Supplement to
THE AMERICAN JOURNAL OF
Tropical Medicine and Hygiene
Official Journal of the American Society of Tropical Medicine and Hygiene

Volume 83 November 2010 Number 5 Supplement

Abstract Book

American Society of Tropical Medicine and Hygiene

59th Annual Meeting

59
ASTMH fifty-ninth
ANNUAL MEETING

November 3–7, 2010

Atlanta Marriott Marquis

Hilton Atlanta

Atlanta, Georgia, USA

Supplement to

The American Journal of
Tropical Medicine and Hygiene

1

ARTEMISININ-BASED COMBINATIONS FOR TREATING UNCOMPLICATED MALARIA IN AFRICAN CHILDREN: THE 4ABC TRIAL

Umberto D'Alessandro[1], 4 Artemisinin Based Combinations Study Group[2]

[1]Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium, [2]Multicentre, Multicentre, Belgium

Artemisinin-based combination therapies (ACTs) are the recommended treatments for uncomplicated *Plasmodium falciparum* malaria. An increasing number of malaria endemic countries in sub-Saharan Africa have adopted ACTs as first line treatments, the most commonly used being arthemeter-lumefantrine (AL) and artesunate-amodiaquine (ASAQ). Such decision was based on a relatively limited information. A clinical trial with the aim of making a 'head-to-head' comparison of four different ACTs was carried out in 10 sites distributed in 7 African countries (Burkina Faso, Gabon, Mozambique, Nigeria, Rwanda, Uganda and Zambia), representing different levels of malaria endemicity. Between July 2007 and December 2008, 4114 children 6-59 months old with clinical malaria (fever and/or history of fever, *P. falciparum* monoinfection with density between 2,000-200,000/µl and Hb ≥ 7.0 g/dl) were recruited and randomized to either AL, ASAQ, dihydroartemisinin-piperaquine (DHAPQ) or chlorproguanil-dapsone plus artesunate (CD-A). They were actively followed up for 28 days and then passively for the next 6 months. AL was administered to 1226 children, ASAQ to 1002, DHAPQ to 1473 and CD-A to 413. Recruitment for the CD-A arm was interrupted in February 2008 following the decision to stop its development. Preliminary results show that day 28 PCR-uncorrected cure rates were 91.1% for DHAPQ, 81.7% for ASAQ, 73.4% for AL and 57.2% for CD-A, with major variations between sites. Day 28 PCR-adjusted cure rates were 98.5% for DHAPQ, 98.0% for ASAQ, 96.8% for AL and 87.4% for CD-A. Using the predefined rule of non-inferiority, a 10% absolute difference in the 28 PCR-adjusted cure rates recalculated to an odds-ratio scale and pooled over the study sites, DHAPQ, AL and ASAQ show equivalence while CD-A appears to have a lower efficacy. Thirteen deaths were recorded during the whole study period, 4 during the first 28 days, one for each study arm. These results show that DHAPQ, AL and ASAQ have similar and excellent efficacy though DHAPQ may exert a stronger post-treatment prophylactic effect.

2

EARLY AND LATE EFFECTS OF TWO ARTEMISININ BASED COMBINATION THERAPIES IN THE TREATMENT OF *FALCIPARUM* MALARIA IN NIGERIAN CHILDREN

Obaro S. Michael[1], Titilope Okuboyejo[1], Onikepe A. Folarin[2], Grace O. Gbotosho[1], Akintunde Sowunmi[1], Ayoade M. Oduola[3], Christian T. Happi[2]

[1]Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria, [2]Malaria Research Laboratories, IMRAT, University of Ibadan, Ibadan, Nigeria, [3]World Health Organization/TDR Special Program on Tropical Diseases, Geneva, Switzerland

Most antimalarial studies are designed to evaluate peripheral blood smears on a daily basis. This makes it impossible to observe the effects of Artemisinin based therapies in the first 24 hours after therapy. We designed a protocol to evaluate the early effects and the standard measures of efficacies of two ACTs on asexual parasitemia during a drug efficacy study. Children aged 12 months to 132 months were randomized to receive Artemether-Lumefanthrine (AL) or Artesunate-Amodiaquine (AA). Peripheral blood smears were made at 0, 1, 2, 4, 8, 16, and 24 hour, and daily on days 2-7, 14, 21, 28, 35, and 42 for microscopic identification and quantification of asexual *Plasmodium falciparum*. A total of 193 children were randomized to receive either AL (97) or AA (96). A proportion of the children (42% AL, 36.7% AA, p-value 0.377) had a significant rise in peripheral parasitemia that peaked at 1 hour after treatment, followed by rapid decline and clearance. This rise in parasitemia was significant (p= 0.007) and suggests a mobilization of asexual parasites from the deep tissues to the periphery. This finding was unexpected. The other children had the expected pattern of rapid fall in asexual parasitemia until clearance. Fever and parasite clearance times for AL and AA were 29.9 ± 18.4, 28.6 ± 18.4 hours and 28.9 ± 12.7, 24.0 ± 15.4 hours (p= 0.630, 0.067) respectively. There was no record of early treatment failure and cure rates at day 42 was >95% for both drugs. In conclusion, the study showed high efficacy of AL and AA in Nigerian children. Results from this study also suggest a mobilisation effect on parasites from deeper tissues to the peripheral blood in the early hours after drug administration. The biological and pharmacological importance of this observation is yet to be understood.

3

LONGITUDINAL TRIAL OF CHLOROQUINE MONOTHERAPY AND COMBINATION THERAPY FOR UNCOMPLICATED *FALCIPARUM* MALARIA IN CHILDREN IN BLANTYRE, MALAWI

Miriam K. Laufer[1], Fraction K. Dzinjalamala[2], Don P. Mathanga[3], Philip C. Thesing[1], Osward M. Nyirenda[4], Rhoda Masonga[4], Joseph Kanyangalika[4], Terrie E. Taylor[5], Christopher V. Plowe[6]

[1]Center for Vaccine Development, University of Maryland, Baltimore, MD, United States, [2]University of Malawi College of Medicine, Blantyre, Malawi, [3]Malaria Alert Centre, College of Medicine, Blantyre, Malawi, [4]Blantyre Malaria Project, Blantyre, Malawi, [5]Michigan State University, College of Osteopathic Medicine, East Lansing, MI, United States, [6]HHMI/ Center for Vaccine Development, University of Maryland, Baltimore, MD, United States

We previously reported that chloroquine-susceptible malaria returned and predominates in Malawi after the successful removal of chloroquine drug pressure. To investigate strategies for the rational combination of drugs to deter the emergence and spread of resistance, we conducted a longitudinal trial of chloroquine alone and in combination with partner drugs with different pharmacokinetic and pharmacodynamic properties. Children ages six to 59 months who presented to the district health center with clinical, microscopy-confirmed *Plasmodium falciparum* malaria were enrolled in the study and randomized to receive one of the four treatment arms: chloroquine monotherapy, or chloroquine in combination with artesunate, azithromycin or atovaquone-proguanil. Each time a participant developed symptomatic malaria during one year of follow up, they were treated with the same regimen. The primary outcome was incidence of malaria. Other important outcomes included the efficacy of the treatment at the first and subsequent episodes of malaria and the effect of each treatment on hemoglobin at the end of the study period. Six hundred forty children were enrolled. Preliminary results suggest that all treatment arms, including chloroquine monotherapy, were highly efficacious for the treatment of *P. falciparum* malaria and maintained efficacy throughout the trial period. Assessment of the incidence of malaria and of the effect of each treatment regimen on the prevalence of anemia and the re-emergence of chloroquine-resistant genotypes are underway and will be presented. Potential future uses of chloroquine in Africa will be discussed.

