case-patient were selected from non-ARF/RHD patients examined during 2011-2012. We calculated ARF 2011-2012 incidence and RHD prevalence by using 2010 U.S. Census data. We used univariate statistical tests and conditional logistic regression for case-control comparisons. During 2013, RHD prevalence was 3.2 cases/1,000 children. ARF incidence was 1.1 (2011) and 1.5 (2012) cases/1,000. Of 65 children diagnosed with ARF during 2011-2012, a total of 32 (49%) subsequently received RHD diagnoses. Median ARF diagnosis age was 11 (range: 2-18) years. Pharyngitis history was more common among case-patients (18%) than control subjects (0%; P <0.01), but preceding pyoderma was not. Post-ARF penicillin prophylaxis compliance (65%) was suboptimal. RHD causes considerable childhood morbidity in AS. Although the pyoderma-ARF association remains unclear, attempts to curb AS's RHD burden should address improved pharyngitis diagnosis and treatment and increased ARF prophylaxis compliance.

1879

REPRODUCTIVE TRACT INFECTIONS AMONG PRIMARY SCHOOLGIRLS IN RURAL WESTERN KENYA

Newton Otecko[1], Emily Kerubo[1], Elizabeth Nyothach[1], Alex Morwabe[1], Harrison Fredrick[1], Kelvin Oruko[1], Clifford Oduor[1], Preston Owiti[1], John Vulule[1], Frank Odhiambo[1], Kayla Laserson[2], Clement Zeh[2], Penelope Phillips-Howard[3]

[1]Kenya Medical Research Institute-Centers for Disease Control and Prevention Research and Public Health Collaboration, Kisumu, Kenya, [2]Centers for Disease Control and Prevention, Kisumu, Kenya, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Reproductive tract infections (RTIs) among adolescent girls remain a great public health concern in developing countries. However, the burden of RTIs in this key population is insufficiently understood. This study presents preliminary analysis of RTIs' symptoms reporting and laboratory detection rates among girls aged 14-16 (median 14) years, in 30 primary schools, enrolled in a feasibility study on acceptance, use and safety of menstrual products in western Kenya. Vaginal self-swabbing samples were prospectively collected during symptom guided RTI testing (SGT) (March-October 2013) and cross-sectional end-of-study screening (EOSS) (November 2013). Samples were analyzed for Bacterial vaginosis (BV), Chlamydia trachomatis (CT), Neisseria gonorrhea (NG), Trichomonas vaginalis (TV) and Candidiasis. Infected girls were referred for treatment. Data were analyzed using SPSS v.21.0. Overall, 532 girls (SGT: 17, 3.2%; EOSS: 453, 85.2%; and overlap in both: 62, 11.7%) were included. Of a total 79 girls in SGT group, BV 13 (16.5%), Candidiasis 11 (13.9%), TV 5 (6.3%) and CT 2 (2.5%) were test confirmed. None tested positive for NG. BV was the most common in EOSS, 94 (18.3%), followed by Candidiasis 44 (8.5%), TV 13 (2.5%), CT 13 (2.5%) and NG 3 (0.6%). Of 62 girls in both STG and EOSS, RTI detection rates varied (SGT-EOSS) for BV (19.4%-14.5%), Candidiasis (14.5%-12.9%), TV (6.5%-0%), CT remained constant. While only 82 (15.9%) girls reported symptoms for RTI at EOSS, laboratory testing showed 146 (28.3%) had at least one RTI. In conclusion, high detection rate of RTIs was observed among the rural adolescent schoolgirls. Symptom-based diagnosis of RTIs poorly predicted RTI in this population. These findings offer important insights for treatment and prevention of RTIs among schoolgirls.

1880

ACCURACY OF THE INTEGRATED MANAGEMENT OF CHILDHOOD ILLNESS (IMCI) ALGORITHM IN IDENTIFYING CULTURE-CONFIRMED DIARRHEAL PATHOGENS REQUIRING ANTIBIOTIC THERAPY

Patricia B. Pavlinac[1], Frankline M. Onchiri[1], Grace C. John-Stewart[1], Jaqueline M. Naulikha[1], Denno M. Donna[1], Elizabeth A. Odundo[2], Benson O. Singa[3], Lisa E. Manhart[1], Judd L. Walson[1]

[1]University of Washington, Seattle, WA, United States, [2]United States Army Medical Research Unit, Kericho, Kenya, [3]Kenya Medical Research Institute, Nairobi, Kenya

Diarrhea is the second leading cause of death in children under 5, with most deaths occurring in settings where microbiology facilities are unavailable or cost prohibitive. The World Health Organization (WHO) developed the Integrated Management of Childhood Illness (IMCI) guidelines to manage sick children based on clinical signs and history. For children with diarrhea, the guidelines recommend empiric antibiotics for children with suspected shigellosis (presence or history of bloody stool) or suspected cholera (age ≥2 years, severe dehydration, and living in a cholera endemic area). We assessed the diagnostic performance of the IMCI guidelines for diarrhea management as compared to stool bacterial culture. Children aged 6 months to 5 years presenting to two Western Kenya District hospitals between December 2011 and September 2013 with acute diarrhea were enrolled. Stool samples were tested using standard methods for bacterial culture. Multiplex PCR was used to further classify diarrheagenic *Escherichia coli*. Among 973 enrolled children,

median age was 17 months (interquartile range 10-34), 16.5% were stunted, and 4.4% were HIV-infected. The most predominate bacterial isolate was EAEC (14.1%), followed by *Campylobacter* (6.6%), EPEC (6.2%), *Shigella* (4.6%), and ETEC (4.4%). IMCI correctly classified 3 of 45 lab-confirmed *Shigella* cases (sensitivity 6.7%), 2 cases of *Shigella flexneri* and 1 *S. dysenteriae*. Among 928 children without shigellosis, IMCI correctly classified 871 (specificity 93.9%). Of the 57 children incorrectly diagnosed with *Shigella* by IMCI, 73.7% had no other bacterial pathogen identified. Cholera was not detected although 11 (1.1%) children were classified as having suspected cholera based on IMCI criteria (specificity 98.9%); of these 11, 36.4% had no isolated bacteria. The IMCI guidelines appear reasonably specific but not sensitive in identifying children requiring antibiotic therapy. IMCI guidelines should be adapted to enhance sensitivity and to account for additional enteric pathogens associated with increased morbidity and mortality.

1881

EVALUATION OF INTEGRATED MANAGEMENT OF ADOLESCENT AND ADULT ILLNESS DISTRICT CLINICIAN MANUAL EMPIRIC ANTIMICROBIAL THERAPY RECOMMENDATIONS FOR SEVERE INFECTIONS IN NORTHERN TANZANIA

Matthew P. Rubach[1], Venance P. Maro[2], John A. Bartlett[1], John A. Crump[1]

[1]Duke University, Durham, NC, United States, [2]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania

We assessed the effectiveness of Integrated Management of Adolescent and Adult Illness District Clinician Manual (IMAI) empiric antimicrobial therapy recommendations for septic shock, severe respiratory distress without shock, and severe pneumonia in hospital settings in northern Tanzania. IMAI recommended empiric therapies were retrospectively evaluated against laboratory-confirmed etiology of illness data for participants in a febrile illness cohort study who met IMAI criteria for the three clinical syndromes. Therapies evaluated included IMAI emergency antibacterials (ceftriaxone or ampicillin plus gentamicin) for septic shock and severe respiratory distress without shock, and ceftriaxone plus a macrolide for severe pneumonia. Among 423 participants hospitalized with febrile illness, 171 cases met IMAI criteria for the three syndromes: 25 septic shock, 37 severe respiratory distress without shock, and 109 severe pneumonia. Forty-four (10%) of 423 participants died in-hospital. Ceftriaxone was the single-most effective agent in all three syndromes, being effective for 12 (48%) septic shock, 5 (14%) severe respiratory distress without shock, and 18 (17%) severe pneumonia illnesses. For each syndrome 17-27% of participants had an etiologic diagnosis non-responsive to ceftriaxone, but responsive to other available antimicrobial regimens, namely amphotericin for cryptococcosis and histoplasmosis; anti-tuberculosis therapy for bacteremic disseminated tuberculosis; or tetracycline therapy for rickettsioses and Q fever. IMAI recommendations for empiric ceftriaxone to treat septic shock, severe respiratory distress without shock, and severe pneumonia are warranted. Etiologies not explicitly addressed in IMAI guidance for these syndromes, such as cryptococcosis, histoplasmosis and tetracycline-responsive bacterial infections, were common. Prospective assessments of IMAI are needed to confirm these results and improve syndromic management algorithms.

1882

IMPACT OF FUTURE CLIMATIC CONDITIONS ON VIRAL, BACTERIAL AND PROTOZOAN ENTERIC PATHOGENS

Karen Levy, Sharia M. Ahmed, Rebecca Philipsborn, Lily M. Tyndall, Berry J. Brosi

Emory University, Atlanta, GA, United States

Understanding the seasonality of infectious diseases is important, in order to prepare for case loads, plan vaccination campaigns, and to anticipate impacts of climate change. Diarrheal diseases are often cited as one of the major health impacts of climate change, but there much uncertainty remains in the estimates associated with the relationship between climatic drivers and diarrheal disease. One source of this uncertainty is the variety of pathogens associated with infectious diarrhea, each of which has different life cycle characteristics such as survival outside of the host. Therefore it is important to understand how the seasonality of different etiological agents of diarrheal disease vary. In this systematic review and meta-analysis, we examined the impact of climatic variability on three representative diarrheal disease pathogens of different taxa: pathogenic E. coli, norovirus, and cryptosporidium. Incidence data for each pathogen were taken directly from tables or extracted from graphs in the published papers. Year-specific monthly temperature and precipitation from each location at the time of disease data collection were assembled from publicly available datasets. We examined the relationship between climatic variables and incidence of each pathogen for each location using generalized log-linear Poisson regression models, and we also pooled all datasets for each pathogen to calculate an overall association between monthly cases and mean monthly temperature, using a generalized estimating equation. We then used the model results to examine what proportion of the total of cases attributable to these three pathogens would be attributable to any one of the pathogens given increases in temperature of 1-4°C, in increments of one degree. We found that a one-unit increase in temperature was associated with increases in incidence of pathogenic E. coli (IRR = 1.08, 95% CI = 1.04-1.11) and Cryptosporidium (IRR=1.03, 95% CI = 1.03-1.04) and decreases in Noroviurs (IRR = 0.92, 95% CI: 0.90-0.94). These results highlight the importance examining taxa-specific climate-disease relationships for enteric diseases. As temperatures increase under future warming scenarios, bacterial and protozoan pathogens are expected to represent an increasingly large fraction of the burden of diarrheal disease. This has important implications for development of control strategies.

1883

FLY ME TO THE PLUME: VIDEO-TRACKING ANALYSIS OF *ANOPHELES GAMBIAE* FLIGHT BEHAVIOR AT HUMAN-BAITED BEDNETS

Josephine Parker[1], Natalia Angarita-Jaimes[2], Mayumi Abe[1], Fabian Mashauri[3], Jackline Martine[3], Catherine E. Towers[2], David Towers[2], Philip J. McCall[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Optical Engineering Group, School of Engineering, University of Warwick, Coventry, United Kingdom, [3]National Institute for Medical Research, Mwanza, United Republic of Tanzania

Understanding how mosquitoes interact with insecticide-treated bednets (LLINs) is fundamental to advancing the design and performance of LLINs, and to ensuring they continue to be effective and sustainable tools for malaria prevention. We have developed an innovative video system that enables nocturnal flight activity of multiple mosquitoes at a human occupied bednet to be captured at high resolution, and individual mosquito flight paths and details of movements to be tracked and analysed over periods of 60 minutes or more. Following initial laboratory studies, the system has been deployed in an experimental hut at a field site in Tanzania, where we are investigating behaviour of local *Anopheles* sp. populations entering the hut in response to human baits in untreated and insecticide-treated bednets. Analysing flight tracks, we have classified mosquito activity into four broad types, termed 'swooping', 'visiting', 'bouncing' and 'resting'. Mosquitoes flew more slowly and flight paths were more tortuous when nets were baited. Responding to human bait, most activity was spent in flight. The majority of contacts made with the net surface were very brief (duration less than 4 seconds) and activity occurred primarily on the top surface of the net over the sleeper's torso, with less activity seen at the supine human's feet. This finding is consistent with previous studies suggesting that hostseeking mosquitoes orient towards a 'plume' of host attractants, funnelled upwards by the 'chimney' effect of the bednet walls. We compared activity on untreated nets with Permanet 2.0 (deltamethrin-treated LLINs) to examine how treatment

influenced mosquito behaviour. Results investigating flight patterns and visiting patterns at the net surfaces, changes in activity patterns over time and LLIN repellency will be presented and implications for current and future LLIN-based approaches will be considered.

1884

SWARMING BEHAVIOR OF *ANOPHELES GAMBIAE* MALES INCREASES FEMALE INSEMINATION RATE IN CAGED POPULATIONS: AN OPPORTUNITY TO STUDY MOSQUITO MATING SYSTEMS

Luca Facchinelli[1], Laura Valerio[1], Clelia Oliva[2], Rosemary S. Lee[2], Tania Persampieri[1], C. Matilda Collins[3], Andrea Crisanti[1], Roberta Spaccapelo[1], Mark Q. Benedict[1]

[1]University of Perugia, Perugia, Italy, [2]Polo d'Innovazione Genomica, Genetica e Biologia S.C.a.R.L., Perugia, Italy, [3]Imperial College London, London, United Kingdom

Long-term control of *Anopheles gambiae*, the major malaria vector in Africa, is difficult to achieve and maintain with traditional control methods. Genetically Modified (GM) *A. gambiae* strains that bear sterility genes are potentially powerful new tools to control the disease. Natural male mating behavior is a key component in GM mosquito control strategies in order to spread transgenes into the wild type population. Mating behavior involves the ability of males to form swarms, which is crucial to inseminate females in the wild. In spite of its biological importance and relevance for vector control, swarming is a poorly understood process because it is hard to study in the wild and difficult to stimulate under laboratory conditions. Here we describe features that promote male *A. gambiae* G3 strain swarming in large cages. In 15.6 m3 cages, a dark foreground and contrasting illuminated background with a contrasting mark on the ground stimulated swarm formation during artificial twilight. G3 males have not lost their capability to swarm although this strain has been colonized since 1975. We asked whether swarming behavior would affect mating performance of wild-type (WT) G3 and I-PpoI transgenic *A. gambiae* sexually sterile males competing for G3 females. We performed competitive matings and recorded female insemination rate and proportion of matings by WT and GM males. The presence of swarming stimuli was associated with an increase in mating frequency from 77.4 to 97.4 %. There was no change in competitiveness by transgenic males as a function of swarming stimuli. The increase of mating frequency in the presence of swarming stimuli highlights the importance of swarming in *A. gambiae* mating behavior. Reproducing *A. gambiae* swarms in controlled conditions provides the possibility to dissect the mating behavior of this species and explain the mechanisms controlling it, which is innovative in mosquito research. We will discuss the results and the possible applications of our findings to investigation of *A. gambiae* biology and to support vector control strategies

1885

NOVEL INSIGHTS INTO GENETIC CONTROL USING EXPERIMENTAL AND MATHEMATICAL SIMULATIONS OF LATE-ACTING LETHAL EFFECTS ON POPULATIONS OF *AEDES* SPP. MOSQUITOES

Mark Q. Benedict[1], Alessandro Bocci[1], Michael A. Robert[2]

[1]University Perugia, Perugia, Italy, [2]University New Mexico, Albuquerque, NM, United States

Mathematical models have been used to predict that late-acting lethal transgenic mosquitoes provide enhanced control of target mosquito populations by maintaining competition in the larval stage. The expected effect is to diminish larval survival among the wild-type larvae, and in comparison with situations in which such competition is not present (e.g. conventional sterile insect technique - SIT), improved control. Mathematical simulations often include simplifying assumptions that might not reflect biological realities and yet provide useful frameworks for predicting the relative value of various control approaches - that is, if they include critical effects of the control measure. While it is usually not possible to simulate large populations with laboratory experiments, it useful to test critical predictions experimentally when such methods can be devised. We will describe experiments conducted to determine whether a previously published model of late-acting lethal transgenic mosquitoes adequately includes critical biological factors of the technology, specifically effects on development rates and survival. We performed laboratory simulations of late-acting lethality and conventional sterile insect technique to determine the effect on the development rate and survival of two *Aedes* species larvae. We also considered the results and novel experimental variables in the context of previous models of control of mosquito populations using late-acting lethals in comparison with SIT.

1886

ANTIMALARIAL AND ANTI-DENGUE PROPERTIES OF A NATURAL *CHROMOBACTERIUM* MOSQUITO MIDGUT COMMENSAL

Raúl G. Saraiva, Yuemei Dong, Abhai Tripathi, José L. Ramirez, George Dimopoulos

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Plasmodium and dengue virus, the causative agents of the two most devastating vector-borne diseases, malaria and dengue, are transmitted by the *Anopheles gambiae* and *Aedes aegypti* mosquito vectors, respectively. We have identified a novel *Chromobacterium* species in adult *A. aegypti* collected in Panama, *Csp_P*, that can effectively colonize the midgut of *A. gambiae* and *A. aegypti* mosquitoes when introduced through an artificial nectar meal. We have shown that this isolate exerts entomopathogenic activity against both of the mosquito species along with *in vivo* and *in vitro* anti-*Plasmodium* and anti-dengue activities. Interestingly, the well characterized *Chromobacterium violaceum* does not exert such effect. Upon bioassay-guided fractionation of supernatants of a *Csp_P* culture, we were able to map the antiparasitic and antiviral properties to a fraction significantly enriched in a previously characterized cyclic dehydropeptide lactone. This bacterial secondary metabolite was previously pursued as an antifungal, being part of a complex of closely related molecules. We have produced *n*-butanol-based extracts of *Csp_P* cultures that retain *in vitro* activity against blood-stage *Plasmodium* and dengue virus, as well as against the yeast *Saccharomyces cerevisiae*. We are currently pursuing mass spectrometry analysis to characterize the compounds behind the antipathogenic activity of our extracts, along with efforts to identify the gene cluster responsible for production of such compounds by means of both comparative genomics and a transposon-mediated random mutagenesis screening. To our knowledge, this is the first identified bacterium that exerts broad spectrum entomopathogenic and antipathogenic activities, thereby rendering it an interesting candidate for the development of novel vector-borne disease control strategies.