4

EFFICACY OF IPTI WITH SULPHADOXINE/PYRIMETHAMINE COMBINED WITH EITHER AMODIAQUINE OR ARTESUNATE ON MALARIA-RELATED MORBIDITY IN AN AREA OF PAPUA NEW GUINEA WITH SIGNIFICANT LEVEL OF NON-*FALCIPARUM* INFECTIONS

Nicolas Senn[1], Patricia Rarau[2], Danielle Stanisic[3], Doris Manong[2], Mary Salib[2], John Aponte[4], Peter Siba[5], Stephen Rogerson[6], John Reeder[7], Ivo Mueller[5]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [3]Walter and Eliza Hall Institute, Melbourne, Australia, [4]Centre of International Health, Spain, Barcelona, Spain, [5]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea, [6]University of Melbourne, Melbourne, Australia, [7]Burnet Institute, Melbourne, Australia

Intermittent Preventive Treatment of malaria in infants (IPTi) is one of the most promising preventative interventions to reduce the burden of malaria in one of the highest risk groups in endemic countries. The concept is to deliver antimalarial treatment to infants at regular intervals, alongside the expanded program of immunization, regardless of the parasitemia. This intervention has been exclusively investigated in Africa where *Plasmodium falciparum* (Pf) is the predominant species. The potential benefits of this intervention in areas with significant levels of non-Pf infections (mainly Pv) is not known. Between June 2006 and June 2010 we conducted a 3 arm randomized controlled trial (part of the IPTi consortium) to investigate the efficacy of IPTi with sulfadoxine/pyrimethamine (SP, single dose) associated to 3 days of either amodiaquine (AQ) or artesunate (ART) compared to placebo in Papua New Guinea (PNG), a country highly endemic for both Pf and Pv. In total, 1125 infants were enrolled and followed-up from 3 to 27 months. As of April 2010, >1800 clinical malaria episodes confirmed by rapid diagnostic antigen testing (RDT) were observed of which *P. vivax* accounting for just over 50%) and 287 serious adverse events (SAE) occurred including 9 deaths (none of them study related). Final results on the efficacy and safety of IPTi for the prevention *P. falciparum* and *P. vivax* clinical malaria, anaemia and hospitalisation will be presented. The results of this study will provide the first evidence for the efficacy of IPTi in settings endemic for both *P. falciparum* and *P. vivax* and have important implication for the role of IPTi as an intervention against malarial outside Africa.

5

EXTENDED PARASITES CLEARANCE TIME AMONG PATIENTS TREATED WITH ARTEMETHER/LUMEFANTRINE OR AMODIAQUINE PLUS ARTESUNATE AT ONE SENTINEL SITE IN TANZANIA

Deus Ishengoma[1], Samuel Sembuche[1], Filbert Francis[1], Ezekiel Malecela[1], Juma Akida[1], Vito Baraka[1], Rashidi Madebe[1], Renata Mandike[2], Alex Mwita[2], Watoky Nkya[3], Johannes Kataraihya[4], Michael Alifrangis[5], Ib Bygbjerg[5], Martha Lemnge[1]

[1]National Institute for Medical Research, Tanga, United Republic of Tanzania, [2]National Malaria Control Programme, Dar es Salaam, United Republic of Tanzania, [3]Mbeya Consultant Hospital, Mbeya, United Republic of Tanzania, [4]Bugando Medical Centre and Weill Bugando University College of Health Sciences, Mwanza, United Republic of Tanzania, [5]University of Copenhagen, Copenhagen, Denmark

Drug resistance which is usually preceded by an intermediate stage whereby parasites become tolerant to therapeutic levels of the drugs has presented many challenges to malaria control programmes. Resistance can be detected using standard methods while tolerance can be indirectly revealed by *in-vitro* tests or examining increased parasite clearance time *in-vivo*. Due to high resistance to SP, Tanzania adopted artemether-lumefantrine (ALu) in November 2006 as a first line antimalarial and monitoring of drug resistance is regularly done at sentinel sites to provide data for updating antimalarial drug policy. A study to assess *in-vivo* efficacy of ALu and amodiaquine plus artesunate (AQ+AS) was conducted between April and December 2007 at Mkuzi and Ujiji sentinel sites. Children with uncomplicated *falciparum* malaria (aged 6-59 months) and meeting inclusion criteria were randomized to receive either ALu (n=233) or AQ+AS (n=232); and were followed-up for 28 days. Filter paper blood spots were collected for PCR analysis to distinguish re-infection from recrudescence. At Mkuzi, 82.8% of the cases on ALu and 87.5% in the AQ+AS group had not cleared parasites by day 2; and on day 3, 39.8% in ALu and 25% in AQ+AS still had parasites. At Ujiji, 94% of the cases (in both arms) had cleared parasites by day 2 and only 1 case in AQ+AS group had parasites on day 3. The median parasite clearance time was 3 days at Mkuzi and 1 day at Ujiji. On day 28, PCR corrected adequate clinical and parasitological response (ACPR) in the ALu groups was high but similar at both sites (98.6% at Mkuzi and 100% at Ujiji, p=0.321). In the AQ+AS groups, PCR corrected ACPR on day 28 was significantly lower at Mkuzi than Ujiji (93.4% at Mkuzi *vs* 100% at Ujiji, p=0.033). In conclusion, extended parasite clearance time at Mkuzi in both drug combinations, might indicate early signs of parasite tolerance. However, ALu was efficacious at both study sites while AQ+AS was less efficacious at Mkuzi where malaria transmission is high. Further studies are needed to monitor possible development of parasite tolerance/resistance to ACTs at these and other sites.

6

POPULATION PHARMACOKINETICS OF ANTIMALARIAL DRUGS IN THE TREATMENT OF PREGNANT WOMEN WITH UNCOMPLICATED MALARIA

Joel Tarning[1], Richard Hoglund[2], Palang Chotsiri[1], Warunee Hanpithakpong[1], Aung Phae Pyo[3], Vincent Jullien[4], Niklas Lindegardh[1], Marcus Rijken[3], Ishag Adam[5], Francois Nosten[3]

[1]Mahidol-Oxford Research Unit, Bangkok, Thailand, [2]Goteborg University, Goteborg, Sweden, [3]Shoklo Malaria Research Unit, Mae Sot, Thailand, [4]Service de Pharmacologie Clinique, Groupe Hospitalier Cochin-Saint-Vincent de Paul, Paris, France, [5]University of Khartoum, Khartoum, Sudan

Pregnancy has considerable effects on the pharmacokinetic properties of many of the drugs used to treat uncomplicated *falciparum* malaria. Several studies have shown reduced antimalarial drug concentrations in later pregnancy. The reductions are often substantial, and as a result, antimalarial cure rates in pregnancy tend to be lower. Unfortunately, pregnant women are especially vulnerable to malaria and the fetus is adversely affected. No reports have described the pharmacokinetic properties of piperaquine, amodiaquine or desethylamodiaquine in pregnant women with uncomplicated malaria. A pharmacokinetic study were conducted in Thailand (24 pregnant and 24 non-pregnant women) and in Sudan (12 pregnant and 14 non-pregnant women). These studies investigated the pharmacokinetic properties of piperaquine after a standard oral three-day fixed dose regimen of dihydroartemisinin-piperaquine in patients with uncomplicated *falciparum* malaria. Pharmacokinetics of amodiaquine and its principal biologically active metabolite desethylamodiaquine was investigated in the treatment of vivax infections in 28 pregnant women during pregnancy and again after delivery. Dense venous plasma samples were collected and drug measurements conducted according to published methods. Concentration-time profiles were characterized using nonlinear mixed-effects modeling. Different structural models and the impact of different covariates on pharmacokinetic parameters were investigated in full for all three antimalarials. Population pharmacokinetics of piperaquine, amodiaquine and desethylamodiaquine will be described using a population pharmacokinetic modeling approach. These results will be compared with available literature for a full understanding of any potential pregnancy related changes on pharmacokinetics and the impact of these on the pharmacodynamics.

7

POPULATION PHARMACOKINETICS OF ARTESUNATE AND DIHYDROARTEMISININ FOLLOWING A SINGLE ORAL DOSE OF ARTESUNATE DURING THE 2ND AND 3RD TRIMESTER OF PREGNANCY

Carrie Morris[1], Marie A. Onyamboko[2], Antoinette K. Tshefu[2], Jef Atibu[2], Vicky Lokomba[2], Steven R. Meshnick[3], Macaya Douoguih[4], Jennifer Hemingway-Foday[5], Matthew A. Koch[5], Edmund Capparelli[6], Robert Ryder[6], Carl Bose[3], Linda Wright[7], David Wesche[8], Lawrence Fleckenstein[1]

[1]University of Iowa, Iowa City, IA, United States, [2]Kinshasa School of Public Health, Kinshasa, The Democratic Republic of the Congo, [3]University of North Carolina, Chapel Hill, NC, United States, [4]Aeras Global TB Vaccine Foundation, Rockville, MD, United States, [5]Research Triangle Institute, Research Triangle Park, NC, United States, [6]University of California, San Diego, San Diego, CA, United States, [7]Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD, United States, [8]David Wesche Consulting LLC, Ann Arbor, MI, United States

The World Health Organization endorses the use of artemisinin-based combination therapy for treatment of uncomplicated *falciparum* malaria in the second and third trimesters of pregnancy. However, the effects of pregnancy on the pharmacokinetics of artemisinin derivatives, such as artesunate (AS), are poorly understood. We analyzed the population pharmacokinetics of oral AS, and its active metabolite dihydroartemisinin (DHA), in pregnant and non-pregnant women with *falciparum* malaria at the Kingasani Maternity Clinic in the DRC. Data were obtained from 26 pregnant women in the late second or in the third trimester of pregnancy and from 25 non-pregnant female controls. All subjects received 200 mg AS. Plasma AS and DHA were measured using a validated LC-MS method with a lower limit of quantification of 1 ng/mL for both AS and DHA. Estimates for pharmacokinetic and variability parameters were obtained through nonlinear mixed effects modeling with NONMEM 7 software. A simultaneous parent-metabolite model was developed consisting of mixed zero-order, lagged first-order absorption of AS, a one-compartment model for AS, and a one-compartment model for DHA. Complete conversion of AS to DHA was assumed. The model displayed satisfactory goodness-of-fit, predictive ability, and stability. Apparent clearance and volume estimates, with 95% bootstrap confidence intervals, were as follows: 195 L (145 - 316 L) for AS V/F, 895 L/h (782 - 1052 L/h) for AS CL/F, 91.4 L (78.1 - 109 L) for DHA V/F, and 64.0 L/h (55.0 - 75.9 L/h) for DHA CL/F. The effect of pregnancy on DHA CL/F was determined to be significant, with a pregnancy-associated increase in DHA CL/F of 42.3% (18.8 - 69.3%). DHA CL/F did not differ substantially between the late second and the third trimesters of pregnancy. The pregnancy-associated increase in DHA clearance detected in this analysis suggests that higher AS doses would need to be used to maintain similar DHA levels in pregnant patients as achieved in non-pregnant controls.

8

EVIDENCE FOR RIBOSOMAL FRAMESHIFTING AND A NOVEL OVERLAPPING GENE IN THE GENOMES OF INSECT-SPECIFIC FLAVIVIRUSES

Bradley J. Blitvich[1], Andrew E. Firth[2], Norma M. Wills[3], Aaron C. Brault[4], Cathy L. Miller[1], John F. Atkins[3]

[1]Iowa State University, Ames, IA, United States, [2]University of Cambridge, Cambridge, United Kingdom, [3]University of Utah, Salt Lake City, UT, United States, [4]Centers for Disease Control and Prevention, Fort Collins, CO, United States

Many viruses harbor sequences that induce a portion of ribosomes to shift -1 nucleotide and continue translating in the new reading frame. The -1 frameshift site typically consists of a 'slippery' heptanucleotide fitting the consensus motif N NNW WWH (where NNN represents any 3 identical nucleotides, WWW represents AAA or UUU, H represents A, C or U, and spaces separate zero-frame codons) followed by a 'spacer' region of 5-9 nt then a stable RNA secondary structure (e.g. a hairpin or pseudoknot). Recently, a ribosomal frameshift site was identified in the Japanese encephalitis virus (JEV) serogroup of flaviviruses that gives rise to a protein (designated NS1') whose origin had previously been an unsolved enigma. The identification of programmed frameshifting in the JEV serogroup of flaviviruses prompted us to look at other flaviviruses resulting in the discovery of a novel coding sequence (designated Fairly Interesting Flavivirus ORF; *fifo*) of 253 to 295-codons that overlaps the NS2A-NS2B coding sequence in the -1/+2 reading frame of all insect-specific flaviviruses. Similar coding regions are not present in the NS2A-NS2B regions of any other flaviviruses. Application of blastp to FIFO revealed no similar sequences in the GenBank database. A conserved G GAU UUY slippery heptanucleotide and 3'-adjacent stable RNA secondary structure at the 5' end of the coding region provide a classical motif for -1 ribosomal frameshifting. The stable RNA secondary structure is either a RNA hairpin (*Culex* flavivirus, Quang Binh virus and Nakiwogo virus) or pseudoknot (Cell fusing agent virus, Kamiti River virus and Aedes flavivirus). Additional evidence for the presence of a novel overlapping gene and subsequent expression of a frame-shift product was provided in (i) reporter assays which confirmed the viability of the proposed translation mechanism and (ii) immunofluorescence assays performed with two separate FIFO-specific antibodies which revealed the presence of proteins containing FIFO antigens in CxFV-infected mosquito cells.