1887

THE EFFICACY OF LONG-LASTING NETS WITH DECLINING PHYSICAL INTEGRITY MAY BE COMPROMISED IN AREAS WITH HIGH LEVELS OF PYRETHROID RESISTANCE

Eric O. Ochomo[1], Nabie M. Bayoh[2], Edward D. Walker[3], Bernard O. Abong'o[2], Maurice O. Ombok[2], Collins Ouma[1], Andrew K. Githeko[4], John M. Vulule[4], Guiyun Yan[5], John E. Gimnig[6]

[1]Maseno University, Kisumu, Kenya, [2]KEMRI/Centers for Disease Control and Prevention, Kisumu, Kenya, [3]Michigan State University, East Lansing, MI, United States, [4]Kenya Medical Research Institute, Kisumu, Kenya, [5]University of California, Irvine, CA, United States, [6]Centers for Disease Control and Prevention, Atlanta, GA, United States

Long-lasting insecticide-treated mosquito nets (LLINs) are a primary malaria prevention tool in sub-Saharan Africa but emergence of insecticide resistance threatens their effectiveness. Cross-sectional surveys of LLINs were conducted in houses of seven and four villages in Gem and Bungoma

Districts in western Kenya, respectively in May 2013. LLIN condition (number and area of holes), number and species of mosquitoes resting inside, and insecticidal activity of LLINs were quantified. Mosquitoes collected inside nets were allowed to lay eggs and the progeny were tested for susceptibility to deltamethrin and permethrin, pyrethoids commonly deployed in LLINs in western Kenya. In Gem, 83.3% of LLINs were less than three years old and 32.4% had at least one hole of any size; while in Bungoma, 92% were less than three years old and 48% had at least one hole. No anopheline and five Culex spp. mosquitoes were found resting inside LLINs in Gem (N=216) regardless of the number and size of holes, while 552 *Anopheles gambiae* s.l., five *An. funestus* s.l. and 137 *Culex* spp. were found inside LLINs (N=216) in Bungoma. The number of mosquitoes resting inside LLINs increased with hole areas >50 cm^2 in Bungoma. In WHO resistance assays, f1 offspring of fed or gravid females collected in nets in Bungoma had 6% and 35% mortaltiy to deltamethrin and permethrin, respectively. LLINs from Bungoma retained strong activity against a susceptible laboratory strain achieving >90% mortality in all bioassays (N=99), but mortality of f1 offspring of field-collected *An. gambiae* s.s. in cone tests was <60% in all assays (N=99) All *An. gambiae* s.s. samples collected in LLINs were homozygous for the kdr genotype L1014S. In conclusion, LLINs develop holes within three years of distribution. In areas with pyrethroid resistance, mosquitoes are able to enter LLINs and survive. LLINs with >50cm^2 of damage were more likely to harbour mosquitoes than nets with no holes. The data indicate that a small amount of damage could compromise the protective efficacy of nets in areas with high levels of pyrethroid resistance.

1888

INDOOR USE OF ATTRACTIVE TOXIC SUGAR BAIT (ATSB) FOR CONTROL OF MOSQUITOES AND FOR RESISTANCE MANAGEMENT

Mark Rowland[1], Zachary Stewart[1], Richard Oxborough[1], Patrick Tungu[2], Matthew Kirby[1], Seth Irish[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]National Institute for Medical Research, Tanga, United Republic of Tanzania

Attractive toxic sugar bait (ATSB), a mixture on insecticide and sugar solution, sprayed onto vegetation has been successful in controlling *Anopheles* mosquitoes outdoors. Indoor application of ATSB has yet to be explored. This study determined whether ATSB stations positioned inside the home would kill host-seeking mosquitoes and constitute a new approach to control of malaria. Classes of insecticides new to malaria vector control were mixed with sugar solution and tested as toxic baits against *Anopheles* in feeding bioassay tests. The most promising ATSB candidates were then trialed in experimental huts in Tanzania against free flying, host seeking mosquitoes. The ATSB stations were hung from ceilings of huts next to untreated mosquito nets occupied by human volunteers. In feeding bioassays, chlorfenapyr (a pyrrole), boric acid and tolfenpyrad (a mitochondrial electron transport inhibitor), mixed in a guava juice-based bait, each killed more than 90% of pyrethroid-susceptible *An. gambiae* s.s. and pyrethroid-resistant An. arabiensis at less than 1% w/v. In the experimental hut trial, the mortality rates of the three ATSB treatments were comparable to long lasting insecticidal nets (LLINs) tested against the same species in the same area. Indoor ATSB constitute a novel application method for insecticide classes that act as stomach poisons and have not been exploited for mosquito control hitherto. Combined with LLIN, indoor use of ATSB has the potential to serve as a strategy for managing insecticide resistance.

1889

FACTORS MEDIATING MATING SUCCESS IN MALE *ANOPHELES GAMBIAE* MOSQUITOES

Adam South[1], Paolo Gabrieli[2], Evdoxia Kakani[1], Flaminia Catteruccia[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Università degli Studi di Perugia, Terni, Italy

Human malaria, a major public health burden in tropical and subtropical countries, is transmitted exclusively by female *Anopheles* mosquitoes. Malaria control strategies aimed at inducing sexual sterility in natural vector populations are an attractive alternative due to increasing levels of insecticide resistance. However, the development of these strategies is hampered by a profound lack of knowledge regarding the most basic elements of *Anopheles* mating ecology. Females mate only once, and the suite of mating induced physiological and behavioral changes are predicated in large part upon the transfer of a mating plug containing steroid hormones (SH). Here we report mechanisms of pre and peri-copulatory sexual selection in *An. gambiae* mosquitoes and a role for SH as a possible selective mechanism. High-speed video analysis of mosquito mating swarms revealed definitive evidence of female choice, as females employ specific rejection and acceptance behaviors. Furthermore, video analysis revealed behavioral mechanisms of male competition. We show that successfully mating males are not only larger, but through an ELISA assay we demonstrate that they have significantly higher SH titers in their reproductive accessory glands relative to their unsuccessful counterparts. The mechanisms behind female discrimination is currently being investigated. Additionally, females mated to males with reduced SH levels have lower fecundity and fertility compared to females mated with controls. Given that previous work has demonstrated the importance of male SH in female reproductive phenotypes, fitness in both sexes of *An. gambiae* appears at least partially SH dependent. This work provides critical insights into the mating ecology of a major disease vector and implicates SH as a key factor determining fitness across sequential episodes of sexual selection. Moreover, these results extend our understanding of swarming and monogamous insect mating systems.

1890

STAGE-SPECIFIC, STRUCTURAL PROTEOMES AND THE NODULAR SECRETOME FROM *ONCHOCERCA OCHENGI*, THE CLOSEST RELATIVE OF THE HUMAN RIVER BLINDNESS PARASITE

Stuart D. Armstrong[1], Germanus S. Bah[1], Henrietta F. Ngangyung[2], Dong Xia[1], Samuel Wanji[3], Peter A. Enyong[4], Vincent N. Tanya[2], David W. Taylor[5], Mark L. Blaxter[5], Jonathan M. Wastling[1], **Benjamin L. Makepeace**[1]

[1]University of Liverpool, Liverpool, United Kingdom, [2]Institut de Recherche Agricole pour le Développement, Ngaoundéré, Cameroon, [3]Research Foundation for Tropical Diseases and Environment, Buea, Cameroon, [4]Tropical Medicine Research Station, Kumba, Cameroon, [5]University of Edinburgh, Edinburgh, United Kingdom

The bovine filaria *Onchocerca ochengi* is the closest extant relative of the human river blindness parasite, *Onchocerca volvulus*, and has been used as a natural model of onchocerciasis for two decades. The close parallels between these species include the propensity of the adult worms to form collagenous nodules and their obligate symbiosis with supergroup C strains of *Wolbachia*. To date, in-depth proteomic analysis of filarial structural proteomes has been restricted to a single species, *Brugia malayi,* which has a fundamentally different lifestyle to *O. volvulus.* Here, we report stage-specific proteomes of *O. ochengi* from intrauterine microfilariae, vector-derived L3, and adult female and male worms; alongside host and parasite excretory-secretory products identified in nodule fluid *ex vivo*. We applied a combination of anion exchange fractionation and geLC-MS with interrogation of a draft *O. ochengi* genome assembly to identify >4,600 filarial proteins and 176

proteins from *Wolbachia* strain *w*Oo (33% and 27% of their theoretical proteomes, respectively). Of the filarial proteins, 1,038 (22%) were common to all stages, whereas microfilariae exhibited the greatest number of stage-specific proteins (~920), despite direct harvesting of this material from adult female uteri. Proteins identified by geLC-MS alone accounted for <20% of the total for any single stage, but showed enrichment for membrane transporters, polyubiquitin and respiratory chain components. Preliminary analyses suggested that the relative abundance of galectins, calponins, myosins and antioxidant proteins varied between lifecycle stages. In nodule fluid, >2,000 proteins were identified (77% bovine, 23% filarial, 0.1% bacterial), with strong representation of bovine antimicrobial proteins and filarial transthyretin-like proteins. These data provide a rich resource for comparative analyses of filarial protein expression throughout the lifecycle, as well as supporting research efforts directed at the development of a filarial vaccine, new drugs and diagnostic biomarkers.

1891

TOWARDS IDENTIFICATION AND VALIDATION OF BIOMARKERS FOR THE QUANTIFICATION OF *LOA LOA* MICROFILARIAE (MF) USING PROTEOMIC ANALYSES OF BODY FLUIDS FROM MICROFILAREMIC *LOA*-INFECTED INDIVIDUALS

Papa M. Drame[1], Sasisekhar Bennuru[1], Zaojing Meng[2], Jesica A. Herrick[1], Zhou Ming[2], Thomas B. Nutman[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]National Institutes of Health, Frederick, MD, United States

Among parasitic helminths, *Loa loa* (Ll) presents a challenge for the mass drug administration programs in areas co-endemic for *Wuchereria bancrofti* and *Onchocerca volvulus* because of the severe adverse events (SAE's) in cases of very high Ll microfilaraemia. To identify microfilarial-derived Ll-specific biomarker(s) that could provide the basis for a microfilarial quantitative immunoassay, we characterized the excretory/secretory (E/S) proteome of Ll mf as well as the Ll-specific proteins found in urine and plasma of Ll-infected and -uninfected individuals using LC MS/MS. From 20 x 10^6 mf purified from the blood of Ll-infected patients and cultured *in vitro,* 1273 proteins (representing 8.2% of the *Ll* putative proteome) were identified. Among the most abundant proteins identified were endochitinase, cyclophilins, and a phosphatidyl ethanolamine binding protein. In addition several hypothetical proteins unique to *Ll* were identified. To further identify if any of these ES proteins were present in body fluids, proteomic analyses of urine and plasma of Ll-infected individuals (depleted of the top 12 to 20 human abundant proteins in plasma) resulted in the identification of 18 (from urine) and 29 (from plasma) Ll proteins found only in Ll-infected individuals that were identified by having at least 2 unique peptides. 4/18 antigens found in urine and 13/20 found in plasma have been selected for biomarker validation based on limited homology to other filarial species and, specific reactivity to polyclonal antibody raised to *Ll* mf ES. In addition, 9 of these tested to date were found to be immunogenic in humans (based on antigen-specific IgG4 reactivity by serum from mf+ Ll-infected plasma (n=30) and not by those from uninfected plasma (n=20)). Development and testing of rapid antigen capture immunoassays are underway to provide an alternative to more standard methods of mf quantification.

1892

VACCINATION WITH *BRUGIA MALAYI*-103 AND *BRUGIA MALAYI*-RAL-2 CONFER SIGNIFICANT PROTECTION AGAINST SUBCUTANEOUS CHALLENGE OF *B. MALAYI* INFECTIVE LARVAE IN MONGOLIAN GERBILS

Sridhar Arumugam[1], Bin Zhan[2], Junfei Wei[2], David Abraham[3], Danielle Ward[1], Sara Lustigman[4], Maria Bottazzi[2], Peter Hotez[2], Thomas Klei[1]

[1]Louisiana State University, Baton Rouge, LA, United States, [2]Baylor College of Medicine, Houston, TX, United States, [3]Thomas Jefferson University, Philadelphia, PA, United States, [4]New York Blood Center, New York, NY, United States

Two *Brugia malayi* proteins, Bm-103 and Bm-RAL-2, are orthologous proteins of confirmed vaccine candidates of *Onchocerca volvulus*. The Ov-103 was identified first as a microfilariae surface associated protein, but later was found to be also expressed in the cuticle, hypodermis and mutivesicular bodies of infective stage larvae of both filarial parasites. Ov-RAL-2 and Bm-RAL-2 are immunodominant proteins expressed in the hypodermis of all stages. Bm-103 was cloned and expressed in *Pichia pastoris* and Bm-RAL-2 was cloned and expressed in Escherichia coli. These recombinant proteins were tested for their efficacy as a vaccine in the *B. malayi* - Mongolian gerbil animal model of lymphatic filariasis. Vaccination was via 3 intraperitoneal injections separated by 2 week intervals. Animals were challenged subcutaneously with 100 infective larvae third stage larvae (L3) and worm recovery was performed 42 or 90 days post infection. Vaccination with Bm-103 administered with alum showed 40% worm reduction in comparison to alum controls. Vaccination with Bm-RAL-2 showed 43% worm reduction in comparison to controls. A fusion protein of Bm-103 and Bm-RAL-2 was created, cloned and expressed in E. coli. Vaccination of gerbils with the Bm-103-Bm-RAL-2 fusion protein induced a 51% worm reduction in comparison to controls. Vaccination of gerbils with the two antigens, Bm-103 and Bm-RAL-2, each injected separately resulted in worm reduction of 69%. The development of embryograms to study the fecundity of female worms harvested from control and vaccinated gerbils are currently underway and will bring insights on impact of vaccination on fertility of female worms. In all vaccination experiments, a strong antigen-specific IgG response was detected by ELISA to the recombinant proteins. Moreover, *in vitro* killing assays using peritoneal exudates cells (PEC) in the presence of gerbil anti-serum against Bm-103 and Bm-RAL-2 showed active killing of L3 larvae in comparison to L3 larvae cultured with appropriate controls sera suggesting that an antibody dependent cell mediated cytotoxicity (ADCC) maybe a potential mechanism of protection. The results suggested that further experiments using these proteins alone or in combination are warranted.

1893

A COMPARATIVE STUDY OF POST-DIETHYLCARBAMAZINE TREATMENT REACTIONS IN ONCHOCERCIASIS AND LOIASIS

Senbagavalli Prakash Babu[1], Jacqueline Herold[1], Fanny Legrand[1], Jesica Herick[1], Eric Ottesen[2], Joseph Kamgno[3], Raceline Gounoue[3], Thomas Nutman[1], Amy D. Klion[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]The Taskforce for Global Health, Decatur, GA, United States, [3]Center for Filariasis and Other Tropical Disease Research, Yaounde, Cameroon, South Africa

Severe post-treatment reactions occur following diethylcarbamazine (DEC) treatment of filarial infections, including onchocerciasis and loiasis. Although release of the intracellular bacteria, Wolbachia, has been implicated in the pathogenesis of these reactions, *Loa loa* does not contain Wolbachia. The aim of this study was to compare eosinophil, neutrophil and cytokine responses post-DEC treatment in subjects with *L. loa* infection to those in patients infected with *Onchocerca volvulus*. The study included three groups: Group I [LOA-NIH], subjects with microfilaremic (MF+) loiasis treated with DEC (8-10 mg/kg/day for 21 days) at NIH; Group II [LOA-CAM], subjects with MF+ loiasis treated with DEC

(8 mg/kg in a single dose) in Cameroon; Group III [ONCHO], subjects with MF+ onchocerciasis treated with DEC (200 mg/day for 7 days) in Ghana. Complete blood counts and previously collected serum were available at 0h, 4h, 8h, 1-7d and 14d post-initiation of treatment for LOA-CAM and ONCHO and at variable time points for LOA-NIH. The early pattern of eosinophilia post-DEC (a decrease from baseline during the first 24 hours followed by a significant increase over the next 3-5 days) was similar in all 3 groups and the rise was preceded by a transient increase in serum IL-5 levels. In contrast, the % baseline ANC increased significantly post-DEC only in the ONCHO group ($P<0.05$ at days 1,2 and 3 compared to LOA-CAM and LOA-NIH. Serum IL-10 levels increased transiently in all 3 groups, reaching peak values at 1-2 days post-DEC. Although serum TNF-alpha, levels increased at 1-2 days post-DEC in all subjects in the ONCHO group, there was no consistent pattern in the subjects with loiasis. To conclude, parasite antigen release and the resultant Th2-driven eosinophilia may be a major driver of post-DEC reactions in both onchocerciasis and loiasis. The increased TNF-alpha and neutrophilia seen post-DEC in onchocerciasis is likely due to the concomitant release of Wolbachia during microfilarial killing.

1894

ENDOTHELIAL CELLS RELEASE SOLUBLE FACTORS THAT PROLONG THE SURVIVAL OF FILARIAL WORMS *IN VITRO*

Holly Evans, Edward Mitre

Uniformed Services of the Health Sciences, Bethesda, MD, United States

A major barrier for current mass drug administration (MDA) efforts to control lymphatic filariasis is the inability of current medications to kill adult worms when given as a short course. The development of novel drugs is complicated by the inability to maintain worms for long periods of time *in vitro*, making effective screening of new drugs difficult. In an attempt to improve *in vitro* culture methodology for filarial worms, we have conducted a series of experiments using microfilariae (MF) obtained from gerbils infected with Litomosoides sigmodontis, a filarial parasite of rodents. While the culture of L. sigmodontis MF in Dulbecco's Modified Eagle Medium supplemented with 10% FBS results in an average survival of only 7 days, co-culturing MF with a mouse endothelial cell line (EOMA) expanded survival to 40 days. Not all cell lines have this property, as MF co-cultured with a rat basophilic cell line (RBL-2H3) survived for only 5 days. Culturing EOMA cells in transwell plates extended MF survival to the same degree as direct co-culture, suggesting that the factors microfilariae require are soluble in nature. Heat inactivation of EOMA conditioned media at 56°C reduced MF survival by approximately 50%. However, heat inactivation at 100°C reduced survival to 3 days, signifying that MF require both heat labile and heat stable factors. EOMA cells require FBS to produce these factors, as conditioned media collected from EOMA cells grown in the absence of FBS fail to prolong survival. Importantly, these findings also pertain to adult worms. Both rodent L. sigmodontis and human *Brugia malayi* adult worms also show significantly extended survival when cultured in EOMA conditioned media. We are poised to begin biochemical and comparative analyses to elucidate the chemical nature of these essential factors. Identification of such factors will advance our ability to cultivate filarial pathogens *in vitro* and may provide insights for the development of new anti-filarial compounds.