9

CULEX FLAVIVIRUS AND WEST NILE VIRUS IN *CULEX QUINQUEFASCIATUS* POPULATIONS IN THE SOUTHEAST UNITED STATES

Rebekah J. Kent[1], Kristen Burkhalter[1], Daniel Mead[2], Rosmarie Kelly[3], Jeffrey Brown[4], Wendy Varnado[4], Alma Roy[5], Barry Miller[1], Roger Nasci[1]

[1]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [2]Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia, Athens, GA, United States, [3]Georgia Division of Public Health Epidemiology Branch, Atlanta, GA, United States, [4]Environmental Services, Mississippi State Department of Health, Jackson, MS, United States, [5]Louisiana Veterinary Medical Diagnostic Laboratory, Louisiana State University, Baton Rouge, LA, United States

Strains of *Culex* flavivirus (CxFV) and related insect-only flaviviruses have been discovered in mosquitoes worldwide. However, little is known of the interactions between the insect-only flaviviruses, other arboviruses and their mosquito hosts, or the potential public health significance of these associations. The specific aims of this study were to 1) describe the geographic distribution, local prevalence, and seasonal distribution of CxFV and West Nile virus (WNV) in *Cx. quinquefasciatus* in the Southeastern U.S., 2) investigate the potential association between CxFV prevalence and WNV disease incidence in the southeastern USA, and 3) describe the phylogenetic relationship of CxFV isolates from the region. Using ArboNET records, 12 locations were selected across Georgia, Mississippi, and Louisiana that have programs routinely collecting *Cx. quinquefasciatus* for arbovirus surveillance and that represent a range of WNV human case incidence levels. Mosquitoes were collected by CDC light traps and gravid traps from the same sites between July and October 2009. Aliquots of homogenized *Cx. quinquefasciatus* pools were screened for flaviviruses by RT-PCR and subjected to virus isolation on C6/36 cells. Georgia experienced the most CxFV activity of all three states. In Georgia, CxFV infection rates (MLE) increased between July and October, and infection rates were highly variable between and within counties as well as seasonally. CxFV infection rates were highest in Fulton County, GA, reaching 200 infected *Cx. quinquefasciatus* per 1000 at some sites. WNV infection rates (MLE) in Georgia were <1 throughout the summer. CxFV infection rates were not significantly different between Georgia and Mississippi in July, however CxFV was not detected in Mississippi after July, and no CxFV was detected in *Cx. quinquefasciatus* in Louisiana in

any month. CxFV isolates from Georgia were 98% identical to CxFV from Japan, Iowa, and Houston in the NS5 gene. Interactions between WNV and CxFV prevalence will be presented.

10

MOLECULAR CHARACTERIZATION OF EPIDEMIC AND NON-EPIDEMIC ST. LOUIS ENCEPHALITIS VIRUS (SLEV) STRAINS ISOLATED IN ARGENTINA

Luis Adrián Diaz[1], Sandra Goñi[2], Javier Iserte[2], Cristopher Logue[3], Amber Singh[3], Ann Powers[3], Marta S. Contigiani[1]

[1]Instituto de Virología ¨Dr. J. M. Vanella¨ - Universidad Nacional de Córdoba, Córdoba, Argentina, [2]Laboratorio de Ingeniería Genética, Biología Celular y Molecular, Universidad Nacional de Quilmes, Quilmes, Argentina, [3]Centers for Disease Control and Prevention, Fort Collins, CO, United States

SLEV (Flavivirus) is an emerging/reemerging arbovirus in South America causing isolated encephalitis human cases or outbreaks in Argentina and Brazil. In the city of Córdoba (Argentina), during a human SLE outbreak, two genotype III SLEV strains were isolated, belonging to the same genotype isolated 27 years ago in Province of Santa Fe (Argentina). Preliminary results show some biological differences among epidemic (Ep) and non-epidemic (NEp) strains. The factors which lead this emergence are not known. The aim of this project was to characterize and compare molecularly both Ep (CbaAr-4005) and NEp (79V-2533) SLEV strains. A complete genome strategy was designed for both Ep and NEp SLEV strains. A bioinformatic analyze was carried out in order to detect cleavage protease site, genetic distance (GD), nucleotide and aminoacidic substitutions and relative homologies index (RHI). Finally a phylogenetic analyze was realized including 26 SLEV strains nearly complete genome sequence available at GenBank. SLEV complete genome consists of 10963 ntds (ORF=3429 aas). Proteins C, PrM, NS2A, NS2B and NS4B have wide regions with RHI greater than >0.80. The most variables proteins (GD) were NS4B (2.7), NS1 (1.7) and M (1.3). A total of 49 conservative and 20 non-conservative aminoacidic substitutions and one deletion were detected in reference with Kern217 SLEV strain (NC_007580.2) sequence. Among Ep and NEp viral strains we detected 17 aminoacidic changes, which 8 of them were non-conservative and located in proteins E, NS1, NS3 and NS5. It is unknown if detected aminoacidic differences would be related to the biological differences previously observed. The development of reverse genetic system will allow us to understand the meaning of such substitutions. The phylogenetic analysis shows two big clades: North and Central America (NCA) and South America (SA). The analyzed strains isolated in Argentina constitute a subgroup inside the NCA group. Likely, ancestors of genotype III could be introduce and originated the SLEV strains circulating in North and Central America.