1895

THE IMPACT OF MATERNAL HELMINTH INFECTIONS ON TH2 RESPONSES AND ATOPIC SENSITIZATION OF INDONESIAN YOUNG CHILDREN

Yenny Djuardi[1], Taniawati Supali[1], Erliyani Sartono[2], Maria Yazdanbakhsh[2]

[1]Department of Parasitology, Faculty of Medicine University of Indonesia, Jakarta, Indonesia, [2]Department of Parasitology, Leiden University Medical Center, Leiden, Netherlands

Immune responses to helminth infection and allergy are both characterized with TH2 responses. Manifestation of allergies in children of low-to-middle income countries were much lower compared to those of affluent countries. We aimed to investigate the impact of maternal helminth infections and other factors on child's development of type 2 responses and atopic sensitization at 4 years of age in an area endemic for filaria and soil-transmitted helminths. Data were collected from pregnant mothers on helminth infections, total IgE and *Ascaris*-specific IgE, education and socioeconomic status (SES). Total IgE and IL-5 in response to mitogen, and helminth antigens were measured in children at 2, 5, 12, 24 and 48 months of age. *Ascaris* and allergen-specific IgE and skin prick testing (SPT) were determined at 4 years of age. Strong TH2 responses were seen at 5 months of age and increased with time. Child's helminth-antigen specific TH2 responses increased significantly with age and were associated with maternal filarial infection, while the increasing of child's general TH2 responses with age were more associated with higher maternal total and *Ascaris*-specific IgE, as well as with low maternal education or SES. Child's *Ascaris*-specific IgE were both associated with child's general and helminth-specific TH2 responses. At 4 years of age when allergen reactivity was assessed by SPT, the high general TH2 responses did not translate into higher SPT. The risk factor for SPT reactivity was low maternal education which decreased the risk of SPT positivity to allergens (adjusted OR, 0.32; 95% CI, 0.12 - 0.87) independently of maternal filarial infection which tended to reduce the child's risk for being SPT positive (adjusted OR, 0.35; 95% CI, 0.07 - 1.70). In conclusion, young children living in areas endemic for helminths developed a strong TH2 responses which was influenced by maternal or child's exposure to helminth infections, but did not translate to a higher SPT reactivity to allergens. This result might explain why the prevalence of allergies in low-to-middle income countries were much lower compared to the more affluent countries.

1896

TRANSCRIPTIONAL PROFILE OF THE *DIROFILARIA IMMITIS* LIFE CYCLE

Ashley N. Luck[1], Christopher C. Evans[2], Molly D. Riggs[2], Laurence Ettwiller[1], Jeremy M. Foster[1], Andrew R. Moorhead[2], Barton E. Slatko[1], Michelle L. Michalski[3]

[1]New England Biolabs, Ipswich, MA, United States, [2]University of Georgia, Athens, GA, United States, [3]University of Wisconsin Oshkosh, Oshkosh, WI, United States

Dirofilaria immitis (Di), or canine heartworm, is a filarial nematode evolutionarily related to those responsible for human parasitic diseases. The *D. immitis* genome, along with the genome of its obligate endosymbiont, *Wolbachia* (*wDi*), was recently completed and published. We initiated a series of transcriptional profiling experiments to better understand the temporal transcriptional activity of *Di* and *wDi* throughout the nematode life cycle. Over 215 million single-end 50 bp reads were generated from total RNA from five *Di* life cycle stages. Based on hierarchical clustering of expression data, nearly 60% of all *Di* genes display stage-specific transcriptional patterns. Pairwise comparison of adult male (AM) and adult female (AF) samples reveals that over 9,000 genes display sex-biased transcriptional patterns. The L3 to L4 transition, which occurs upon entering the mammalian host, is critical to the *Di* life cycle and a potential point of intervention. Among all five life cycle stages examined, a significant portion of *Di* genes are L4-

associated (3525 transcripts), whereas only 65 transcripts show L3-biased expression. Pairwise comparison of the L3 and L4 stages reveals 3157 significantly differentially expressed genes (1170 L3 upregulated, 1987 L4 upregulated) and provides important information regarding transcriptional changes required for this transition. As anticipated, significantly fewer reads mapped to *wDi* genes than to *Di* genes in each life cycle stage. Interestingly, synthesis of the critical metabolite, heme, by *wDi* appears to be synchronized with the production of heme-binding proteins in *Di* in a stage-specific manner. Comparative analysis to human filarial nematodes provides further information on the evolutionary biology of these parasites, while also highlighting opportunities for further drug targeting initiatives. A better understanding of how these genomes function in concert with one another is required for unraveling the complex relationship of the nematode with its endosymbiont, *Wolbachia*, as well as with its canine and mosquito hosts.

1897

EXPERIMENTAL *PLASMODIUM FALCIPARUM* GENETIC CROSSES IN HUMAN LIVER-CHIMERIC MICE

Ashley M. Vaughan[1], Nelly Camargo[1], Matthew Fishbaugher[1], Lisa A. Checkley[2], Richard S. Pinapati[2], Sebastian A. Mikolajczak[1], Michael Ferdig[2], Stefan H. Kappe[1]

[1]Seattle Biomedical Research Institute, Seattle, WA, United States, [2]University of Notre Dame, Notre Dame, IN, United States

Forward genetic studies using experimental genetic crosses are an incredibly powerful tool to pinpoint the genetic basis of phenotypic variation and are typically more powerful than population analysis. For *Plasmodium falciparum* however, only three experimental crosses have been carried out due to the hurdles associated with the process – for ethical reasons humans can not be used and until recently, only splenectomized chimpanzees allowed for the transition from the sporozoite stage to the asexual blood stage of the life cycle necessary for progeny amplification and analysis. Nevertheless, the recombinant progeny from the three crosses led to the discovery of genetic determinants for drug resistance, erythrocyte invasion and blood stage parasite growth. The recent decision by the NIH to cease chimpanzee biomedical research suggests further forward genetic studies are impossible. However, we have shown that a mouse harboring human hepatocytes (the FRG KO huHep mouse) infused with human erythrocytes can support *P. falciparum* sporozoite infection, the completion of liver stage development and the transition to asexual blood stage replication. This thus suggests that *P. falciparum* experimental crosses can be achieved in the FRG KO huHep mouse and here we show here that this is indeed possible and thus this mouse model can replace the previously essential chimpanzee. To achieve our goal, we generated *P. falciparum* gametocytes from the NF54 chloroquine sensitive and GB4 chloroquine resistant parasite lines and used these in mixed feeds to mosquitoes to generate recombinant sporozoite progeny – produced after zygote formation and sexual recombination. Sporozoites were injected into FRG KO huHep mice harboring human erythrocytes and after the liver stage-to-blood stage transition, blood stage parasites were maintained *in vitro*. Parasite cloning and downstream microsatellite analysis revealed the presence of unique recombinant progeny. Furthermore, drug selection demonstrated the creation of recombinant progeny with unique drug resistance patterns not shared by the parental populations. Thus we provide evidence of successful experimental genetic crosses. This methodology should allow for *P. falciparum* "systems genetics" – the study of complex genetic traits in which genomic data and clinical phenotypes are obtained using global "omic" technologies.

1898

MOLECULAR BASIS FOR SIALIC-ACID DEPENDENT RECEPTOR RECOGNITION BY THE *PLASMODIUM FALCIPARUM* INVASION PROTEIN ERYTHROCYTE-BINDING ANTIGEN-140 (EBA-140/BAEBL)

Brian Malpede, Dan Lin, Niraj Tolia

Washington University School of Medicine, St. Louis, MO, United States

Erythrocyte-binding antigen 140 (PfEBA-140/BAEBL) is a *Plasmodium falciparum* erythrocyte invasion ligand that engages Glycophorin C (GPC) on host erythrocytes during malaria infection. PfEBA-140 is a member of the erythrocyte-binding ligand (EBL) family, which contains the four sialic acid dependent invasion proteins utilized by *P. falciparum*. Each of these ligands recognizes a different erythrocyte receptor despite being composed of a highly conserved domain architecture. To elucidate the foundations of receptor specificity within the EBL family and define the structural basis of GPC engagement, we determined two crystal structures of the PfEBA-140 minimal binding domain unbound and in complex with a glycan containing the essential sugar component of GPC that is recognized during erythrocyte engagement. The two domains composing the minimal binding region contain unique structural elements that are likely determinants of receptor specificity. Two glycan binding pockets were observed, one per domain, and the bound sialic acid was modeled into each site. Erythrocyte binding experiments elucidated important glycan contact residues and identified distinct functional roles for the individual sugar binding sites. Our studies provide a structural framework for GPC recognition, form a foundation for future studies of the interaction between PfEBA-140 and erythrocytes, and offer insight into deficient receptor binding and putative receptor switching described for polymorphisms in PfEBA-140. Preventing erythrocyte engagement is an excellent opportunity to inhibit merozoite invasion. Our results will thus aid in the design of rational therapeutics and vaccines that target erythrocyte invasion ligands.

1899

EVOLUTION BEFORE OUR EYES: GENOME MUTATION IN *PLASMODIUM FALCIPARUM*

William L. Hamilton, Antoine Claessens, Mihir Kekre, Thomas D. Otto, Adnan Faizullabhoy, Julian C. Rayner, Dominic Kwiatkowski

Wellcome Trust Sanger Institute, Hinxton, United Kingdom

Genetic mutation is a central process in evolution, and is involved in the emergence of drug resistance and the generation of antigen sequence diversity in the malaria parasite *Plasmodium falciparum*. We studied *P. falciparum* mutation by regularly sub-cloning parasites cultured *in vitro* to isolate single infected red blood cells, so that mutations arising in asexually dividing cells could be detected using whole genome sequencing. We produced the most comprehensive description of *P. falciparum* mutation to date, studying four lab strains, artemisinin resistant Cambodian field isolates and parasites with experimentally induced mutations in DNA repair genes. In total, we analysed >300 genomes from parasites cultured for a total of >1,000 days, capturing hundreds of mutations. We found that: (1) Point mutations are distributed throughout the genome and occur at a similar rate between strains regardless of the drug sensitivity status of the line, contrary to previous studies suggesting that drug resistant parasites are hypermutable; (2) There is a strong mutation bias with G/C to A/T transition mutations over-represented. We estimate this would equilibrate at a similar AT ratio to that observed in the *P. falciparum* genome (~80%); (3) InDels occur predominantly in AT rich low-complexity regions at a higher rate than point mutations, likely due to DNA polymerase slippage events; (4) Structural variation is focused in and around var genes, which encode highly polymorphic PfEMP1 surface-expressed antigens, and this mitotic recombination generates sequence diversity by producing mosaic var genes. With 10^10 parasites in a single infected individual, our data indicate that every nucleotide in the *P. falciparum* genome will undergo point mutations and millions of new mosaic var genes will be produced

every 48-hour life cycle. In summary, we have produced a comprehensive catalogue of *P. falciparum* mitotic genome mutation at all scales from point mutations to interchromosomal translocations, adding considerably to our understanding of parasite genomics and evolution.

1900

IDENTIFYING NOVEL TRAFFICKING COMPONENTS OF THE *PLASMODIUM FALCIPARUM* VIRULENCE FACTOR PFEMP1 THROUGH QTL ANALYSIS

Aaron T. Neal[1], Lisa C. Ranford-Cartwright[2], Richard T. Eastman[1], Chris I. Newbold[3], Rick M. Fairhurst[1]

[1]National Institutes of Health, Rockville, MD, United States, [2]University of Glasgow, Glasgow, United Kingdom, [3]University of Oxford, Oxford, United Kingdom

Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) is the main parasite virulence factor due to its central role in mediating the cytoadherence of infected red blood cells (iRBCs) to the host microvascular endothelium. Directly targeting PfEMP1 as a therapeutic strategy is greatly limited due to the protein's hypervariable nature, which gives rise to approximately 60 different variants. However, interfering with the trafficking of PfEMP1 to the iRBC surface is an attractive approach, as hypervariability becomes inconsequential and previous studies have demonstrated that reduced surface PfEMP1 levels significantly weaken cytoadherence, likely lessening the severity of malaria symptoms and permitting parasite clearance by the spleen. Interestingly, the *in vitro* culture-adapted parasite line 3D7 is inherently defective in exporting PfEMP1 to the iRBC surface. Presuming that PfEMP1 export from the parasite to the iRBC surface is controlled at the genetic level, we hypothesized that 3D7 harbors one or more genetic determinants of impaired PfEMP1 trafficking. To test this possibility, we examined the surface PfEMP1 levels of 17 progeny clones from the genetic cross between 3D7 and the 'trafficking-competent' parasite line HB3. This was assessed using Western blotting and a two-color, triple-layer flow cytometry assay with plasma from malaria-immune Malian adults. Normalized to HB3, we found that 3D7 displays 75% less PfEMP1 on the iRBC surface, with progeny phenotypes ranging from 37% more to 88% less PfEMP1. QTL analysis using 3,597 genome-wide SNP markers identified a significant locus with a LOD score of 4.963 on chromosome 12 that explains approximately 50% of the phenotypic variance. This locus contains a single gene, *Pf3D7_1245600*, encoding a putative kinesin. The role of this gene in the trafficking of PfEMP1 is being confirmed in allele-exchange experiments, where the defect is rescued in 3D7 and introduced in HB3. The results of this study may strengthen our understanding of malaria pathogenesis and provide new targets for much needed therapeutics.

1901

PROTEOMIC COMPOSITION AND SURFACE ACCESSIBLE TARGETS OF *PLASMODIUM* SPOROZOITES

Scott E. Lindner[1], Kristian E. Swearingen[2], Lirong Shi[3], Anke Harupa[4], Ashley M. Vaughan[4], Robert L. Moritz[2], Stefan H. Kappe[4], Photini Sinnis[3]

[1]Pennsylvania State University, University Park, PA, United States, [2]Institute for Systems Biology, Seattle, WA, United States, [3]Johns Hopkins University, Baltimore, MD, United States, [4]Seattle Biomedical Research Institute, Seattle, WA, United States

The transmission of salivary gland sporozoites from an *Anopheline* mosquito initiates the malarial infection of a new mammalian host, which has prompted the development of various therapeutic interventions targeted against sporozoites. While several sporozoite proteins have been historically well studied, systematic proteomic analyses have been hampered by the presence of overwhelming amounts of proteins, nucleic acids, and lipids derived from the dissected mosquito vector. We have overcome this obstacle by developing streamlined purification methods that result in fully infectious sporozoites with very low levels of vector, bacterial, and fungal contamination. These approaches have enabled the most comprehensive total proteomics of two human-infective malaria species (*Plasmodium falciparum* and *P. vivax*), as well as the model rodent-infective species *P. yoelii*. Moreover, these purified sporozoites are also sufficiently devoid of soluble mosquito material to permit the assessment of the surface-accessible proteome of the salivary gland sporozoite. This was done using an amine-reactive crosslinker bearing a cleavable biotin group, which enables high affinity purification and yet leaves a covalent modification of accessible lysines for high confidence identification. We have developed an extensive and stringent washing strategy to minimize the binding of non-specific proteins, which has yielded a greatly expanded surface-accessible proteome above and beyond our previously published list. Several of these candidates have been confirmed with transgenic parasites and the generation of specific antisera. Finally, we have treated purified sporozoites with molecular mimics of mammalian body conditions to observe any differences in protein accessibility or secretion onto the parasite surface in response to these stimuli. Taken together, these characterizations provide a sizeable list of surface accessible proteins that may be valuable new targets for antibody-based interventions.

1902

AGE SPECIFIC INCIDENCE RATES OF MALARIA SUGGEST DIFFERENT RATES OF NATURALLY ACQUIRED IMMUNITY TO MALARIA ACROSS HUMAN HOST GENOTYPES

Oscar A. Nyangiri[1], Meghna Desai[2], Godfrey Bigogo[3], Deron Burton[4], Billy G. Ogwel[3], Allan Audi[3], Joel M. Montgomery[5], Simon Kariuki[3], Thomas N. Williams[6]

[1]Kenya Medical Research Institute-Wellcome Trust Research Programme, Kilifi, Kenya, [2]Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Kenya Medical Research Institute-Centers for Disease Control and Prevention, Kisumu, Kenya, [4]National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention, Office of Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]Global Disease Detection Branch, Division of Global Health Protection, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States, [6]Imperial College, London, United Kingdom

In endemic areas, the incidence of clinical malaria declines with age, reflecting the role of naturally acquired immunity (NAI) in modulating malaria. Asembo is a malaria endemic area in western Kenya. We investigated whether NAI develops at different rates among children carrying a range of malaria-associated genes by comparing age-specific malaria incidence in children, stratified by genotype. We recruited a birth cohort in March 2012-December 2013 (n=700) from a population under passive surveillance for clinical malaria since August 2006. Additionaly, we recruited birth cohort member siblings <12 years of age into a sibling cohort (n=780) for a combined cohort of 1480 children. Clinical malaria was defined as fever with a malaria-positive blood film in the absence of bacterial co-infections causing fever. Participants were typed for polymorphisms in 40 malaria-associated genes. Age-specific malaria incidence was significantly lower for sickle cell trait (AS) compared to sickle normal (AA) individuals (P<0.001), marginally significant for homozygous(-α/-α) compared to normal ($\alpha\alpha/\alpha\alpha$) alpha thalassemia individuals (P=0.04) and not significant for $\alpha\alpha/\alpha\alpha$ compared to heterozygous(-$\alpha/\alpha\alpha$) individuals . Incidence rates peaked earlier in AA (4-5 years, 0.77 episodes per child per year)compared to AS children (9-10 years, 0.8episodes per child year). For alpha thalassemia, incidence rates peaked earlier in -$\alpha/\alpha\alpha$ (2-3 years, 0.73 episodes per child year), compared to -α/-α individuals (6 years, 1 episode per child per year) and $\alpha\alpha/\alpha\alpha$ individuals (4-5 years, 0.79 episodes per child year). Analyses for the 40 malaria susceptibility genes are ongoing. Preliminary results show shifts in the peaks of age-specific incidence rates by genotype. These findings suggest different rates of NAI among children of different genotypes. Results may be useful in understanding genotype-specific effects of interventions such as malaria vaccines that can modulate NAI.