11

PHYLOGENY OF TICK-BORNE ENCEPHALITIS VIRUSES IN CENTRAL EUROPE

Gerhard Dobler[1], Gudrun Zöller[1], Martin Pfeffer[2], Frank Hufert[3], Manfred Weidmann[3], Sandra Essbauer[1]

[1]Bundeswehr Institute of Micorbiology, Munich, Germany, [2]Institute of Animal Hygiene and Public Animal Health, University of Leipzig, Germany, [3]Institute of Virology, University of Göttingen, Germany

Tick-borne encephalitis virus (TBEV) is a member of the genus Flavivirus in the family Flaviviridae. It is transmitted in nature by ticks. As far as known, three subtypes are cirvculating in Europe and Asia. Within the particular subtypes, members exhibit little variability in nucleotide and amino acid sequences. By sequencing complete viral genomes we identified a variable region in the NS2a gene which was used for more detailled phylogenetic characterization of TBEV strains in Southern Germany. Ten new TBEV strains were isolated from ticks of four different regions in South-Eastern Germany. All TBEV strains belong to the Western subtype of TBEV. Furthermore, the phylogeny of sequences of E and NS2a genes (as far as available) of other TBE viruses and of Slovak, Czech and Austrian TBE virus strains were used for comparative analysis. One of the new strains, AS33, exhibited unique nucleic acid and amino acid patterns of the E gene with two amino acid exchanges at positions (E51D; T128I) which have not been detected so far in any other known TBEV strain known to occur in Germany or in any other TBEV strain listed in data bases. A TBEV strain (Haselmühl-1) isolated only in 10 km distance to the AS33 focus did not show the described unique amino acid pattern. About 40 km distant to the east, another TBE focus was detected and two TBEV strains were recovered from ticks. A fourth natural TBE focus was detected some 140 km east from the other foci described foci near the city of Passau at the frontier to Czech Republic and to Austria. This South-Eastern part of Germany is assumed to be one of the two most active TBE endemic areas in Germany. However so far, no sequence data on the circulating TBEV strains were available. The generated sequences of TBEV show that the NS2a gene may be better used for comparative phylogenetic analysis of TBEV than the E gene hwich is currently used for most of these analyses. Using the generated sequence data, several genetic clusters of TBEV in Southern Germany can be distinguished. These data imply that TBEV strains were independantly introduced from Slovak Republic and Czech Republic several times. Furthermore, TBE foci seem to be established independantly from each other, even in closely located regions.

12

POTENTIAL MARKERS OF ATTENUATION OF YF VIRUS AFTER INFECTION OF STEM CELL-DERIVED HUMAN HEPATOCYTES WITH WILD-TYPE ASIBI OR LIVE-ATTENUATED YF17D VIRUS

Nathalie Mantel[1], Sandrine Gulia[1], Thomas Vial[1], Sarah Lafon[1], Josefina Edsbagge[2], Petter Björquist[2], Véronique Barban[1]

[1]sanofi pasteur, Marcy l'Etoile, France, [2]Cellartis AB, Goteborg, Sweden

Yellow fever virus (YFV) is an arthropod-borne virus belonging to the Flaviviridae family, which causes acute febrile illness with symptoms ranging from mild, non specific syndrome to hemorrhagic fever involving all organs, including the liver in which hepatic lesions are accompanied by an intense inflammation. With the objective of developing an in vitro model to evaluate YFV hepatotropism, we compared the infection of hepatocytes derived from human embryonic stem cells (hES-Hep™002) by wild-type YFV or by the attenuated YF-17D vaccine strain. hES-Hep™002 cells were infected by each virus at an M.O.I of 0.01 or 2. Viral replication was quantified at regular intervals in culture supernatants and expression of cytokines and transaminases (ALT/AST - GST) were followed by ELISA and enzymatic assays. Apoptosis and cell infection rates were measured by flow cytometry analysis and a transcriptomic analysis was done using PCR arrays, focusing on cytokines, apoptosis, cellular stress, and drug metabolism pathways. The PCR arrays identified different markers. At low M.O.I. 17D-infected cells were found to express more IL-5 and IFN-γ than Asibi-infected cells and several genes involved in drug metabolism were activated, while activation of the apoptotic pathway was increased in Asibi-infected cells. At high M.O.I. Asibi-infected cells expressed more IFNα, IL-5 and TNF than 17D-infected cells, and genes involved in cellular toxicity were massively activated. At high M.O.I., cytokine expression differences were also highlighted by ELISA: production of IL-6 and IL-8 were up regulated following infection by YF-17D.

Using a hES-Hep™002 cell model we identified differences in the antiviral responses to infection by pathogenic wild-type YFV or the attenuated 17D strain as well as potential markers predictive for YFV attenuation. The relevance of the hES-Hep™002 cell model needs to be established in comparison with primary hepatocytes.

13

PERSISTENCE OF ANTIBODIES ONE YEAR AFTER A SINGLE INJECTION OF LIVE ATTENUATED JAPANESE ENCEPHALITIS CHIMERIC VIRUS VACCINE AT 12-18 MONTHS OF AGE

Emmanuel Feroldi[1], Chitsanu Pancharoen[2], Veerachai Watanaveeradej[3], Alain Bouckenooghe[4]

[1]sanofi pasteur, Marcy l'Etoile, France, [2]Chulalongkorn Hospital, Bangkok, Thailand, [3]Department of Pediatrics, Phramongkutklao Hospital, Bangkok, Thailand, [4]sanofi pasteur, Singapore, Singapore

Live attenuated Japanese encephalitis chimeric virus vaccine (JE-CV) was developed to replace first mouse-brain derived JE vaccines. An adult trial has shown that 87% of the participants who were seroprotected at month 6 after JE-CV vaccination were still protected at month 60. The objective of this study was to document persistence of JE-CV-induced antibodies in children. In a 5-year follow-up to a phase 3 trial where 1200 JE-naïve Thai and Filipino children aged 12-18 months were randomized 11:1 to receive a single injection of JE-CV (Sanofi Pasteur, Lyon, France) or a control, we are following a cohort of ~600 JE-CV-vaccinated children to assess long term antibody persistence. Plaque reduction neutralization test (PRNT50) antibody titers against homologous JE-CV will be tested in annual samples. Children with titers >/=1:10 are considered seroprotected against JE. Here we present the first data, obtained one year after vaccination. The seroprotection rate in all 1100 children vaccinated with JE-CV in the phase 3 trial was 95% 28 days after vaccination. Of these, 591 children were enrolled in this follow-up trial in August and September 2009. In this subset, the seroprotection rate 28 days after JE-CV vaccination was 100% (95%CI: 99.4-100) and the geometric mean titer (GMT) was 253 (95%CI: 225-284). One year after vaccination (12±1 month), 88.2% (85.3-90.7) of the 591 children were still seroprotected and the GMT was 77.2 (67.7-88.0). No cases of Japanese encephalitis and no vaccine related SAEs occurred during the year after vaccination. In conclusion, all the subjects enrolled in this long-term follow-up presented with seroprotective antibody titers one month after a single injection of JE-CV between the ages of 12 and 18 months; more than 88% of them were still seroprotected one year later. This ongoing follow-up study is documenting the long term persistence of antibodies against JE after a single injection of the new JE-CV vaccine. Data obtained each successive year will enable us to refine the antibody persistence curve.

14

SURVEILLANCE OF ARBOVIRUSES IN FIVE PRIMARY HEALTH CENTERS IN JAKARTA, INDONESIA (2005-2006)

Agus Suwandono[1], Herman Kosasih[2], Susana Widjaja[2], Nurhayati N[2], Ungke Antonjaya[2], Patrick J. Blair[2], Maya William[2]

[1]National Institute of Health Research and Development, Jakarta Selatan, Indonesia, [2]Naval Medical Research Unit 2, Jakarta, Indonesia

Although dengue and chikungunya viruses belong to different families, both share the same vector *Aedes aegypti*. While dengue virus (DENV) is known to be hyper-endemic in Jakarta metropolitan, little is known about the endemicity of chikungunya virus (CHIKV). A one-year surveillance was conducted at five primary health centers in Jakarta, representing the West, East, North, South and Central districts from May 2005 to June 2006. A total of 377 febrile participants were enrolled into the study. DENV infections were identified in 57 (15.1%) of cases whereas CHIKV infections in 60 (15.9%). All dengue virus serotypes were identified, most predominantly DENV-2 (35.1%), followed by DENV-3 (24.6%), DENV-4 (12.3%) and DENV-1 (7%). The cross-sectional prevalence of DENV infections prior to the current illness was 89.3% and of CHIKV infections was 10.1%. Unlike DENV cases that were identified all year round (endemic) in all districts, CHIKV cases were only found in December 2005 and February 2006 in South Jakarta and May 2006 in East Jakarta. No DENV and CHIKV co-infections occurred and no fatalities were reported.

15

WORLDWIDE PHYLOGEOGRAPHIC PATTERNS OF DOMESTICATION AND VECTOR COMPETENCE IN *AEDES AEGYPTI*

Julia E. Brown, Jeffrey R. Powell

Yale University, New Haven, CT, United States

Aedes aegypti is a human commensal mosquito that has invaded much of the tropical and subtropical world over the past few centuries. As the principal vector of both dengue fever and yellow fever, this species is enormously important from a public health standpoint. Though *A. aegypti* is often treated as a homogenous species in its role as a disease vector, in reality, the species displays vast morphological and ecological heterogeneity. The two described subspecies of *A. aegypti* (*Aedes aegypti aegypti* and *Aedes aegypti formosus*) differ markedly in their association with human habitats, as well as in their ability to transmit dengue viruses. We have used 12 microsatellite markers, as well as nuclear sequence data, to describe the worldwide population genetics of *A. aegypti* and explore the evolution of human association in this species. Data have been collected from over 30 populations across five continents. Our results suggest that the African sylvan subspecies, *A. a. formosus*, is indeed ancestral to the worldwide domestic form of the species, but that close human association has likely evolved multiple times independently. We have found that most populations of domestic *A. aegypti* across Africa are genetically more similar to *A. a. formosus* than to the worldwide form, *A. a. aegypti*, even when morphologically identified as *A. a. aegypti*. As *A. a. formosus* is known to be significantly less competent for dengue viruses, these results may help elucidate differing patterns of epidemic dengue activity in Africa as compared to tropics in other regions of the world. High-throughput capture sequencing is currently being used to explore patterns of nucleotide sequence differences in candidate genes for dengue competence across worldwide populations of the mosquito. In addition, our microsatellite markers can reliably assign individual mosquitoes back to their population of origin, which could be used in conjunction with information about vector competence for dengue viruses to determine the public health significance of new *A. aegypti* introductions.

16

THE IMPACT OF DENSITY-DEPENDENCE ON NATURAL LARVAL POPULATIONS OF *AEDES AEGYPTI*: A TWO YEAR FIELD STUDY IN TAPACHULA, MEXICO

Rachael K. Walsh[1], Luca Facchinelli[2], Laura Valerio[2], J. Guillermo B. Compean[3], Janine R. Willoquet[3], Fred Gould[1]

[1]North Carolina State University, Raleigh, NC, United States, [2]University of California Davis, Davis, CA, United States, [3]Centro Regional de Investigaciòn en Salud Pùblica/CISEI3, Instituto Nacional de Salud Pública, Tapachula, Mexico

Dengue fever is transmitted by *Aedes aegypti*. The larvae of this mosquito develop in containers of water in and around houses. In order to design more efficient approaches for controlling *Ae. aegypti* it is critical to understand the factors that regulate larval density within water-filled containers. Although many studies of intra-specific competition have been conducted using larvae of *Ae. aegypti* in the laboratory, few studies have been done in the natural environment of *Ae. aegypti*, and no published studies have critically examined density dependence in natural containers at normal field densities. Additionally, mathematical models that predict *Ae. aegy*pti populations currently lack empirically-based functions for density-dependence. We performed field experiments in Tapachula, Mexico where dengue is a significant public health concern. Data were acquired during 1 dry season and 2 rainy seasons. Containers with natural food and water which already contained larvae were taken from local houses. Containers were divided in half with a tightly fitted piece of Styrofoam, enabling the natural water to be divided between the two sides in equal volume. Larvae from the container were separated by stage

and divided into a low and high density treatment. Larvae were counted and pupae were removed daily. Once adults emerged, wings were cut and measured to determine body size. Results from the first two seasons showed that density had a significant impact on larval survival, resulting in a 15 percent decrease in survival from the low density treatment to the high density treatment. Adults in the low density treatment were significantly larger than adults in the high density treatment. The last season of data will be acquired from June through August, 2010. These data will be added to the previous data and analyzed using paired t-tests. We will also look at differences between the three seasons. The data collected will then be used to assess and improve the density-dependence function in a detailed *Ae. aegypti* model.

17

IMPACT OF ROAD NETWORKS ON THE DISTRIBUTION OF DENGUE FEVER CASES IN TRINIDAD, WEST INDIES

Ron S. Mahabir, Dave D. Chadee

The University of the West Indies, St. Augustine, Trinidad and Tobago

This study was undertaken to investigate the impact of road networks on the distribution of dengue fever cases in Trinidad, West Indies. Confirmed cases of DHF for the year 1998 were collected and spatially located using a GIS road map of Trinidad. A new digital geographic layer representing these cases was created and the distances from these cases to the nearest classified road category (5 classifications based on a functional utility system) was examined. The road layer was then decomposed into 5 subsets, each representing 1 of 5 assigned road classifications. The distance from each spatially located DHF case to the nearest road in each of the 5 road subsets was then calculated and placed into 1km bins depending on their distance value. A threshold representing the maximum number of bins was determined by examination of each of the 5 layer's distance histogram. The distances from each spatially located DHF case to the nearest forest land cover was also calculated and divided in 1km bins and then further subdivided into the nearest road class to each DHF case within each bin. Statistical ANOVA and T-tests were performed to determined significance relationships between DHF cases and their distances from the different classifications of road. A positive correlation was found between road networks and DHF cases. More specifically, results showed that dengue cases are more associated with close proximity to minor motorways and greater distances away from forests, especially 3rd and 4th road classifications than with major motorways, 1st and 2nd roads classifications. In conclusion, minor motorways and distance away from forest provide conducive conditions for *Aedes aegypti* dispersal, finding suitable habitats and blood meals required for completion of their gonotrophic cycles. It is recommended that health authorities take these findings into consideration when planning and implementing strategies for the eradication of *Ae. aegypti* in Trinidad.