1903

QUANTIFYING THE INDEPENDENT EFFECTS OF AGE AND EXPOSURE ON TWO COMPONENTS OF MALARIA IMMUNITY: RESTRICTION AND TOLERANCE

Isabel Rodriguez-Barraquer[1], Emmanuel Arinaitwe[2], Prasana Jagannathan[3], Isaac Ssewanyana[2], Agaba Katureebe[2], Moses Kamya[4], Sarah Staedke[5], Grant Dorsey[3], David L. Smith[1], Bryan Greenhouse[3]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Infectious Diseases Research Collaboration, Kampala, Uganda, [3]Department of Medicine, University of California San Francisco, San Francisco, CA, United States, [4]Department of Medicine, Makerere University, Kampala, Uganda, [5]London School of Hygiene & Tropical Medicine, London, United Kingdom

While epidemiologic data consistently suggest that clinical immunity to *Plasmodium falciparum* develops over time in exposed children, lack of accurate measures of exposure and protection have limited our capacity to understand the development of immunity to infection and disease. The objective of this study was to define the acquisition of immunity against *P. falciparum* malaria among children living in endemic areas of varying malaria transmission. In particular, we were interested in measuring the development of clinical tolerance (lack of symptomatic disease given parasitemia, evaluated here by parasite density at which subjects developed objective fever) and parasite restriction (ability to kill or otherwise control the growth of parasites, evaluated here by parasite density). We used data from representative cohort studies being conducted in 100 households from each of three sub-counties in Uganda: Walakuba (aEIR= 3.3), Kihihi (aEIR=31.5) and Nagongera (aEIR=315). The study comprises continuous passive surveillance, active surveillance every 3 months, and monthly mosquito collections in all households. Thus, the dataset used for this analysis included data on over 3400 episodes of clinical malaria and 1400 episodes of asymptomatic parasitemia occurring in 739 children aged 6 months to 11 years of age over two years of follow up. Results from generalized additive models, allowing for flexible interactions between variables of interest, are consistent with strong independent effects of both age and exposure on the development of both tolerance and restriction. Tolerance develops gradually beginning early in life and is not strongly modified by variable exposure. In contrast, restriction starts to develop later in life (4-6 years of age) and depends strongly on cumulative exposure. Further analyses will explore the role of recent and persistent exposure on both of these components of immunity. These findings provide unprecedented insight about the roles of age and exposure on the development of immunity to malaria.

1904

ESTIMATING MALARIA FORCE OF INFECTION ACCOUNTING FOR HETEROGENEITY IN THE RISK OF INFECTION

Levi Mugenyi[1], Steven Abrams[2], Sarah Staedke[3], Niel Hens[2]

[1]Infectious Diseases Research Collaboration, Kampala, Uganda, [2]Centre for Statistics, Interuniversity Institute for Biostatistics and Statistical Bioinformatics, Hasselt University, Diepenbeek, Belgium, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom

The burden of malaria and the intensity of its transmission have been estimated using clinical incidence, parasite rate, and entomological inoculation rate. Less emphasis has been placed on the rate at which individuals become infected, or the force of infection (FOI), the number of new infections per person per unit of time. The above parameters are often estimated without accounting for the intrinsic variability among individuals in the risk of infection. However, such heterogeneity exists due to variability in risk factors, including proximity of residence to mosquito breeding sites, housing structure, density of mosquitoes and frequency of biting, use of prevention measures, differences in surface area between adults and children and in human sweat components, and antimalarial immunity. Here we have proposed approaches for estimating malaria FOI accounting for unobserved heterogeneity using data collected from August 2011 to August 2013 in a cohort of children aged less than 11 years in three sites in Uganda (Tororo, Kanungu and Jinja) with variable malaria transmission intensities. We applied the statistical methodology using linear and nonlinear mixed effects models to estimate both a constant and time-dependent FOI at each site, while accommodating for individual heterogeneity in the acquisition of malaria, and accounting for re-infections. Differences in the FOI were more pronounced between households (variance=2.25) than between children (variance=0.67). The FOI did not vary with time, but differed between the three study sites with higher risk in Tororo (FOI=4.0, 95%CI: 3.4 - 4.6), followed by Kanungu (FOI=0.6, 95%CI: 0.4 - 0.8), and by Jinja (FOI=0.2, 95%CI: 0.1 - 0.3). The FOI was also higher in children above five years of age (FOI=1.5, 95%CI: 1.2 - 1.7), those with symptomatic infection (FOI=0.7, 95%CI: 0.6 - 0.9) and those with anemia (FOI=2.2, 95%CI: 1.7 - 2.7). Therefore, housing structure, individual differences, location of an area, age, symptomatic status and anemia are important factors to consider when estimating the burden of malaria.

1905

SEVERE MALARIAL THROMBOCYTOPENIA: A RISK FACTOR FOR MORTALITY

Daniel A. Lampah[1], Tsin Yeo[2], Michael Malloy[3], Enny Kenangalam[4], Nicholas M. Douglas[2], Paulus Sugiarto[5], Julie A. Simpson[3], Nicholas M. Anstey[2], Nicholas M. Anstey[2], **Ric Price**[2]

[1]Papuan Health and Community Development Foundation, Timika, Indonesia, [2]Menzies School of Health Research, Darwin, Australia, [3]Melbourne School of Population and Global Health, Melbourne, Australia, [4]Mimika District Health Authority, Papua, Indonesia, [5]Rumah Sakit Mitra Masyarakat, Papua, Indonesia

The significance of thrombocytopenia to the morbidity and mortality of malaria is poorly defined. We compared the platelet profiles of patients with and without malaria in southern Papua, Indonesia. Between April 2004 and December 2012 data were available on patient demographics, malaria diagnosis, haematological investigations and clinical outcome in a referral hospital. Of 922,120 patient episodes a total of 215,479 (23.4%) were associated with a platelet measurement, of whom 66,421 (30.8%) had clinical malaria. Patients with *Plasmodium falciparum* monoinfection had the lowest platelet counts with an adjusted odds ratio (AOR) for severe thrombocytopenia (platelet count <50,000 µl-1), compared to those without malaria, of 6.03 [95% Confidence Interval (CI) 5.77-6.30]. The corresponding risks were 5.4 [95% CI 5.02-5.80] for mixed infections, 3.73 [95% CI 3.51-3.97] for *P. vivax* and 2.16 [95% CI 1.78-2.63] for *P. malariae*; p<0.001. In total 1.3% (2,701/215,479) of patients died. Compared to patients with neither severe anemia nor severe thrombocytopenia, those with severe anemia alone had an AOR for death of 5.21 [95%CI 4.53-5.98], those with severe thrombocytopenia alone had an AOR of 4.65 [95%CI 4.10-5.28] and those with both risk factors an AOR of 16.44 [95%CI 13.70-19.74]; p<0.001. In conclusion, severe thrombocytopenia is associated with malarial related mortality. Prospective studies are warranted to define its utility in defining the clinical management of patients with malaria.

1906

EXTENDING THE AGE RANGE FOR SEASONAL MALARIA CHEMOPREVENTION (SMC): EFFECTIVENESS OF SMC IN CHILDREN UNDER 10 YEARS OF AGE DELIVERED THROUGH THE DISTRICT HEALTH SERVICE IN SENEGAL

Elhadji K. Ba[1], B. Cisse[2], C. Pitt[3], J. L. Ndiaye[2], M. Ndiaye[2], J. F. Gomis[2], M. Cairns[3], B. M. Greenwood[3], C. Sokhna[1], O. Gaye[2], P. J. Milligan[3]

[1]Institut de Recherche pour le Developpement, Dakar, Senegal, [2]Universite Cheikh Anta Diop de Dakar, Dakar, Senegal, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom

Seasonal Malaria Chemoprevention (SMC) is recommended for malaria control in children under 5yrs of age where transmission is highly seasonal. In some areas the disease burden may justify extending the age range for SMC. We investigated the effectiveness of SMC in children under 10 years of age in Senegal. SMC with sulfadoxine-pyrimethamine plus amodiaquine was introduced in three districts in a step-wedge design. Fifty-four health posts were randomized to implement SMC starting in 2008, 2009, or 2010, or to remain without the intervention. A surveillance system was established to record all deaths and all malaria cases diagnosed at health facilities, and a pharmacovigilance system was put in place to detect adverse drug reactions. A poisson regression model was used to estimate the effectiveness of SMC in reducing malaria incidence in treated children, with a random effect to account for variation in incidence between health posts. To determine whether SMC was able to reduce malaria transmission, incidence of malaria in age groups too old to receive SMC, was compared between health posts in which SMC was delivered to children, and health posts without SMC, using random-effects poisson regression. SMC was administered to about 14,000 children under 5yrs in 2008, 90,000 children under 10yrs in 2009, and to 155,000 children under 10 yrs in 2010. No serious adverse events attributed to SMC were detected despite a high level of surveillance. Where SMC was delivered, the number of malaria cases in children under 10 years was reduced by 69% (95%CI 65%,72%). Malaria incidence in older age groups was reduced in areas where SMC was delivered to children, by 29% (21%,35%). In conclusion, in some regions of the Sahel and sub Sahel, the age distribution of malaria may justify extending the age range for SMC. Including older children in SMC programmes is safe and effective, and may contribute to reducing transmission.

1907

COMPARISON OF SEASONAL MALARIA CHEMOPREVENTION COVERAGE IN NORTHERN NIGERIA VIA DOOR-TO-DOOR, HEALTH FACILITY AND RETAIL SECTOR DELIVERY

Abigail Ward[1], Justin C. Graves[2], Omowunmi F. Omoniwa[2], Nasir Mohammed Bashir[2], Kathleen Maloney[1], Leslie Emegbuonye[2], Arnaud Le Menach[1], Caitlin F. Dolkart[3], Kabir Masokano[4], Owens Wiwa[2]

[1]Clinton Health Access Initiative, Boston, MA, United States, [2]Clinton Health Access Initiative, Abuja, Nigeria, [3]Clinton Health Access Initiative, Nairobi, Kenya, [4]Kano SMOH, Kano, Nigeria

In 2012, WHO recommended seasonal malaria chemoprevention (SMC) with sulfadoxine-pyrimethamine and amodiaquine (SP-AQ) to prevent malaria in children under five in the Sahel sub-region. As the National Malaria Elimination program in Nigeria plans to apply the recommendation to protect 6 million children in its 9 northern states, identifying the most effective ways to deliver the drug is critical. From August-November 2013, SP-AQ was delivered monthly to under-fives in 3 local government areas of Kano State using 3 delivery mechanisms: door-to-door using community drug distributors, at public health facilities (fixed points), and for a low cost in the private sector. A household survey was conducted in December 2013, one month after the final round of SMC distribution, to assess the coverage achieved through each distribution method. Data were collected on demographics, malaria knowledge, treatment practices, and SMC awareness. We estimated the partial SMC coverage (proportion of under-fives receiving at least one of the four monthly doses) and full coverage (proportion of under fives receiving all doses), and identified factors associated with coverage using multivariable logistic regression models. 176,281 doses of SP-AQ were distributed over four months. The survey collected data from 5,291 children and 3,206 caregivers in 3,079 households. Adjusted partial coverage was significantly higher via door-to-door distribution (86.5%) than via health facility (46.7%) or private sector (27.9%). Full coverage was also highest in the door-to-door delivery arm (56.3%) compared to health facility (19.4%) and private sector (12.2%). Children 1-4 years old were significantly more likely than those <1 year old to receive SMC (p<0.001), and child use of an insecticide-treated bed net was significantly associated with partial coverage (OR=1.4). Door-to-door delivery achieved the highest coverage although a substantial population did not receive SMC. The findings are informing plans for 2014 SMC scale-up across Kano State including community mobilization and sensitization strategies and other fixed-point distribution opportunities.

1908

THE EFFECTIVENESS OF INSECTICIDE TREATED BEDNETS IN HAITI: RESULTS FROM A CASE-CONTROL STUDY

Laura Steinhardt[1], Yvan St. Jean[2], Kimberly Mace[1], Daniel Impoinvil[1], Ryan Wiegand[1], Jean Seme Fils Alexandre[3], Joseph Frederick[2], Roland Oscar[2], Jean Frantz Lemoine[2], Emery Nkuruziza[4], Samuel Jean[4], Brian Wheeler[3], John Barnwell[1], Ellen Dotson[1], Laurence Slutsker[1], S. Patrick Kachur[1], Michelle Chang[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Programme National de la Control de la Malaria, Port-au-Prince, Haiti, [3]Centers for Disease Control and Prevention, Port-au-Prince, Haiti, [4]PSI, Port-au-Prince, Haiti

Insecticide-treated bednets (ITNs) are a cornerstone of malaria prevention, but little evidence exists on their effectiveness in Haiti where the primary vector, *Anopheles albimanus*, has been documented as exophilic and with variable biting times. We conducted a case-control study to assess ITN effectiveness in Haiti, following a national ITN distribution campaign in 2012. Patients presenting to outpatient departments were systematically screened for fever or history of fever. Eligible patients were administered a brief questionnaire and blood was collected for a malaria rapid diagnostic test (RDT) and dried blood spots. From September 2012-February 2014, 9,318 patients, including 379 (4.1%) RDT-positive patients, were enrolled across 17 health facilities in five departments in Haiti. Retrospectively matching up to four RDT-negative controls per RDT-positive case by age group, sex, location of residence, and enrollment period yielded 365 cases and 1,204 RDT-negative controls. Slightly more than half (57.1%) of patients reported owning any bednet, with no difference among matched cases and controls. We found no difference in the proportion of cases and controls who reported using any bednet (34.5% vs. 32.9%, p=0.39) or a campaign ITN (21.9% vs. 19.5%, p=0.30) the previous night, or always using a campaign ITN in the two weeks before their illness (18.4% vs. 18.5%, p=0.84). In a multivariabe conditional logistic regression model, consistent use of a campaign ITN was not related to RDT positivity. The only variable related to RDT positivity in the model included body temperature (Odds Ratio = 1.40, 95% Confidence Interval: 1.25, 1.57 per one-degree Celsius increase). Additional entomologic investigation found that all *Anopheles* mosquitoes tested from the study areas were susceptible to permethrin, the insecticide used on campaign ITNs. Our results based on RDT status do not provide evidence to support ITNs as an effective malaria prevention strategy in Haiti. Additional results using a PCR-based case definition will be presented.

1909

AEDES AEGYPTI FEMALE MOSQUITOES WITH ALANINE AMINOTRANSFERASE DEFICIENCY FACE A STRESSFUL METABOLIC CHALLENGE DURING BLOOD DIGESTION

Jun Isoe[1], Stacy Mazzalupo[1], Virginia Belloni[2], **Patricia Y. Scaraffia**[2]

[1]The University of Arizona, Tucson, AZ, United States, [2]Tulane University, New Orleans, LA, United States

We have recently evaluated the exposure of *A. aegypti* females to L-cycloserine (LCS), a well-known inhibitor of alanine aminotransferase (ALAT) in animals. Our results indicated that 10 mM LCS interferes with *Aedes aegypti* blood metabolism causing motor impairment and a 35% of mortality with an acute effect during the first 6 hours after treatment. Interestingly, only 11% of the total mortality was observed between 24 and 72 hours after feeding. In order to follow up this finding, the expression pattern of two genes encoding ALAT (1 and 2) was first analyzed in sucrose- and blood-fed *A. aegypti* tissues by qRT-PCR. ALAT1 and ALAT2 transcript levels exhibited a distinct expression pattern in mosquito tissues dissected during a gonotrophic cycle. Next, RNAi-mediated gene silencing was used to knock down endogenous levels of each transcript in mosquito tissues. Injection of female mosquitoes with either dsRNA-ALAT1 or dsRNA-ALAT2 or both (dsRNA-ALAT1/2) significantly decreased the expression of ALAT1 or ALAT2 or ALAT1/2 in fat body (FB) and Malphigian tubules (MT) at 24 hours after blood feeding, when compared to dsRNA-firefly luciferase-injected control. As expected, the expression of ALAT1 was not modified in tissues from dsRNA-ALAT2-injected females and vice versa. Western blot analysis demonstrated that the protein levels of ALAT were also significantly reduced in tissues of dsRNA-ALAT-injected females when compared to control mosquitoes. Moreover, the knockdown of *A. aegypti* ALAT1 or ALAT2 or ALAT1/2 caused unexpected phenotypes such as a delay in blood digestion, a massive accumulation of uric acid in the midgut posterior region, and a significant decrease in nitrogen waste excretion during the first 48 hours after blood feeding. Concomitant with these results, the expression of genes encoding both the ammonia transporter and xanthine dehydrogenase were significantly increased in FB and MT of dsRNA-ALAT-injected females. These findings highlight the efficient and complex mechanisms that blood-fed mosquitoes use to avoid ammonia and free radical toxicity.

1910

ANOPHELES GAMBIAE SMALL-RNA PATHWAYS IMMUNITY TO DIFFERENT PATHOGENS

Guillaume Carissimo[1], Emilie Pondeville[1], Emmanuel Bischoff[1], Catherine Bourgouin[1], Anna-Bella Failloux[1], Christophe Antoniewski[2], Alain Kohl[3], Kenneth D. Vernick[1]

[1]Institut Pasteur, Paris, France, [2]Université Pierre et Marie Curie - UPMC, Paris, France, [3]University of Glasgow - MRC Center for Virus Research, Glasgow, United Kingdom

Many mosquito species are vectors of human pathogens, such as viruses responsible for Dengue or Chikungunya, or malaria parasites. Mosquitoes from the *Aedes* genus mainly transmit viruses, whereas the *Anopheles* genus is exclusively responsible for transmission of human malaria. The basis for these pathogen-vector specificities is currently unknown. Mosquito small RNA pathways have many functions such as regulation of genes in the development or immunity. Previous studies in *An. gambiae* have shown that (i) regulation of anti-*Plasmodium* immunity by microRNA was essential for correct mosquito protection, and that (ii) the siRNA pathway is essential for the control of arboviral infection after intrathoracic inoculation. Using next generation sequencing and functional genomics to examine *Anopheles gambiae* mosquitoes infected with O'Nyong Nyong virus and *Plasmodium* parasites, we were able to (i) discover new *Anopheles* microRNAs and characterize specific microRNAs that are regulated upon infection; (ii) show that the siRNA pathway does not contribute to antiviral defense during early arboviral infection in the midgut while it is protective at later stages in the systemic compartment; (iii) and implicate the siRNA pathway in evasion of immunity by malaria parasites in the midgut. These results expand our understanding of the small RNA immunity machinery in an important African vector.

1911

STRUCTURAL DIVERGENCE OF HETEROCHROMATIN BETWEEN INCIPIENT SPECIES OF *ANOPHELES GAMBIAE*

Atashi Sharma, Maria V. Sharakhova, Igor V. Sharakhov

University of Virginia Tech, Blacksburg, VA, United States

The major African malaria vector *Anopheles gambiae* is known to be undergoing incipient speciation into two molecular forms – M and S – recently named *An. coluzzi* and *An. gambiae*, respectively. Heterochromatin plays a vital role in several important biological functions, and is known to be associated with longevity and individual fitness of organisms as well as with postmating reproductive isolation between species. The genome of *Anopheles gambiae* was first sequenced in 2002 and has been updated since but still contains important information gaps with regards to the repetitive DNA content in heterochromatin. In order to achieve a better understanding of differentiation within *An. gambiae*, it is essential to develop a physical map containing information about the repetitive DNA and determine the differences in heterochromatin between the incipient species. Using multiple strains of M and S forms, we mapped repetitive DNA sequences including satellite DNA and ribosomal DNA (rDNA) with respect to bands of pericentric heterochromatin of mitotic chromosomes. Satellite DNA probe Ag53A hybridized to the pericentric heterochromatin/rDNA locus junction in both forms. However, unexpectedly, satellite DNA AgY53B hybridized at the base of proximal band in the M form but at the tip of the band in the S form, indicating a possible shift or inversion in the satellite DNA position during divergence of these forms. Satellite DNA AgY477 depicted a similar pattern, hybridizing to different positions between the forms. Idiograms based on above information were prepared for the M and S forms as well as other members of the *An. gambiae* complex, serving as a tool in better understanding of evolution of the repeat rich regions in the *An. gambiae* genome. Our results revealed that the rapid evolution of heterochromatin is not restricted to species with the postmating reproductive isolation. The structural reorganization of satellite DNA observed between the M and S forms suggests a possible role of heterochromatin in initial diversification of malarial vectors.

1912

X-CHROMOSOME LOCALIZED RECOMBINATION HOTSPOTS UNDERMINE EXISTING MOLECULAR DIAGNOSIS OF *ANOPHELES GAMBIAE* AND *AN. COLUZZII* UNDER HIGH HYBRIDIZATION

Beniamino Caputo[1], Verena Pichler[1], Emiliano Mancini[1], Marco Pombi[1], Josè Vicente[2], Amabelia Rodrigues[3], Joao Dinis[3], Vincenzo Petrarca[4], Joao Pinto[5], Alessandra della Torre[1], David Weetman[6]

[1]Department of Public Health and Infectious Diseases, Università di Roma "La Sapienza", Italy, Rome, Italy, [2]Centro de Malária e outras Doenças Tropicais, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal, [3]Instituto Nacional de Saúde Pública, Ministério da Saúde Pública, Bissau, Guinea-Bissau, [4]Department of Biology and Biotechnology, Università di Roma Sapienza, Rome, Italy, [5]Centro de Malária e outras Doenças Tropicais, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal, [6]Vector Group, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Anopheles gambiae and *An. coluzzii* are defined based on SNPs in the multi-copy IGS chromosome-X linked rDNA region. In most of their range of sympatry, where they rarely hybridize, multiple areas of the genome are differentiated, with highest divergence found toward centromeres. However, genomic divergence varies markedly with levels of introgression.

In a putative secondary contact region in Guinea Bissau, where hybrid females have been observed at frequencies >20%, differentiation is largely limited to a region proximal to the X-centromere. This region may play a critical role in the speciation process, but the evolutionary forces acting upon it are poorly understood. The aim of our work was to evaluate inter-specific genomic differentiation and frequency of recombination along the X-centromere in the Guinean hybridization region, focusing the analysis on X-chromosome hemizygous males to permit unambiguous haplotype analysis. We genotyped 263 males for the IGS diagnostic locus and for two additional markers about 1 Mb from it: i) the insertion of a SINE retrotransposable element specific for *An. coluzzii*, which is widely used as a species diagnostic; and ii) a 57 bp-insertion in intron 4 of cytochrome CYP4G16 (CYP) gene specific for *An. gambiae*. Moreover, using Illumina and Sequenom genotyping we characterised almost 800 SNPs (34 of which are species-specific and located in the X centromeric region) in 59 males. We observed: i) lack of inter-specific differentiation in the overall genome, with the exception of chromosome-X centromere; ii) intra-individual mixed IGS-arrays in 12% of the whole male sample, suggesting the occurrence of introgression events; iii) unexpected recombination among IGS, SINE and CYP in 24% of the males, and between SINE and CYP in 13% of them despite the close proximity of these 2 loci (7 Kb). Moreover, results from SNP-genotyping showed: i) some, although low, levels of recombination in the X-centromere; ii) introgression in the IGS-region in the absence of recombination in the X-centromere; iii) a hot-spot of recombination nearby the SINE-insertion. The results highlight (1) the likely importance of reduced recombination in maintaining integrity of the X-genomic island of divergence with high gene flow, consistent with genetic-hitchhiking-based speciation models and (2) the poor reliability of existing diagnostics (IGS, SINE) for the two species in the secondary contact region.

1913

AN INTEGRATED CHROMOSOME, GENETIC LINKAGE AND GENOME MAP FOR THE SOUTHERN HOUSE MOSQUITO *CULEX QUINQUEFASCIATUS*

Anastasia N. Naumenko[1], Vladimir A. Timoshevskiy[1], Becky S. deBruyn[2], David W. Severson[2], Igor V. Sharakhov[1], Maria V. Sharakhova[1]

[1]Fralin Biotechnology Center, Virginia Tech, Blacksburg, VA, United States, [2]Department of Biological Sciences, Eck Institute for Global Health, University of Notre Dame, Notre Dame, IN, United States

Culex quinquefasciatus, a southern house mosquito, is a member of geographically widespread mosquito complex species with high variation in behavioral patterns and ability to transmit diseases, including lymphatic filariasis and West Nile fever. Only 10.4 % of the 579 Mb genome is currently assigned to the chromosomes based on genetic linkage mapping. Although cytogenetic maps for the polytene chromosomes for this mosquito were developed, their utilization for the genome mapping remains difficult because of the low number of high quality spreads in chromosome preparations. We constructed idiograms for mitotic chromosomes of *Cx. quinquefasciatus* based on their banding patterns at early metaphase. These idiograms represent the first cytogenetic map developed for mitotic chromosomes of *Cx. quinquefasciatus*. Genetic contigs associated with 14 major genetic markers, 18S rDNA and 10 largest contigs were anchored to the exact positions on *Cx. quinquefasciatus* chromosomes using fluorescent *in situ* hybridization. The order of genetic markers was consistent with the previously developed genetic linkage map. Some new insights were provided into chromosome evolution in mosquitoes. For example, FISH result of 18S rDNA suggests an inverted position of the ribosomal locus in chromosome 1 of *Cx. quinquefasciatus* compared with Ae. aegypti. This locus was mapped close to the centromere above the heterochromatin band in *Cx. quinquefasciatus* but in the middle of the 1q arm below the heterochromatin band in Ae. aegypti. Our study in progress linked chromosome and genetic linkage maps with 4.8% of the *Cx. quinquefasciatus* genome.

1914

MICROSATELLITE AND DNA SEQUENCE POPULATION GENETICS EVALUATION OF THE SOUTHWEST PACIFIC MALARIA VECTOR *ANOPHELES KOLIENSIS* (OWEN)

Nigel W. Beebe[1], Robert D. Cooper[2], Weixin Xu[3], Sarah L. Fordyce[4], Luke Ambrose[3]

[1]School of Biological Sciences, University of Queensland and CSIRO, Brisbane, Australia, [2]Australian Army Malaria Institute, Brisbane, Australia, [3]School of Biological Sciences, University of Queensland, Brisbane, Australia, [4]Department of Forensic Medicine, University of Copenhagen, Copenhagen, Denmark

The southwest Pacific malaria vector *Anopheles koliensis* Owen is one of 13 cryptic members of the *Anopheles punctulatus* group that can only be identified by molecular tools. Its distribution in Papua New Guinea (PNG) has only recently been described and it is found predominantly throughout inland lowlands and river valley flood plains below 300m and is common throughout the continual wet Sepik, Ramu, and Markham River valleys on the north side of PNG's central ranges as well as throughout the northern and southern lowland region of PNG's Papuan Peninsula. *An. koliensis* utilizes both natural larval habitat (ground pools and swamps), as well as human modified habitat (vehicle wheel tracks and drains). In this study, we drill into the population genetic of *An. koliensis* in PNG - a region of incredible biogeography - to detail the spatial and genetic connectivity of this malaria vector species. We evaluate nuclear and mitochondrial DNA sequence as well as develop and analyse 12 microsatellites. We find a species with overt genetic and geographic population structure that can be explained, in most cases, by natural barriers. We do not find evidence to support the existence of intraspecific rDNA genotypes previously described but we do find *An. koliensis* to be a single species with a long history in New Guinea.

1915

CHARACTERIZATION OF MALE REPRODUCTIVE FACTORS ESSENTIAL FOR MATING SUCCESS IN THE LIFE CYCLE OF *ANOPHELES GAMBIAE* MOSQUITOES

Evdoxia Kakani[1], Sara Mitchell[2], Paolo Gabrieli[1], Enzo Mameli[1], Ainhoa Mariezcurrena[3], Flaminia Catteruccia[2]

[1]University of Perugia, Perugia, Italy, [2]Harvard School of Public Health, Boston, MA, United States, [3]Imperial College, London, United Kingdom

Mating is a vulnerable step in the lifecycle of *Anopheles gambiae* mosquitoes, as females of this species mate only once. Seminal fluids produced in the male accessory glands (MAGs) and transferred during mating are likely to induce permanent refractoriness to further insemination in females. Moreover, these factors are likely to play a key role in other post-mating processes, including egg laying and fertility. However, the identity of these male molecular triggers is largely unknown. As changes in levels of individual semen components after mating may indicate factors crucial for mating success, we performed a time course transcriptional analysis of MAG genes at 3 time points (3h, 12h, 24h) after mating, representing the period between 2 mating events in the field. Mated tissues were compared to those from virgin males in 4 replicates using whole-genome microarrays. Surprisingly, a total of 4,319 genes were differentially expressed after mating ($p<0.05$ FDR). Gene enrichment analysis revealed a number of functional groups significantly enriched in the dataset. During early time points after mating, genes associated with RNA transcription, translation and post-translational modifications were enriched, suggesting an induction of pathways essential for the replenishment of MAG content. At the latest time point enrichment was observed in genes involved in protein export, indicative of males preparing for the next mating event. Interestingly, genes involved in hormone biosynthesis were also highly enriched, supporting previous findings that male hormones may play a critical role in *An. gambiae* reproduction. We then investigated the function of male hormones that are replenished after mating. Tampering with the synthesis of these hormones in males

had dramatic effects on the reproductive physiology of mated females, which showed strongly reduced fecundity and fertility. These results reveal previously unknown pathways that are key to mosquito reproductive physiology, and offer novel targets for vector control efforts aimed at reducing mosquito reproductive success.

1916

AN INPUT ON PREVENTIVE STRATEGIES FROM THE FIELD: IRON LEVELS AND INTERMITTENT PREVENTIVE TREATMENT (IPTP) CALENDAR ARE ASSOCIATED WITH *PLASMODIUM FALCIPARUM* PARASITEMIA DURING THE FIRST YEAR OF LIFE

Violeta Moya-Alvarez[1], Smaila Ouedraogo[2], Florence Bodeau-Livinec[3], Gilles Cottrell[2], Michel Cot[4]

[1]Institut de Recherche pour le Développement, EHESP, Paris/Benin, France, [2]Institut de Recherche pour le Développement, Cotonou, Benin, [3]Ecole des Hautes Etudes en Santé Publique, Rennes, France, [4]Institut de Recherche pour le Développement, UPMC, Paris, France

Malaria is the disease with the highest infant mortality and morbidity worldwide. In 2012 the WHO reported over 207 million cases and more than 627,000 deaths. In Benin malaria is the leading cause of mortality (23%) among children under 5 years. There are significant differences in *P. falciparum* parasitemia among children during the first year of life. We aim at describing the factors contributing to malaria episodes and to high parasitemia during the first year of life in Benin. Therefore we have investigated the association of maternal exposure to the vector *in utero* (determined by pregnancy associated malaria (PAM) and the intermittent preventive treatment (ITPp)) to infant parasitemia analyzing as well nutritional, environmental and socio-economic risk factors. 1000 pregnant women and 400 of their children were followed during pregnancy and the first year of life in Allada (Benin), between 2010 and 2012. At inclusion socio-demographic status and gyneco-obstetric history were investigated. Extensive medical and biological exams were realized with both doses of IPTp and at delivery for the mothers and at 6, 9, and 12 months for the infants. Further exams were realized at each emergency consultation. All patients were treated in case of disease. Random coefficient models assessed the relationship between the different parasitemia measures in infants and other variables. A novel approach consisting in pathway analysis was used to analyze the evolution pattern of parasitemia. Maternal age at both IPTp doses, infant weight, mother parasitemia at delivery, number of emergency consultations and total body iron were correlated with infant parasitemia. Placental malaria was not correlated with infant parasitemia when adjusting for mother parasitemia at delivery. We find for the first time that IPTp has not only an effect on LBW but also on infant parasitemia. Therefore IPTp calendar should cover extensively the pregnancy and protect both the mother and the infant. Total body iron is also correlated with infant parasitemia. WHO recommends supplements with iron and folic acid when the prevalence of anaemia exceeds 40%. However the Pemba study and a Cochrane review conclude to an increased risk for malaria among supplemented children in the context of limited malaria coverage. Our results confirm the association between iron and malaria and plead for protective measures in the context of iron supplementation

1917

MALARIA-TRANSMISSION INTENSITY AND THE PROTECTIVE EFFECT OF INTERMITTENT PREVENTIVE TREATMENT (IPTP): POLICY IMPLICATIONS FOR THE ANTENATAL CARE OF PREGNANT WOMEN IN SUB-SAHARAN AFRICA

R. Matthew Chico, Jorge Cano, Cono Ariti, Timothy Collier, Daniel Chandramohan, Brian Greenwood

London School of Hygiene & Tropical Medicine, London, United Kingdom

The World Health Organization (WHO) recommends the provision of intermittent preventive treatment with sulphadoxine-pyrimethamine (IPTp-SP) to pregnant women resident in areas of moderate (stable) or high malarial transmission to prevent low birthweight (LBW), neontal deaths and maternal anemia. The incidence of malaria below which IPTp-SP no longer provides a cost-benefit is unknown, but it is important to estimate as countries make progress towards malaria elimination. We conducted a review of IPTp-SP studies and matched the protective efficacy of IPTp-SP against LBW to a proxy measure of malarial incidence in women of the same studies, the prevalence of malaria in children. The latter was calculated in two ways. We first extracted prevalence estimates in children from the Malaria Atlas Project (MAP) database and, for the second measure, we selected and then pooled the point prevalence data that underly the MAP estimates using random-effects models. We then applied meta-regression models of the protective efficacy of IPTp-SP against LBW to the estimates of malarial prevalence in children calculated in both ways, and stratified results by gravidity. Among multigravidae, the protective effect of IPTp-SP against LBW was no longer significant in areas where the malarial prevalence in children was < to 9% when we applied MAP estimates, and < to 8% using our pooled estimates. The latter analysis showed a significant linear trend (P=0.043). Malarial transmission intensity could not explain variations in the efficacy of IPTp-SP among paucigravidae. IPTp-SP no longer protects against the incidence of LBW among multigravidae in geographical areas of 20 sub-Saharan countries where the parasite prevalence among children is < 8%. In contrast, our analysis among paucigravidae suggests that two or more doses of SP is protective against LBW in transmission settings that are below the current recommendation set by the WHO.

1918

MISSED OPPORTUNITIES FOR DELIVERING PREVENTIVE TREATMENT FOR MALARIA IN PREGNANCY DURING ANTENATAL CARE AND COMPARISON WITH DELIVERY OF NEONATAL TETANUS PREVENTION: AN ANALYSIS OF HOUSEHOLD SURVEYS IN SUB-SAHARAN AFRICA

Kathryn Andrews[1], Michael Lynch[2], Erin Eckert[3], **Julie Gutman**[2]

[1]Harvard School of Public Health, Cambridge, MA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]President's Malaria Initiative, United States Agency for International Development, Washington, DC, United States

Coverage of intermittent preventive treatment for malaria during pregnancy (IPTp), a potentially lifesaving intervention, remains low in Africa, despite high antenatal care attendance among pregnant women and recommendations for IPTp to be given at each antenatal care visit after the first trimester. To highlight areas of potential improvement, we assessed trends in IPTp coverage over time, and identified the missed opportunities to deliver IPTp at antenatal clinics. Data from 66 national household surveys conducted in 31 sub-Saharan African countries between 2000 and 2012, with relevant questions on antenatal care (ANC) were used to determine the coverage of ANC visits, IPTp and tetanus toxoid (TT). Missed opportunities for IPTp were calculated by comparing the number of IPTp doses received to the number of ANC visits during which IPTp could be given. To account for visits occurring in the first trimester when IPTp is not given, one visit was subtracted from the total number of visits for women who reported having their first visit in the first trimester. The median proportion of pregnant women receiving at least 2 doses of IPTp was 1.0% (IQR 0-10.3%) during 2000-2007 and 27.2% (IQR 13.9-42.1%) during 2008-2012. Missed opportunities for IPTp delivery occurred in a median 99.2% (IQR 90.2-100%) of ANC visits from 2000-2007 and 76.8% (IQR 65.6-92.9%) from 2008-2012. The median proportion of primigravid women receiving at least 2 doses of TT is much higher: 50.2% (IQR 34.4-64.0%) during 2000-2007, and 59.2% (IQR 48.6-64.9%) during 2008-2012. With the exception of two countries, the proportion of primigravid women receiving at least 2 doses of IPTp is lower than the proportion receiving at least 2 of TT: the median absolute difference is 41.7% from 2000-2007 (IQR 29.2-54.1%) and 36.7% (IQR 23.7-40.6%) from 2008-2012. Although IPTp coverage has increased slightly over time, levels remain disappointingly low, and missed opportunities for IPTp occur at the majority of ANC visits. Although

both are delivered through the ANC, delivery of IPTp occurs much less frequently than delivery of TT, suggesting that barriers to IPTp delivery could be overcome. Further work is required to determine the specific factors that are driving the surprising discrepancies between IPTp and TT coverage, with an eye toward improving IPTp coverage through potential linkage with the TT administration infrastructure.

1919

PHARMACOKINETICS OF ARTEMETHER-LUMEFANTRINE IN PREGNANT AND NON-PREGNANT WOMEN WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN WESTERN KENYA

Elizabeth A. Juma[1], Bernhards R. Ogutu[1], Florence Oloo[2], Tara Barwa[2], Rashid Aman[3]

[1]Kenya Medical Research Institute, Nairobi, Kenya, [2]Centre for Research in Therapeutic Sciences, Nairobi, Kenya, [3]African Centre for Clinical Trials, Nairobi, Kenya

Pregnancy increases the vulnerability to malaria infection in all women living in areas of malaria risk. The World Health Organization recommends the use of artemisinin-based combination therapies (ACTs) for treatment of acute uncomplicated falciparum malaria in the second and third trimesters of pregnancy. The pharmacokinetic properties of antimalarial drugs are often affected by pregnancy, resulting in lower drug concentrations and a consequently higher risk of treatment failure. While artemether-lumefantrine is used in Kenya and most Eastern Africa countries as first line treatment for malaria in pregnancy in the second and third trimesters, its pharmacokinetics in pregnant African women with malaria is not well characterized. This study evaluated the population pharmacokinetics of artemether, dihydroartemisinin, lumefantrine and desbutyl-lumefantrine in 45 pregnant and 25 non-pregnant women with uncomplicated malaria in Western Kenya. All patients were treated with the standard fixed dose artemether-lumefantrine 20/120mg tablets over 3 days. Frequent venous blood sampling was obtained over the treatment period for pharmacokinetic evaluation. Estimates for pharmacokinetic and variability parameters will be obtained through nonlinear mixed effects modeling. Simultaneous modeling of parent drug and metabolite will be used for both artemether and lumefantrine. Absorption and clearance of artemether-lumefantrine in pregnant compared with non-pregnant African women with uncomplicated malaria and the implications of findings will be presented.