18

DENGUE VIRUS INTERACTS WITH MOSQUITO SALIVARY PROTEINS AS MEASURED BY INDIRECT IMMUNOLOGICAL METHODS

Berlin L. Londono[1], Rebecca C. Christofferson[2], Daniel Chisenhall[2], Ann-Marie F. Johnson[2], Christopher N. Mores[2]

[1]Louisiana State University, Universidad de Pamplona, Baton Rouge, LA, United States, [2]Louisiana State University, Baton Rouge, LA, United States

Based on our findings that dengue virus infection in mosquitoes produces an altered array of salivary proteins (many of which were discovered to elicit antibody production in people), we anticipated that some such peptides might be directly involved in the transmission of virus to humans. To investigate this, we performed an ELISA-based technique to measure the level of binding of the four dengue virus serotypes with *Ae. aegypti* salivary proteins. The level of virus-saliva binding was determined by using specific monoclonal antibodies against each dengue serotype, as well as by using serum from people with significant levels of antibodies against Aedes spp. saliva (but were otherwise dengue seronegative). When using monoclonal antibodies, we found that dengue 2 presented the highest binding to salivary proteins followed by dengue 4. Dengue 1 and 3 presented similar binding levels to one another, though moderate when compared to dengue 2 and 4, as measured by optical density (OD) when using these monoclonal antibodies. Additionally, when using human serum, dengue 1 and 3 displayed similar binding levels, and to a much greater degree that was seen in dengue 2. These findings suggest that: a) dengue virus is able to bind mosquito salivary proteins, b) there are differences in the binding levels of each serotype to salivary proteins and, c) some immunogenic salivary proteins to humans can also bind dengue virus. This last conclusion may also indicate direct interaction of some salivary proteins with dengue virus and suggest an active role for salivary proteins in dengue transmission.

19

CULEX TARSALIS BLOOD-FEEDING PATTERNS AND HOST PREFERENCE AT A RURAL SITE IN NORTHERN CALIFORNIA

Tara C. Thiemann, Sarah S. Wheeler, Christopher M. Barker, William K. Reisen

University of California, Davis, Davis, CA, United States

Culex tarsalis is an important vector of West Nile virus in California, particularly in more rural areas. Based on historical data using serological methods and more recent data using molecular methods, it is a generalist feeder and will take bloodmeals from a variety of avian and mammalian hosts. Like other *Culex* mosquitoes, *Cx. tarsalis* seems to feed more frequently on mammals during the late summer months than during the early summer and spring. To further explore this seasonal change in host selection, bloodfed *Cx. tarsalis* were collected at a rural farmstead in Northern California from June 2008 through June 2009. Engorged mosquitoes were found in all months except November and December. Bloodmeals were identified using either a newly developed Luminex®-based assay or the mitochondrial sequence of cytochrome c oxidase I (COI), and host feeding patterns were assessed. In addition, hosts were censused on three occasions, and host feeding indices were determined for these periods. Host composition in summer months was dominated by four species of colonially nesting Ardeids. In addition to these herons, and while the herons were absent from September to May, the site was populated with various passerine species (that changed throughout the seasons) as well as farm animals including chickens, geese, goats, horses and cattle. When herons were present, *Cx. tarsalis* fed predominantly upon these birds, with heron bloodmeals comprising >80% of the total bloodmeals tested. Of the herons, Black-crowned night-herons, a competent host for WNV, were the preferred host. As the herons left the area, *Cx. tarsalis* feeding shifted to other birds as well as mammals, and in the winter months Yellow-billed magpies and House sparrows, both WNV competent, were the predominant hosts. These data demonstrate that host selection is likely based on a combination of host availability and preference and that WNV-competent hosts are fed upon by *Cx. tarsalis* throughout the year.

20

CONSEQUENCES OF THE EXPANDING GLOBAL DISTRIBUTION OF *AEDES ALBOPICTUS* FOR DENGUE VIRUS TRANSMISSION

Louis Lambrechts[1], Thomas W. Scott[2], Duane J. Gubler[3]

[1]Institut Pasteur, Paris, France, [2]University of California, Davis, CA, United States, [3]Duke University-National University of Singapore Graduate Medical School, Singapore, Singapore

The dramatic global expansion of *Aedes albopictus* in the last three decades has increased public health concern because it is a potential vector of numerous arthropod-borne viruses (arboviruses), including the most prevalent arboviral pathogen of humans, dengue virus (DENV). *Ae. aegypti* is considered the primary DENV vector and has repeatedly been incriminated as a driving force in dengue's worldwide emergence. What

remains unresolved is the extent to which *Ae. albopictus* contributes to DENV transmission and whether an improved understanding of its vector status would enhance dengue surveillance and prevention. To assess the relative public health importance of *Ae. albopictus* for dengue, we carried out two complementary analyses. We reviewed its role in past dengue epidemics and compared its DENV vector competence with that of *Ae. aegypti*. Observations from "natural experiments" indicate that, despite seemingly favorable conditions, places where *Ae. albopictus* predominates over *Ae. aegypti* have never experienced a typical explosive dengue epidemic with severe cases of the disease. Results from a meta-analysis of experimental laboratory studies reveal that although *Ae. albopictus* is overall more susceptible to DENV midgut infection, rates of virus dissemination from the midgut to other tissues are significantly lower in *Ae. albopictus* than in *Ae. aegypti*. For both indices of vector competence, a few generations of mosquito colonization appear to result in a relative increase of *Ae. albopictus* susceptibility, which may have been a confounding factor in the literature. Our results lead to the conclusion that *Ae. albopictus* plays a relatively minor role compared to *Ae. aegypti* in DENV transmission, at least in part due to differences in host preferences and reduced vector competence. Recent examples of rapid arboviral adaptation to alternative mosquito vectors, however, call for cautious extrapolation of our conclusion. Vector status is a dynamic process that in the future could change in epidemiologically important ways.

21

ELIMINATION OF A PRIMARY FILARIASIS VECTOR POPULATION AT AN ENDEMIC FIELD SITE: COMPARING THE RELATIVE VECTOR COMPETENCE OF A NATURAL AND INCOMPATIBLE STRAIN OF *AEDES POLYNESIENSIS*

Hervé C. Bossin[1], Annerose H. Kuras[1], David R. Mercer[2], Anne-Marie Legrand[1], Eric W. Chambers[2], Marc Faaruia[1], Jérôme Marie[1], Albert Tetuanui[1], Michel German[1], Limb K. Hapairai[1], Stephen L. Dobson[2]

[1]Institut Louis Malardé, Papeete, French Polynesia, [2]University of Kentucky, Lexington, KY, United States