1920

A STUDY OF THE PHARMACOKINETICS OF PRIMAQUINE IN LACTATING WOMEN AND BREASTFED INFANTS FOR THE RADICAL TREATMENT OF UNCOMPLICATED MATERNAL *PLASMODIUM VIVAX*

Mary Ellen T. Gilder[1], Joel Tarning[2], Niklas Lindegårdh (posthumous)[2], Germana Bancone[1], Warunee Hanpithakphong[2], Hilda Moo[1], Tun Tun Win[1], Nicholas White[3], François Nosten[3], Rose McGready[3]

[1]Shoklo Malaria Research Unit, Mae Sot, Tak, Thailand, [2]Mahidol Oxford Research Unit, Bangkok, Thailand, [3]Centre for Tropical Medicine, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom

Women of reproductive age in malarial areas of the world suffer due to lack of evidence on the safety of effective antimalarials in pregnancy and lactation. *Plasmodium vivax* recurrences are more common during pregnancy but the only widely available medication for radical treatment of *P.vivax*, primaquine, is contraindicated in pregnancy. The postpartum period presents a key opportunity for radical treatment of *P. vivax*, but there are no studies quantifying primaquine excretion in breast milk and the dose that breastfed infants would be exposed to is unknown. We are conducting the first-ever study of the pharmacokinetics of primaquine lactating women and their breastfed infants during a 14-day radical treatment of *P.vivax*. Twenty-four healthy lactating women at risk for recurrent malaria (i.e. with a history of *P. vivax*) and their infants (at least 28 days old) are being recruited for detailed pharmacokinetic study. Prior to enrolment, G6PD deficiency is excluded by rapid qualitative fluorescent spot test and G6PD genotype from PCR spot. Anemic patients are treated and enrolment is delayed until normal HCT is established. Hemoglobin typing is analyzed and fetal hemoglobin in infant blood is quantified. Primaquine is administered to eligible mothers at a dose of 0.5 mg/kg/day and is directly observed. Primaquine and carboxyprimaquine levels are measured by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) on venous and capillary plasma, urine, saliva and breast milk samples from the mothers, as well as capillary plasma samples from the infants. There have been no drug-related adverse events to infants, though several women have experienced mild to moderate methemaglobinemia (not requiring treatment). Preliminary data shows low but measurable levels of primaquine in both breast milk and infant plasma. The final results of this study could have profound impacts on malaria control and women's health in the tropics.

1921

SAFETY OF ARTEMETHER-LUMEFANTRINE EXPOSURE IN EARLY PREGNANCY: AN OBSERVATIONAL COHORT

Dominic Franklin Mosha

Ifakara Health Institute, Dar es salaam, United Republic of Tanzania

There is limited data available regarding safety profile of artemisinins in early pregnancy. They are therefore not recommended by WHO as a first line treatment for malaria in first trimester due to associated embryo-foetal toxicity in animal studies. The aim of the study was to assess birth outcome among pregnant women inadvertently exposed to AL during first trimester in comparison to those of women exposed to other antimalarial drugs or no drug at all during the same period of pregnancy. Pregnant women with gestational age < 20 weeks were recruited from Reproductive and Child Health (RCH) clinic or from monthly house visits (demography surveillance), and followed prospectively until delivery. A structured questionnaire was used to interview participants. 2167 pregnant women were recruited and 1783 (82.3%) completed the study until delivery. 319 (17.9%) used antimalarials in first trimester, of whom 172 (53.9%) used artemether-lumefantrine (AL), 78 (24.4%) quinine, 66 (20.7%) sulfadoxine-pyrimethamine (SP) and 11 (3.4%) amodiaquine. Quinine exposure in first trimester was associated with an increased risk of miscarriage/stillbirth (OR 2.5; 1.3 - 5.1) and premature birth (OR 2.6; 1.3 - 5.3) as opposed to AL with (OR 1.4; 0.8 - 2.5) for miscarriage/stillbirth and (OR 0.9; 0.5 - 1.8) for preterm birth. Congenital anomalies were identified in 4 exposed groups namely AL only (1/164 [0.6%]), quinine only (1/70 [1.4%]), SP (2/66 [3.0%]), and non-antimalarial exposed group (19/1464 [1.3%]). Exposure to AL in first trimester was more common than to any other antimalarial drugs. Quinine exposure was associated with adverse pregnancy outcome, which was not the case following other antimalarial intake. Since AL and quinine were used according to their availability rather than to disease severity, it is likely that the effect observed was related to the drug, and not to the disease itself. Detailed information on developmental milestone up to 12 months is ongoing to rule out any adverse effect on infancy as a result of AL exposure in first trimester. Even with this caveat, a change of policy from quinine to AL for the treatment of uncomplicated malaria during the whole pregnancy period could be already envisaged.

1922

SAFETY AND EFFICACY OF FOUR ARTEMISININ-BASED COMBINATION TREATMENTS IN AFRICAN PREGNANT WOMEN WITH MALARIA

Umberto D'Alessandro[1], Halidou Tinto[2], Linda Kalilani Phiri[3], Michael Nambozi[4], Harry Tagbor[5], Maminata Traore[2], Innocent Valea[2], Victor Mwapasa[3], Gertrude Kalanda[2], Modest Mulenga[4], Joris Menten[6], Raffaella Ravinetto[6], Yves Claeys[6], Maaike De Crop[6], Theonest Mutabingwa[7]

[1]Medical Research Council, Fajara, Gambia, [2]Clinical Research Unit, Nanoro, Burkina Faso, [3]College of Medicine, University of Malawi, Blantyre, Malawi, [4]Tropical Diseases Research Centre, Ndola, Zambia, [5]Kwame Nkrumah University of Science and Technology, Ku, Ghana, [6]London School of Hygiene & Tropical Medicine, London, United Kingdom, [7]Hubert Kairuki Memorial University, Dar es Salaam, United Republic of Tanzania

Pregnant women are at increased risk of *Plasmodium falciparum* malaria which is associated with increased maternal, foetal and neonatal morbidity and mortality. Thus, malaria-infected pregnant women need prompt and effective treatment. Artemisinin-based combination treatments (ACT) are recommended for pregnant women in their second and third trimesters of pregnancy though information on their safety and efficacy in African pregnant women is limited. A Phase 3, non-inferiority, multicentre, randomized, open-label clinical trial compared the efficacy and safety of four ACTs, namely amodiaquine-artesunate, dihydroartemisinin-piperaquine, artemether-lumefantrine, and mefloquine-artesunate, in women with malaria and in the second or third trimester of pregnancy. A total of 3,423 pregnant women were recruited in Burkina Faso, Ghana, Malawi and Zambia. After being treated with one of the 4 ACTs at day 0, 1 and 2, women were reviewed at days 3, 7, 14, 21, 28, 35, 42, 49, 56 and 63, and whenever they were sick. There were 3 early treatment failures, 2 in Malawi and 1 in Zambia. Eight hundred twenty five women (24.1%) had a recurrent infection during the follow up, 81 (2.4%) of them identified as recrudescences after genotyping. No major safety problems were observed during the follow up. This is the largest trial on ACT use during pregnancy ever done in sub-Saharan Africa. Its preliminary results are reassuring.

1923

EPIDEMIOLOGICAL AND MOLECULAR FEATURES OF DENGUE, ZIKA AND CHIKUNGUNYA CONCURRENT OUTBREAKS IN THE PACIFIC, 2014

Van-Mai Cao-Lormeau[1], Claudine Roche[1], Anita Teissier[1], Eric J. Nilles[2], Didier Musso[1]

[1]Institut Louis Malardé, Papeete - Tahiti, French Polynesia, [2]World Health Organization, Division of Pacific Technical Support, Suva, Fiji

During more than a century dengue has been the only mosquito-borne virus considered as major public health concern for Pacific nations. However, during the past 5 years the epidemiology of arboviruses in the Pacific region has shown terrific changes. The situation gradually switched from the predominant circulation of a single dengue virus (DENV) to active transmission of multiple DENV serotypes and genotypes as observed in French Polynesia from 2013 and in New Caledonia and Fiji since the beginning of 2014. In the mean time, Chikungunya virus (CHIKV) appeared for the first time in New Caledonia with autochtonous cases sporadically reported from 2011 up to 2013, and large outbreaks occurring in Papua New Guinea in 2012, Yap Island in 2013 and in Tonga in 2014. Another unexpected event was the emergence of Zika virus (ZIKV) in French Polynesia at the end of 2013. ZIKV caused in French Polynesia the largest outbreak ever documented, and in a context of active circulation of DENV serotypes 1 and 3. At the beginning of 2014, ZIKV outbreaks also emerged in New Caledonia and Cook Islands. As of April 2014, outbreaks of "dengue-like illnesses" were under investigation in several other Pacific islands suggesting that the situation was evolving from bad to worse. We will describe here the early laboratory investigations that contributed to the identification of the aetiological agents of the outbreaks that recently occurred in the Pacific, notably based on the use of filter paper-spotted serum and saliva collected on cotton swab as a source of viral RNA. Based on phylogenetic data we will discuss how these viruses were introduced from continental regions into the Pacific and how they spread from one Pacific island country to another. We will also discuss the particular features of these outbreaks, notably in the occurrence of unusual clinical manifestations, like observed in French Polynesia during the ZIKV outbreak.

1924

POTENT ANTI-MERS COV (MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS) FULLY HUMAN ANTIBODIES FROM TRANSCHROMOSOMIC BOVINES FOR PASSIVE IMMUNOTHERAPY

Thomas Luke[1], Kanakatte Raviprakash[1], Eddie Sullivan[2], Stanley Perlman[3], Jincun Zhao[3], Hua Wu[2], Jin-An Jiao[2], Lisa Hensley[4], Reed Johnson[5], Gabriel Defang[1], Peter Jahrling[4], Maya Williams[1]

[1]Naval Medical Research Center, Silver Spring, MD, United States, [2]SAB Biotherapeutics, Sioux Falls, SD, United States, [3]University of Iowa, Iowa City, IA, United States, [4]Integrated Research Facility, National Institute of Allergy and Infectious Diseases, Fort Detrick, MD, United States, [5]Emerging Viral Pathogens Section, National Institute of Allergy and Infectious Diseases, Fort Detrick, MD, United States

No specific treatments of proven effectiveness for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infections are currently available. The International Severe Acute Respiratory & Emerging Infection Consortium (ISARIC) identified passive immunotherapy with neutralizing antibodies as a treatment approach that warrants priority study. A platform technology using transchromosomic bovines (Tc-bovines) that produce fully human, antigen specific, polyclonal IgG antibody (Tc-pAb) of all subclasses following immunization has been developed. The quantity of Tc-pAb that can be derived from each animal after plasmapheresis ranges from 150 to 300 grams per month. Purified Tc-pAb for intravenous or intramuscular administration has extremely low quantities of bovine proteins and no evidence of adventitious agents. The Tc-bovine platform can also rapidly produce a diverse repertoire of fully human monoclonal antibodies. Two experimental anti-MERS CoV Tc-pAb immunoglobulins were produced in Tc-bovines hyperimmunized with inactivated whole virion Jordan strain virus (clade A) or a recombinant spike protein derived from an Al-Hasa strain (clade B). Both Tc-pAb immunoglobulins, termed SAB-300 and SAB-301, demonstrated 50% plaque reduction neutralizing antibody titers > 10e4/ml and cross neutralized other MERS-CoV strains. SAB-300/SAB-301 were evaluated in recombinant mice expressing the DPP-4 receptor (5 mg/kg and 25 mg/kg IP as a single dose 12 hours before intranasal challenge) and SAB-300 in marmosets (80 mg/kg in 4 divided doses IV starting 24 hours after intratracheal challenge). Control infected mice had a lung viral titer of ~6.0 $\log_{10}$ PFU/mg through day 5 post inoculation but treated mice approached, or were below, the limit of detection (2.0 $\log_{10}$ PFU/mg) by 24-72 hours and displayed no toxicity. Treated marmosets displayed no toxicity and the clinical/virologic data will be presented. Because of these encouraging pre-clinical findings, an IND application is in development.

1925

CLINICAL STUDIES OF DNA VACCINES FOR HEMORRHAGIC FEVER WITH RENAL SYNDROME

Connie Schmaljohn[1], Jay W. Hooper[1], Drew Hannaman[2], Kristopher M. Paolino[3]

[1]United States Army Medical Research Institute for Infectious Diseases, Fort Detrick, MD, United States, [2]Ichor Medical Systems, San Diego, CA, United States, [3]Walter Reed Army Institute of Research, Silver Spring, MD, United States

Hemorrhagic fever with renal syndrome (HFRS) is endemic in Asia, Europe and Scandinavia, and is caused by infection with the hantaviruses Hantaan (HTNV), Seoul (SEOV), Puumala (PUUV), or Dobrava (DOBV) viruses. We developed candidate DNA vaccines for HFRS expressing Gn and Gc genes of HTNV or PUUV and evaluated them in an open-label, single-center Phase 1 study. Three groups of nine subjects each were vaccinated on days 0, 28 and 56 with the DNA vaccines for HTNV, PUUV, or mixture of both vaccines using the Ichor Medical Systems TriGridTM Intramuscular Delivery System (TDS-IM). All vaccinations consisted of a total dose of 2.0 mg DNA in an injected volume of 1 mL saline. For the combined vaccine, the mixture contained equal amounts (1 .0 mg) of each DNA vaccine. There were no study-related serious adverse events (SAEs). Neutralizing antibody responses were detected in 5/9 and 7/9 of individuals who completed all three vaccinations with the HTNV or PUUV DNA vaccines, respectively. In the combined vaccine group, 7/9 of the volunteers receiving all three vaccinations developed neutralizing antibodies to PUUV. The three strongest responders to the PUUV vaccine also had strong neutralizing antibody responses to HTNV. These results demonstrate that the HTNV and PUUV DNA vaccines delivered by TDS-IM separately or as a mixture are safe. In addition, both vaccines were immunogenic, although when mixed together, more subjects responded to the PUUV than to the HTNV DNA vaccine, suggesting immunological interference. Consequently, we have developed an optimized HTNV DNA vaccine that shows no interference in hamsters when mixed with the PUUV vaccine. A Phase 2a clinical study will be initiated in 2014 to assess dose and schedule with the combined, optimized HTNV and PUUV DNA vaccines. An additional Phase 1 study is being planned to compare intradermal and intramuscular delivery of the mixed DNA vaccines.

1926

AN INEXPENSIVE SYSTEM FOR PRODUCING STRUCTURALLY STABLE REPLICATIVE RNA VIRUS-BASED NANOPARTICLE VACCINES

Yiyang Zhou, Christopher Kearney

Baylor University, Waco, TX, United States

Tropical infectious disease vaccine development requires particular attention to cost-efficiency and minimized storage conditions to be optimally useful in third world countries. In this study, the use of a plant-produced, trans-encapsidated non-pathogenic RNA virus as a vaccine vector addresses these issues. Plant-based manufacturing allows large scale production with greatly reduced expense. Transencapsidation with *Tobacco mosaic virus* (TMV) coat protein provides superior chemical and environmental protection to any RNA sequence containing a TMV encapsidation site. A transencapsidated RNA virus that expresses vaccine antigens in human cells would be highly attractive for vaccine use due to its ability to induce innate immune activation pathways to aid immunogenicity of its antigen payload. To avoid the potential safety concerns using a human virus, we have used the insect RNA virus, *Flock House virus* (FHV), which replicates in both human and plant cells but is not pathogenic to either. The RNA2 of the bipartite FHV genome codes for FHV coat protein and is not necessary for replication. We created an FHV RNA1/eGFP vector and noted strong expression of eGFP in inoculated *Nicotiana benthamiana* plants. This was followed by inserting the TMV encapsidation site into the FHV RNA1 and inoculating plants in combination with a high expression vector derived from *Foxtail mosaic virus* to express TMV coat protein. The production of *in planta* nanoparticles was verified by transmission electron microscopy and these yielded an immune response in vaccinated mice that was superior to that of *in vitro* assembled nanoparticles. In this study, we validated the use of *in planta* encapsidated RNAs as an immune activator in the absence of adjuvants. We can now use this system to create sturdy and inexpensive vaccines for tropical infectious diseases.

1927

SEROPREVALENCE OF NGARI AND BUNYAMWERA VIRUSES IN SELECT PARTS OF RIFT VALLEY AND NORTHERN KENYA

Collins O. Odhiambo[1], Marietjie Venter[2], Robert Swanepoel[2], Rosemary Sang[1]

[1]International Centre for Insect Physiology and Ecology, Nairobi, Kenya, [2]University of Pretoria, Pretoria, South Africa

Ngari and Bunyamwera viruses are among the few mosquito-borne human pathogens in the Orthobunyavirus genus, family Bunyaviridae, associated with febrile illness. Ngari virus has been associated with hemorrhagic fever during Rift Valley fever outbreaks in Africa. Ngari virus is a reassortant virus composed of the S and L segments from Bunyamwera and the M segment from Batai virus. While isolations of both viruses have been made from mosquito and tick vectors in the transmission foci in Kenya, no human serosurveys have been conducted. We report findings from a retrospective serosurvey of febrile ill patients attending three health facilities located in Sangailu, Kotile (in Garissa) and Naivasha in Kenya. Bunyamwera and Ngari virus specific antibodies were detected by plaque reduction neutralization tests in 84 (24.3%) of 345 persons tested; Prevalence rates were 11.9% for Bunyamwera virus and 15.9% for Ngari virus. Multivariable analysis revealed age and location as risk factors for Bunyamwera and Ngari virus infections. Patients presenting with febrile illness in identified endemic regions should be vigorously investigated to determine the public health impact of these infections especially during seasons of high mosquito abundance.

1928

ITAYA VIRUS: A NOVEL *ORTHOBUNYAVIRUS* ASSOCIATED WITH HUMAN FEBRILE ILLNESS IN PERU

Carolina Guevara[1], Jesus Silvas[2], Robert Hontz[1], Wilma Casanova[3], Nikos Vasilakis[2], Douglas Watts[4], Tadeusz Kochel[1], Julia S. Ampuero[1], Hideki Ebihara[5], Patricia V. Aguilar[2]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Department of Pathology, Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, TX, United States, [3]Direccion Regional de Salud Loreto, Iquitos, Peru, [4]University of Texas at El Paso, El Paso, TX, United States, [5]Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, MT, United States

The *Orthobunyavirus* genus in the family *Bunyaviridae* comprises more than 170 viruses, at least 30 of them associated with human disease. Caraparu virus, a member of the group C (*Orthobunyavirus* genus), was first isolated from a sentinel monkey in Brazil and subsequently isolated from febrile patients in Bolivia, Brazil, Peru and Trinidad. Febrile surveillance studies conducted by the U.S. Naval Medical Research Center Unit No. 6 identified Caraparu virus (and other group C viruses) as an important cause of febrile illness in the Amazon region of Peru. We conducted genetic analyses of previously uncharacterized bunyavirus strains isolated from febrile patients in Peru, and identified a novel reassortant virus containing the S and L segment of Caraparu virus and the M segment of an unidentified Group C virus. Neutralization test using mouse antisera prepared against the prototype Caraparu strain BeAn 3994 and the novel reassortant virus showed that there was more than a 4-fold difference in titer between these viruses, indicating that the new reassortant was serologically distinct from the prototype Caraparu strain. Serological analyses also confirmed that the novel reassortant was antigenically

distinct from Peruvian Caraparu strains. This new reassortant virus, which we named Itaya virus, was first isolated during 1999 from a 25-year-old male febrile patient in Iquitos, Peru, and subsequently isolated during 2006 from a 59-year-old male febrile patient in Yurimaguas, another city within the Amazon region of Peru. Geographical distance between these two cases indicates that Itaya virus may be widely distributed within the Peruvian Amazon. The recognition of a new *Orthobunyavirus* human pathogen in the Amazon region of Peru reinforces the need to continue and expand viral disease surveillance activities in tropical regions of South America.

1929

MAPPING THE ZOONOTIC NICHE OF EBOLA VIRUS DISEASE IN AFRICA

David Pigott[1], Nick Golding[2], Adrian Mylne[2], Zhi Huang[2], Andrew Henry[2], Daniel Weiss[2], Oliver Brady[2], Moritz Kraemer[2], David Smith[2], Catherine Moyes[2], Samir Bhatt[2], Peter Gething[2], Peter Horby[2], Isaac Bogoch[3], John Brownstein[4], Sumiko Mekaru[4], Andrew Tatem[5], Kamran Khan[3], Simon Hay[2]

[A]Department of Zoology, [1]University of Oxford, Oxford, United Kingdom, [2]University of Toronto, Toronto, ON, Canada, [3]Boston Children's Hospital, Boston, MA, United States, [4]University of Southampton, Southampton, United Kingdom

Ebola virus disease (EVD) is a complex zoonosis that is highly virulent in humans. The largest recorded outbreak of EVD is ongoing in West Africa, outside of its previously reported and predicted niche. We assembled location data on all recorded zoonotic transmission to humans and Ebola virus infection in bats and primates (1976-2014). Using species distribution models these occurrence data were paired with environmental covariates to predict a zoonotic transmission niche covering 22 countries across Central and West Africa. Vegetation, elevation, temperature, evapotranspiration and suspected reservoir bat distributions define this relationship. At-risk areas are inhabited by 22 million people, however the rarity of human outbreaks emphasises the very low probability of transmission to humans. Increasing population sizes and international connectivity by air since the first detection of EVD in 1976 suggest that the dynamics of human-to-human secondary transmission in contemporary outbreaks will be very different to those of the past.

1930

USE OF A NOVEL CHAGAS URINE NANOPARTICLE TEST (CHUNAP) FOR DIAGNOSIS OF CONGENITAL CHAGAS DISEASE

Yagahira E. Castro[1], Robert Gilman[2], Gerson Galdos-Cardenas[2], Lisbeth Ferrufino[3], Gerardo Sánchez[1], Edward Valencia Ayala[1], Lance Liotta[4], Caryn Bern[5]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Johns Hopkins University, Bloomberg School of Hygiene and Public Health, Baltimore, MD, United States, [3]Hospital Universitario Japonés, Santa Cruz, Plurinational State of Bolivia, [4]Center for Applied Proteomics and Molecular Medicine, George Mason University, Manassas, VA, United States, [5]Global Health Sciences, Department of Epidemiology and Biostatistics School of Medicine, University of California San Francisco, CA, United States

Detection of congenital *Trypanosoma cruzi* transmission is considered one of the pillars of control programs of Chagas disease, because 25% of new infections occur by this route with an estimated of 15,000 infected infants per year in Latin America. Current programs to detect congenital Chagas disease in Latin America utilize microscopy early in life and serology after 6 months. These programs suffer from low sensitivity by microscopy and high loss to follow-up later in infancy. We developed a Chagas urine nanoparticle test (Chunap) to concentrate, preserve and detect *T. cruzi* antigens in urine for early, non-invasive diagnosis of congenital Chagas disease. This is a proof-of-concept study to provide an initial indication that Chunap allows for the early diagnosis of congenital Chagas disease. Poly N-isopropylacrylamide nano-particles functionalized with trypan blue were synthesized by precipitation polymerization and characterized with photon correlation spectroscopy. We evaluated the ability of the nanoparticles to capture, concentrate and preserve *T. cruzi* antigens. Urine samples from congenitally infected and uninfected infants were then concentrated using these nanoparticles. The antigens were eluted and detected by Western Blot using a monoclonal antibody against *T. cruzi* lipophosphoglycan. The nanoparticles concentrated *T. cruzi* antigens by 100 fold (western blot detection limit decreased from 50 ng/ml to 0.5 ng/ml). The sensitivity of Chunap in a single specimen at one month of age was 91.3% (21/23, 95% CI: 71.92%-98.68%), comparable to PCR in two specimens at 0 and 1 month (91.3%) and significantly higher than microscopy in two specimens (34.8%, 95% CI: 16.42%-57.26%). Chunap specificity was 96.5% (71/74 endemic, 12/12 non-endemic specimens). Particle-sequestered *T. cruzi* antigens were protected from trypsin digestion. Chunap has the potential to be developed into a simple and sensitive test for the early diagnosis of congenital Chagas disease.

1931

A THERAPEUTIC NANOPARTICLE VACCINE AGAINST *TRYPANOSOMA CRUZI* IN A MOUSE MODEL OF CHAGAS DISEASE

Meagan A. Barry, Qian Wang, Coreen M. Beaumier, Kathryn M. Jones, Brian P. Keegen, Maria Elena Bottazzi, Michael J. Heffernan, Peter J. Hotez

Baylor College of Medicine, Houston, TX, United States

Chagas disease is a neglected tropical disease of great importance in the Americas, with 7-8 million people infected. The causative agent is *Trypanosoma cruzi*, and results in an acute febrile illness that progresses to chronic chagasic cardiomyopathy in 30% of patients. In endemic areas, Chagas disease is the leading cause of cardiovascular death between ages 30-50. Current pharmacological treatments are plagued by significant side effects, poor efficacy, and are contraindicated in pregnancy. There is an urgent need for new treatment modalities. A therapeutic vaccine for Chagas disease has potential advantages that include cost savings, reduced adverse effects, and the potential to be used as a replacement for current therapies or when paired with chemotherapy. Prior work in mice has identified an efficacious *T. cruzi* antigen (Tc24). To elicit a protective cell-mediated immune response to the Tc24 protein, we have utilized a nanoparticle delivery system in conjunction with CpG motif-containing oligodeoxynucleotides (ODN) as an immunomodulatory adjuvant. When tested in a BALB/c mouse model, a dose response study demonstrated a positive relationship between dose of vaccine and Tc24-specific IFN-γ response. Our nanoparticle vaccine, comprised of Tc24 and CpG ODN encapsulated in poly(lactic-co-glycolic acid) (PLGA) nanoparticles, produced the most robust T_H1-mediated $CD8^+$ T cell immune response. When tested for therapeutic efficacy in *T. cruzi* infected BALB/c mice, improved survival was seen in the vaccine group compared to the control groups. Additionally, there was a significant reduction in the number of parasites in the cardiac tissue of the vaccine group compared to the PBS sham vaccine group, indicating protection from parasite-driven cardiac damage. The mice that survived to the end of the study had almost undetectable numbers of parasites in the cardiac tissue. These data demonstrate the immunogenicity and efficacy of a Tc24/CpG ODN nanoparticle vaccine and are convincing evidence for a potential new therapeutic vaccine against Chagas disease.

1932

ASSEMBLING NEW CHEMICAL BOXES AS AN OPEN SOURCE OF STARTING POINTS FOR DRUG DISCOVERY AGAINST KINETOPLASTID PARASITES CAUSING NEGLECTED TROPICAL DISEASES

Julio J. Martin[1], Emilio Alvarez[1], Ana I. Bardera[1], Juan Cantizani[1], Gonzalo Colmenarejo[1], Jose M. Fiandor[1], Albane Kessler[1], Maria P. Manzano[1], Julio Alonso-Padilla[2], Imanol Peña[1], Ana Rodriguez[2]

[1]GlaxoSmithKline, Tres Cantos, Spain, [2]New York University School of Medicine, New York, NY, United States

Neglected Tropical Diseases (NTDs) are a group of infectious diseases categorized by the particular neglect they have suffered in terms of investment in control measures, when compared with malaria and tuberculosis, "the big two" within the diseases. The NTDs encompass a broad range of viral, bacterial, and parasitic infections. Kinetoplastids are a group of flagellated protozoans that include the species *Leishmania* and *Trypanosoma*, some of which are human pathogens with devastating health and economic effect. The most common human diseases caused by kinetoplastids are included within the list of 17 core NTDs declared by WHO. They are human African trypanosomiasis, caused by subspecies of *T. brucei;* Chagas disease, caused by the infection with *T. cruzi*; and various clinical manifestations of leishmaniasis, caused by more than 20 species of *Leishmania*. All NTDs have been categorized as "tool ready," yet also "tool deficient" because many of these tools (i.e. drugs and diagnostics) and implementation strategies are inadequate to achieve the desired goals. New effective, safe, and affordable drugs, preferably oral, are needed. The general neglect that these diseases have encountered by the pharmaceutical industry has meant that basic research findings have not found their way into a drug discovery pipeline. In this paper we present an integral approach to the early drug discovery for the three major kinetoplastid NTDs, i.e. visceral leishmaniasis, Chagas disease and sleeping sickness. The GSK 1.8 million compounds diverse collection has been screened phenotypically against their causative parasites, respectively *L. donovani*, *T. cruzi* and *T. brucei*, using the state-of-the-art methodologies available in high throughput screening. As a result of this effort, three anti-kinetoplastidal boxes of approximately 200 compounds each have been assembled, which represent all the chemical and biological diversity identified and are intended to serve as an open source of starting points for further lead discovery programs.

1933

A FULLY INTEGRATED PARTNERSHIP PERFORMING DRUG DISCOVERY TOWARDS VISCERAL LEISHMANIASIS: PART 1

Paul G. Wyatt

University of Dundee, Dundee, United Kingdom

GSK Kinetoplastid DPU and the Drug Discovery Unit, University of Dundee have formed a partnership to conduct drug discovery within kinetoplastid diseases (Visceral Leishmaniasis, Chagas disease and human African trypanosomiasis). The collaboration, with support from the Wellcome Trust has made significant progress, in building new methods and infrastructure to carry out drug discovery for these parasites. Our advances has resulted in the identification of a lead optimisation series for Visceral Leishmaniasis through phenotypic optimisation. Estimates suggest that Visceral Leishmaniasis worldwide causes 51,000 deaths per year. The current drugs are not fit for purpose, suffering from many issues including poor efficacy and unacceptable levels of toxicity. Part 1, by Paul Wyatt from the Drug Discovery Unit, will describe the transition of a T. brucei GSK3 kinase inhibitor series into a series that fulfils lead optimisation criteria for Visceral Leishmaniasis. This novel series is one of the few reported globally to show oral efficacy in an acute *in vivo* mouse model against Visceral Leishmaniasis. Part 2, by Tim Miles from GSK, will concentrate on the lead optimisation and progression of this series. As a number of issues were highlighted through critical path screening that have been overcome (i.e. solubility and exposure). Hence a discussion of medicinal chemistry strategies to solve these issues within a phenotypic screening setting will be discussed. The current set lead compounds within this series are being evaluated for pre-candidate selection.

1934

CLINICAL EVALUATION OF CL DETECT™ RAPID TEST FOR CUTANEOUS LEISHMANIASIS: PERFORMANCE CHARACTERISTICS WHEN COMPARED TO SMEAR MICROSCOPY AT MULTIPLE TEST SITES

Afif Ben Salah[1], Nabil Bel Haj Hamida[1], Amor Zaatour[1], Adel Gharbi[1], Jihene Bettaieb[1], Wissem Ghawar[1], Amina Khedher[1], Aicha Boukthir[1], Nathalie Ben Messaoud[1], Evelyne Guedri[1], Nathalie Ben Mbarek[1], Nasri Mbarek[1], Rabiaa Harrabi[1], Chadhi Dawahi[1], Hongjing Chen[2], Stan Morkowski[2], James Needham[2], Annabel Tsai[2], Yvonne Stevens[2], Syamal Raychaudhuri[2], Stuart Kahn[2], Janet Ransom[3], Charles Scott[3], Giselle Singer[4], Danielle Baum[4], Shelby Jim On[4], Mark Richardson[4], William McCarthy[5], Max Grogl[5], Mara Kreishman-Deitrick[5], Jeanne Norwood[5], Raymond Houghton[2], Mark Lebwohl[4]

[1]Institut Pasteur de Tunis, Tunis, Tunisia, [2]InBios International Inc., Seattle, WA, United States, [3]Fast-Track Drugs and Biologics, LLC, North Potomac, MD, United States, [4]Icahn School of Medicine at Mount Sinai, New York, NY, United States, [5]Office U.S. Army Medical Materiel Development Activity, Fort Detrick, MD, United States

This study focused on establishing the performance characteristics of the CL Detect™ rapid immunoassay when compared to smear microscopy. The assay is intended for the diagnosis of Cutaneous Leishmaniasis in far-forward, rugged environments. The test is based on the detection of the thiol specific antioxidant protein (TSA, Peroxidoxin) present in amastigotes and promastigotes of L. major and other Leishmania spp. It uses a capture polyclonal antibody to TSA in combination with a gold conjugated monoclonal antibody directed to L. major amastigotes but reactive with TSA. Testing was performed on samples from skin lesions collected with a dental broach device. In each case dipstick reactivity was compared to the lesion parasite load determined by microscopy and quantified using the WHO scale. A total of 168 patients ranging in age from 18-79 years with suspected CL lesions were enrolled with written informed consent at 2 sites endemic for L. major infections in central Tunisia (Sidi Bouzid, Gafsa). 149 were positive by CL Detect™ and microscopy while 16 were negative by both tests and 3 were positive by dipstick but not microscopy. Of these three, 1 was positive by culture. Of the 16 negatives by dipstick and microscopy 2 were positive by culture. In the Icahn School of Medicine, Mount Sinai specificity study, 150 samples were tested by CL Detect™ and microscopy. These included patients ranging in age from 18-92 years with other skin lesions and non-CL infections. In the specificity study 144 of 150 were true negatives for parasites by both CL Detect™ and microscopy for a specificity of 96.0%. Six samples negative by microscopy were low positive by rapid test but negative by microscopy. Cross reactivity studies with other bacteria, parasites, viruses and fungi confirmed specificity for Leishmania spp. Interference, stability and reproducibility studies indicate that CL Detect™ is a robust assay. The pairing of this test with a safe and easy to use drug treatment has the potential to greatly enhance the management of CL patients in far-forward rugged environments.

1935

ACCESS TO DIAGNOSIS AND TREATMENT FOR CHAGAS DISEASE IN THE UNITED STATES: A HEALTH SYSTEMS PERSPECTIVE

Jen Manne-Goehler[1], Michael R. Reich[2], Veronika J. Wirtz[3]

[1]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States, [2]Department of Global Health and Population, Harvard School of Public Health, Boston, MA, United States, [3]Boston University Center for Global Health and Development, Boston, MA, United States

Chagas disease, caused by infection with *Trypanosoma cruzi*, is a vector-borne disease with an estimated 300,000 cases in the United States (US). Screening of blood donors for infection with *T. cruzi* was started in 2007 in the US. Currently, benznidazole and nifurtimox are available to treat this infection through direct request from the US Centers for Diseases Control and Prevention (CDC) under compassionate use protocols. This study analyzed epidemiological trends in diagnosis and treatment for Chagas disease in the US and assessed national and state barriers to access. Data on the distribution of cases of Chagas disease identified in blood donors and drug releases were obtained from the AABB and CDC respectively. Semi-structured in-depth interviews were conducted with 30 key informants at the national level and in 6 high-burden states (CA, FL, VA, NY, MA, and TX) where treatments were provided. Interview responses were analyzed according to the health system's dimensions of regulation, financing, payment, organization, and persuasion. Data indicate that 1,908 cases were identified in the blood donation system from 2007-2013 and that CDC provided 422 courses of benznidazole or nifurtimox during this period. Interview data revealed that local ad-hoc procedures were used by individual physicians with an interest in the disease to increase access to medicines for Chagas disease, especially through cross-financing of patient care activities using grants and donations. The primary barriers to access at the national level include limited diagnostic and institutionalized referral and care processes (Organization), lack of financing for patient care activities in most states (Financing), and limited awareness and training among physicians and patients (Persuasion). This study demonstrates that access to treatment for Chagas disease in the US is limited. The lack of licensing for the two medications used in treatment was only one of several barriers to access, highlighting the need for a health systems perspective when scaling up access to these essential medicines.

1936

IMMUNOGENICITY OF *TRYPANOSOMA CRUZI* VACCINE CANDIDATE ANTIGENS TSA-1 AND TC24 IN MEXICAN CHAGASIC PATIENTS

Liliana Villanueva-Lizama, Amaru Aguilar-Cetina, Vladimir Cruz-Chan, Maria Jesus Ramirez-Sierra, Miguel Rosado-Vallado, Eric Dumonteil

Universidad Autonoma de Yucatan, Merida, Yucatan, Mexico

Chagas disease affects 8-10 million persons worldwide, and at least 1-2 millions in Mexico. Current drugs have a limited efficacy and the development of a preventive and/or therapeutic vaccine is an important goal. Based on previous work in mouse and dog models, trypomastigote surface antigen-1 (TSA-1) and 24-kDa-trypomastigote secretion/excretion antigen (Tc24) are good candidates for a vaccine against *Trypanosoma cruzi*. We evaluated here the recall immune response against these vaccine antigens in Chagas disease patients, as a first step to assess their immunogenicity in humans. We used peripheral blood mononuclear cells (PBMC) from chagasic patients (n=8) and healthy controls (n=8) that were stimulated *in vitro* with TSA-1 and Tc24 recombinant antigens. After 120 hours of stimulation, we evaluated cell proliferation, identified CD4+ and CD8+ memory cell subpopulations, and IFN-gamma and IL-10-producing cells by flow cytometry. We observed a specific proliferative response to TSA-1 and to a lesser extent to Tc24, with a central memory T cell phenotype and antigen-specific INF-gamma and IL-10 production in several Chagas disease patients. Additional patients and controls will be enrolled until September 2014. These preliminary results suggest that the selected antigens are immunogenic in humans and may thus be good candidates for further development of a Chagas disease vaccine

1937

EARLY IL-10 PRODUCTION BY CD4+ T CELLS IN THE SKIN IS FUNCTIONALLY SUPPRESSIVE

David Sanin, Catriona T. Prendergast, Adrian P. Mountford

Centre for Immunology and Infection, Department of Biology, University of York, York, United Kingdom

Communities in endemic areas affected by Schistosomiasis, a parasitic disease caused by *Schistosoma mansoni*, are repeatedly infected when they come into contact with contaminated water. *S. mansoni* cercariae penetrate the skin and elicit a strong inflammatory response, which after repeated exposure becomes both exacerbated and skewed towards type 2 immunity, with high levels of IL-10, IL-4, eosinophilia and alternative activation of macrophages. In compass with this phenomenon at the site of infection, local skin draining draining lymph node (sdLN) cells progressively lose their ability to respond to *S. mansoni* cercarial antigens, becoming unable to proliferate or produce cytokines. Our evidence shows that the effect multiple infections have on sdLN responsiveness is mediated by IL-10, which is found at significantly higher levels in the skin after repeated exposure to the parasite. sdLN cells from IL-10 deficient mice retained their ability to respond to schistosomula antigens, while the immune response in the skin was significantly more pro-inflammatory. After the initial exposure to the parasite, $CD4^+$ T cells and $F4/80^+MHC\text{-}II^{high}$ monocytes produced most of the IL-10 in the skin. Strikingly, $CD4^+$ T cells in the skin made IL-10 as early as day 1 after the initial exposure. This initial response was directed against commensal antigens that would penetrate the skin during cercariae invasion. However, by day 4 after the first exposure, non-regulatory $CD4^+$ T cells respond to *S. mansoni* antigens, expanding considerably after multiple infections and accounting for most of the detected IL-10. Furthermore, IL-10 producing $CD4^+$ T cells from the skin have the ability to inhibit the proliferation of sdLN $CD4^+$ cells. In summary, $CD4^+$ T cells in the skin produce IL-10 and prevent cells in the lymph nodes from responding to repeated infections with the parasite, whilst they contribute to a type 2 immune response environment in the skin. Skin commensals are partly responsible for this type of response, as they penetrate the skin when *S. mansoni* cercarieae invade the tissue.

1938

A CENTRAL ROLE FOR TYPE I IFN IN THE INDUCTION OF TH2 RESPONSES BY DENDRITIC CELLS

Lauren Webb[1], Rachel J. Lundie[2], Jessica G. Borger[2], Peter C. Cook[1], Sheila L. Brown[1], Lucy H. Jones[3], Alexander T. Phythian-Adams[1], Andrew S. MacDonald[1]

[1]Manchester Collaborative Centre for Inflammation Research, University of Manchester, Manchester, United Kingdom, [2]Institute of Inflammation and Infection Research, University of Edinburgh, Edinburgh, United Kingdom, [3]Institute of Inflammation and Infection Research, University of Edinburgh, Edinburgh, United Kingdom

Although dendritic cells (DCs) are critical for induction of Th2 immunity against helminths or allergens, relatively little is known about how they become activated and function in response to Th2-polarizing antigens. We have discovered a previously unrecognized role for Type I IFN (IFN-I) in the optimal activation and function of DCs following exposure to strongly Th2-polarizing antigens from the parasitic helminth *Schistosoma mansoni*. To date, IFN-I has primarily been associated with anti-viral immunity, and its role in Th2 settings is currently unclear. DCs lacking the IFN-I receptor displayed a dramatically impaired ability to induce Th2 cytokines *in vivo*, but unimpaired ability to support Th2 polarization *in vitro*. Further, Th2-promoting DCs depended on IFN-I signaling for efficient migration to the draining LN. We are now investigating whether IFN-I is also required for effective localization within the draining LN and interaction with LN-

resident T cells. Together, our data suggest a key role for IFN-I to enable Th2 induction by DCs against helminths *in vivo*. Future work will address the wider role of IFN-I in Th2 inflammation, including during helminth infection, and the activation of allergic responses in the airways.

1939

VSG-SEQ: A QUANTITATIVE METHOD FOR TRACKING THE *IN VIVO* DYNAMICS OF ANTIGENIC VARIATION IN *TRYPANOSOMA BRUCEI*

Monica Mugnier, George Cross, Nina Papavasiliou

The Rockefeller University, New York, NY, United States

Using a repertoire of over 2000 different variant surface glycoprotein (VSG) genes within its genome, *Trypanosoma brucei,* the causative agent of African sleeping sickness, changes its dense VSG surface coat to avoid detection by the immune system of its mammalian host. The dynamics of antigenic variation in *T. brucei* during an infection, however, are poorly understood. How many variants appear over the course of an infection? Is there a pattern to VSG expression over time? Although some of these questions have been broached using Sanger sequencing of VSG cDNA, technical limitations have prevented a high-resolution, quantitative study of VSG expression during *T. brucei* infection. Here we present VSG-seq, the first method for quantitatively examining the diversity of expressed VSGs in a population of trypanosomes, isolated either from culture or from blood. This next-generation sequencing approach requires very little input material and is quite sensitive, detecting VSGs expressed on less than 0.1% of a population of trypanosomes. Using samples isolated from mouse infections, expressed VSG sequences can be assembled accurately *de novo*, demonstrating that this approach can be used for the high-resolution study of VSG expression in any strain of *T. brucei,* whether in the lab or in the field. We have used VSG-seq to study the kinetics of VSG populations throughout *T. brucei* infections. These studies reveal more complex switching dynamics than previously expected and hint at the possibility of new mechanisms for increasing antigenic diversity *in vivo*.

1940

BLOODSTREAM FORM *TRYPANOSOMA BRUCEI* MEMBRANE NANOTUBES AND EXTRACELLULAR VESICLES MEDIATE INTERCELLULAR INTERACTIONS AND HOST ANEMIA

Anthony Szempruch[1], S. E. Sykes[1], J. M. Harrington[1], A. C. Becker[1], A. Gartrell[1], E. Arigi[2], I. C. Almeida[2], S. L. Hajduk[1]

[1]Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, United States, [2]Department of Biology, University of Texas El Paso, El Paso, TX, United States

Trypanosoma brucei cycles between an insect vector and a mammalian host, causing human African sleeping sickness and Nagana in cattle. We identified extracellular vesicles (EVs) from wild type bloodstream form (BF) cells by electron microscopy. Purified vesicles have been shown to propagate a distinctive morphological phenotype when added to wild type cells. Fluorescently labeled EVs bind to the trypanosome flagellar pocket and are subsequently endocytosed. We have developed an RNAi cell line that can be induced to produce an excess of EVs. When used in transwell separation experiments, these cells showed EVs mediate phenotype transfer. Proteomic analysis of EVs from both wild type and RNAi cells revealed ~50 shared proteins. In addition, we observe that purified EVs are capable of membrane fusion and transferring variant surface glycoprotein to human red blood cells. This fusion and protein transfer alters the physical properties of the red blood cell membrane, potentially leading to anemia seen during infection. Imaging of *T. brucei* cells reveal the formation of long membrane nanotubes at the posterior end of the cells that are able to bind other trypanosomes. These membrane nanotubes originate from budding of the flagellar membrane and form a helical wrapping structure that resembles "beads on a string." These "beads" closely resemble the structure of cell-associated EVs. In addition, live cell imaging suggests that these nanotubes can disassociate into what appear to be free vesicles. We hypothesize that membrane nanotubes are the structures with which trypanosomes produce EVs. This demonstrates that *T. brucei* is capable of cellular communication and may have significant impact to understanding infection, immune evasion, and differentiation of this parasite.

1941

MONOCYTE-DERIVED ALTERNATIVELY ACTIVATED MACROPHAGES RETAIN PLASTICITY AFTER ACTIVATION AND DIFFERENTIATION

Natasha Girgis, Uma Mahesh Gundra, Michael A. Gonzales, P'ng Loke

New York University School of Medicine, Department of Microbiology, New York, NY, United States

Alternatively activated macrophages (AAM) induced by type 2 responses promote clearance of helminth parasites, wound healing, and tissue remodeling. AAM can accumulate through recruitment and differentiation of monocytes, or through proliferation of tissue resident macrophages. We have recently shown that AAM derived from monocytes ($_{mono}$AAM) are phenotypically and functionally distinct from AAM that arise from tissue macrophages ($_{tiss}$AAM). $_{tiss}$AAM are F480^{high} and express the mitochondrial protein Ucp1, but low levels of MR1 and PDL2, while $_{mono}$AAM were F480^{int} and expressed high levels of MR1 and PDL2. Here, we find that $_{mono}$AAM recruited to sites of inflammation remain plastic after activation and differentiation. In short-term transfer experiments, $_{mono}$AAM donor cells transferred into naïve recipients retain expression of MR1, but lose expression of PDL2. In long-term transfer experiments, $_{mono}$AAM can convert to F4/80^{high} macrophages with a phenotype similar to $_{tiss}$AAM. Hence, $_{mono}$AAM remain plastic after activation, which is dependent on Stat6 signaling in donor AAM, as well as accessory cells in the recipient mice. We have also found that AAM in the hepatic granulomas of mice infected with the parasitic helminth *Schistosoma mansoni* accumulate primarily through the recruitment of inflammatory monocytes. Future experiments will determine if these $_{mono}$AAM will further adopt the phenotype of $_{tiss}$AAM in the livers of infected mice after long-term residence in the granulomas

1942

METABOLIC REGULATION OF TYPE 2 IMMUNITY CONTROLS TISSUE REPAIR

Wildaliz Nieves, Taylor Oniskey, Li-Yin Hung, De'Broski R. Herbert

Division of Experimental Medicine, Department of Medicine, University of California at San Francisco, San Francisco, CA, United States

Host metabolism is profoundly affected by gastrointestinal (GI) nematodes. Many GI nematodes feed upon the tissues and blood of their hosts, resulting in anemia, malnutrition, and generalized immunosuppression. It is debatable whether these features of worm infection are due to parasite and/or host-derived factors. In this study, we investigated whether adenosine monophosphate kinase (AMPK) controlled the outcome of GI nematode infection through controlling the inflammatory response. AMPK is a heterotrimeric enzyme complex of $\alpha\beta\gamma$ subunits that restores cellular energy through oxidative phosphorylation. Given that AMPK activity is regulated by phosphorylation of the catalytic α subunit, we generated CD11c^{Cre} x AMPKα1$^{flox/flox}$ (DC-AMPK$^{-/-}$) mice to study the importance of AMPK in alveolar macrophage and dendritic cell function. DC-AMPK$^{-/-}$ mice infected with the hookworm *Nippostrongylus brasiliensis* (*N.b.*) generated abnormal Type-2 immune responses and failed to regenerate areas of hookworm-damaged tissue 9 days post-primary infection. In comparison to littermate controls, DC-AMPK$^{-/-}$ mice were unable to generate intestinal goblet cell metaplasia and failed to expel adult worms from the intestine. Moreover, *N.b.*-induced lung injury was more severe in DC-AMPK$^{-/-}$ mice and the restoration of pulmonary function was significantly delayed compared to controls. Dysregulated responses generated in DC-AMPK$^{-/-}$ mice were associated with increased Type-1 responses (IL-12, iNOS), greater numbers of T_H17 cells, and defects in the generation of alternatively activated macrophages. Taken together, our data are consistent with an

important role for host metabolism in shaping inflammatory responses during helminth infection. Thus, AMPK activity within myeloid antigen presenting cells regulates host protection against GI parasites.

1943

THE *TOXOPLASMA* DENSE GRANULE PROTEINS GRA17 AND GRA23 MEDIATE THE MOVEMENT OF SMALL MOLECULES BETWEEN THE HOST AND THE PARASITOPHOROUS VACUOLE

Dan Gold[1], Aaron D. Kaplan[2], Agnieszka Lis[2], Glenna C. Bett[2], Emily E. Rosowski[1], Kimberly M. Cirelli[1], Alexandre Bougdour[3], Saima M. Sidik[4], Josh R. Beck[5], Sebastian Lourido[6], Pascal F. Egea[7], Peter J. Bradley[5], Mohamed-Ali Hakimi[3], Randall L. Rasmusson[2], Jeroen P. Saeij[1]

[1]Massachusetts Institute of Technology, Department of Biology, Cambridge, MA, United States, [2]Physiology and Biophysics, The State University of New York, University at Buffalo, Buffalo, NY, United States, [3]Centre National de la Recherche Scientifique, UMR5163, Laboratoire Adaptation et Pathogénie des Microorganismes, Grenoble, France, [4]Whitehead Institute for Biomedical Research, Cambridge, MA, United States, [5]Department of Microbiology, Immunology and Molecular Genetics, University of California Los Angeles, Los Angeles, CA, United States, [6]Université Joseph Fourier, Grenoble, France, [7]Department of Biological Chemistry, University of California Los Angeles David Geffen School of Medicine, Los Angeles, CA, United States

Toxoplasma gondii is a widespread protozoan pathogen in the phylum Apicomplexa that resides intracellularly within a parasitophorous vacuole (PV) that is selectively permeable to small molecules through an unknown mechanism. We have identified GRA17 as a novel *Toxoplasma*-secreted protein, which localizes to the parasitophorous vacuole membrane (PVM) and is conserved across PV-residing apicomplexans. GRA17 mediates the passive transport of small molecules across the PVM. The PVs of GRA17-deficient parasites have aberrant morphology, reduced permeability to small molecules, and structural instability. GRA17-deficient parasites proliferate slowly and are avirulent in mice. GRA17 functions synergistically with a related protein, GRA23. Exogenous expression of GRA17 or GRA23 alters the membrane conductance properties of *Xenopus* oocytes in a manner consistent with a large non-selective pore. GRA17 and GRA23 provide the first molecular basis to explain the PVM permeability to small molecules.

1944

QUANTIFYING LABILE HEME IN LIVE MALARIA PARASITES USING NOVEL FRET BIOSENSORS

James Abshire, Christopher J. Rowlands, Peter T. So, Jacquin C. Niles

Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, United States

The heme cofactor is centrally important in malaria parasite physiology. On one hand, it is a byproduct generated in large quantities during parasite-mediated hemoglobin degradation during the blood stage of malaria infection. Parasites must detoxify excess heme to limit oxidative damage, and do so by polymerizing liberated heme into nonreactive hemozoin. Several successful antimalarial drugs such as the aminoquinolines inhibit this process, presumably leading to the toxic accumulation of labile heme, although this has not been directly demonstrated or quantified. On the other hand, the parasite genome encodes a complete heme biosynthetic pathway that is essential for other stages of parasite development. This suggests heme is a necessary cofactor that is toxic in excess. Despite the central role of heme to parasite metabolism and its link to antimalarial drug potency, little is known about heme dynamics in normal parasite physiology or how these dynamics change under stresses imposed by heme-interacting drugs. To address these questions, we have developed and characterized a family of novel, genetically-encoded FRET biosensors for quantifying labile heme in live parasites. In vitro spectroscopic characterization of the purified protein sensors demonstrates their ability to reversibly bind heme, and to exhibit significant heme-dependent changes in FRET. Our studies with blood-stage parasite lines expressing these biosensors indicate that micromolar concentrations of labile heme are maintained in the parasite cytosol throughout development. Furthermore, exposure to chloroquine, but not pyrimethamine, leads to accumulation of cytosolic labile heme, thus directly linking heme dysregulation to the in situ effects of aminoquinolines. We believe these studies will advance our understanding of how heme perturbation is linked to antimalarial drug potency, and help to mechanistically inform future drug development efforts.

1945

CONDITIONAL EXPRESSION OF PFRIPR CONFIRMS ITS ESSENTIALITY FOR PLASMODIUM *FALCIPARUM* MEROZOITE INVASION INTO HUMAN ERYTHROCYTES

Alan Yap, Alan F. Cowman

Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

Plasmodium falciparum merozoites actively invade human erythrocytes during blood stage malaria, the stage that causes clinical symptoms. Invasion of erythrocytes is a complex process that involves a cascade of protein-protein interactions between merozoite ligands and erythrocyte receptors. To date, many merozoite ligands have been described as essential based on the inability to knockout the proteins *in vitro*. One of these is *P. falciparum* Rh5 interacting protein (PfRipr). Here we present evidence for the efficient knockdown of PfRipr using a dimerizable Cre recombinase (DiCre) system. PfRipr is an essential invasion protein that forms a complex with *P. falciparum* reticulocyte binding-like homologues 5 (PfRh5). Previous studies have shown that the PfRipr/PfRh5 complex plays a critical role in merozoite attachment and invasion as anti-PfRipr antibodies block merozoite invasion. We generated parasites expressing DiCre, which is activated by the addition of rapamycin leading to the deletion of *Pfripr*. Knockdown of gene and protein levels up to 90% within one cycle of the blood stage (about 48 hours) was achieved. This led to a growth reduction in the following cycles, which relates to a reduction in invasion efficiency as determined by flow cytometry, live-imaging and superresolution microscopy. In summary, conditional regulation of PfRipr confirms the essential role of this protein and further elucidates its functions.

1946

HSP101/PTEX MEDIATES EXPORT OF DIVERSE MALARIA EFFECTOR PROTEINS INTO THE HOST ERYTHROCYTE

Josh Beck, Vasant Muralidharan, Anna Oksman, Daniel E. Goldberg

Washington University School of Medicine, St. Louis, MO, United States

To mediate its survival and virulence the malaria parasite *Plasmodium falciparum* exports hundreds of proteins into the host erythrocyte. To enter the host cell, exported proteins must cross the parasitophorous vacuolar membrane (PVM) within which the parasite resides, but the mechanism remains unclear. A putative Plasmodium translocon of exported proteins (PTEX) has been suggested to be involved for at least one class of exported proteins; however, direct functional evidence has remained elusive. Here we show that export across the PVM requires heat shock protein 101 (HSP101), a ClpB-like AAA+ ATPase component of PTEX. Using a chaperone auto-inhibition strategy, we achieved rapid, reversible ablation of HSP101 function, resulting in a nearly complete block in export with substrates accumulating in the vacuole in both asexual and sexual parasites. Surprisingly, this block extended to all classes of exported proteins, revealing HSP101-dependent translocation across the PVM as a convergent step in the multi-pathway export process. Under export-blocked conditions, association between HSP101 and other components of the PTEX complex was lost while association with exported substrates was maintained, suggesting that HSP101 first recognizes proteins destined for export before feeding them into the translocon. Our results demonstrate an essential and universal role for HSP101 in protein export and provide strong evidence for PTEX function in protein translocation into the host cell.

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