Field evaluation of a novel vector control strategy is underway in French Polynesia. In the approach tested, inundative releases of incompatible *Aedes polynesiensis* male mosquitoes infected with *Wolbachia* should result in the sterilization of wild female populations at a field site endemic for filariasis transmission. Prior to the field trial, experiments have compared the release (CP) strain and field population in their fitness, population dynamics and genetic structure, mating competitiveness, and vector competency. Although only males are to be released, the competency experiment is an appropriate precaution to exclude the risk of an increased vectorial competence through introgression crosses or due to unnatural *Wolbachia* infection occurring in the CP strain. Vector competence of the incompatible CP strain was compared with the lab colony from which it was derived. Female mosquitoes of each strain were fed with blood drawn from *Wuchereria bancrofti* positive donors containing low and high densities of microfilariae. To assess the vector competence, *Wuchereria* parasite development was monitored in both strains. Females were screened for microfilariae immediately after the infectious bloodmeal and for infective larvae at the end of the developmental cycle. The strains showed no significant differences in the number of females infected with infective L3 larvae at day-14. However, there was a significant difference in the mean number of infective L3 larvae per female that each strain had permitted to develop. CP females appear less permissive towards filarial worm infection with a reduced degree of infection when compared to natural populations of Ae. polynesiensis. The significance of these observations will be discussed in the framework of field trials designed to assess the efficacy of cytoplasmic incompatibility as a vector control strategy. If successful, this strategy could adequately complement ongoing mass drug administration to augment ongoing efforts to eliminate Lymphatic Filariasis in the South Pacific.

22

A CAUSAL FRAMEWORK FOR EVALUATING PRE-EXISTING INTERVENTIONS: AN EXAMPLE MOTIVATED BY EFFORTS IN THE WATER, SANITATION AND HYGIENE SECTOR IN RURAL INDIA

Benjamin F. Arnold[1], Ranjiv S. Khush[2], Padmavathi Ramaswamy[3], Alicia London[2], Paramasivan Rajkumar[3], Prabhakar Ramaprabha[3], Natesan Durairaj[3], Alan Hubbard[1], Kalpana Balakrishnan[3], John M. Colford[1]

[1]University of California, Berkeley, Berkeley, CA, United States, [2]Aquaya Institute, San Francisco, CA, United States, [3]Department of Environmental and Health Engineering, Sri Ramachandra Medical College and Research Institute, Chennai, India

Although the gold standard for causal inference from community-level interventions is the community-randomized trial, many interventions cannot be randomized, and the conditions of trials often make their external validity and sustainability difficult to assess. Studies of non-randomized, pre-existing interventions can estimate the average treatment effect among those most likely to receive an intervention from providers who will actually deliver it (a policy-relevant quantity). Pre-existing interventions can also provide useful information about intervention sustainability. However, evaluating such efforts raises challenges for the unbiased estimation of treatment effects. Drawing on a causal inference framework, we developed a matched cohort design to study non-randomized, pre-existing interventions. We illustrate the strengths and limitations of the method with a sanitation mobilization, water supply and hygiene intervention in rural Tamil Nadu, India. In a propensity score matched sample of 25 villages, we enrolled 1,285 children < 5 years, up to 4 years after the end of intervention activities. Over 12 months, we measured sanitation and hygiene practices, water quality, and child health (diarrhea and anthropometry). The matched cohort design resulted in a control group that was extremely similar to intervention villages across numerous covariates. Access to improved water sources was universal in both groups, but intervention households were far more likely than controls to construct toilets (48% v. 15%, p<0.0001). Adults practice daily open defecation in 39% of all households with a private toilet. Diarrhea among children < 5 was rare in all villages: 1.8% combined prevalence over 14,259 child-weeks (adj. longitudinal prevalence difference = -0.002, p=0.69). The stunting prevalence in the cohort was 53%, and there were no detectable anthropometric gains due to the intervention (Z-score differences < 0.07). In concluaion, matched cohort designs can be a useful tool to study non-randomized, pre-existing community interventions that arise in actual development efforts. In some environments, diarrhea prevalence can be very low despite ubiquitous open defecation; even with these surprisingly low diarrhea rates, growth is poor in this cohort.

23

EFFECT OF A LARGE-SCALE SANITATION, HYGIENE EDUCATION AND WATER SUPPLY INTERVENTION IN RURAL BANGLADESH

Tarique M. Huda[1], Leanne Unicomb[1], Amal K. Halder[1], Richard B. Johnston[2], Stephen P. Luby[1]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]UNICEF, Dhaka, Bangladesh

Sanitation Hygiene Education and Water supply in Bangladesh (SHEWA-B) is a large program targeted at 19 million rural people. Between 2007 and 2009, the intervention aimed to change 11 key behaviors related to hygiene, sanitation and water supply. Program activities included household visits, tea stall sessions and courtyard meetings, as well as other social mobilization activities. We assessed indicators at baseline and after 2 years, using 5-hour structured observations of hand washing behavior and spot-checks of water sources, latrines, and waste disposal in 500 randomly selected intervention and 500 control households. To assess the health

impact we established sentinel surveillance in 500 intervention and 500 control households. The proportion of people washing both hands with soap or ash after cleaning a child's anus in intervention areas improved from 22% to 36%, significantly better than in control areas (P=0.048). Households in intervention areas without access to latrine facilities, who practiced open defecation, reduced slightly from 10% to 7% (P=0.017). In the intervention areas the proportion of households that reported hearing hygiene messages increased from 72% to 77%, significantly better than in the control areas (P<0.001). There were no significant improvements found in the practice of child feces disposal, improved latrine use, cleanliness of latrine, storing of drinking water in a covered container and waste disposal. Overall, on monthly visits during the first 21 months, 11% of children under 5 years of age in intervention areas were reported to have diarrhea in the preceding 2 days compared to 10% of children in control areas (P=0.67). In concluaion, the large ambitious SHEWA-B intervention improved only a few of its targeted behaviors, and these changes were sufficiently modest that they did not lead to a measurable reduction in childhood diarrhoea.

24

IMPACT OF BASIC CARE PACKAGE DISTRIBUTION ON THE HEALTH OF PEOPLE LIVING WITH HIV/AIDS--ETHIOPIA, 2009

Ethel V. Taylor[1], Sisay Alemayehu[2], Ribka Fantu[2], Robert Quick[1], Achuyt Bhattarai[1], Barbara Marston[1], Yordanos Belayneh[3], Tegene Sewnet[3], Ciara E. O'Reilly[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]CDC-Ethiopia, Addis Ababa, Ethiopia, [3]Dr. Michael's Public Health Consulting Services, Addis Ababa, Ethiopia

Opportunistic infections (OIs) cause substantial morbidity and mortality among people living with HIV/AIDS (PLWHA) in Ethiopia. To reduce the risk of OIs, a national program in 2009 provided free basic care packages (BCP) - which included water chlorination products, safe water storage containers, soap, condoms, and albendazole - to PLWHA. To evaluate the impact of BCPs, we enrolled PLWHA from antiretroviral treatment (ART) programs at two hospitals and designated enrollees from one hospital as the intervention group and from the other as the comparison group. We conducted a baseline survey and chart review, then provided BCPs to the intervention group followed by biweekly home visits for 14 weeks to both groups to ask about recent onset of diarrhea, respiratory infection, and febrile illness. We enrolled 405 PLWHA from the intervention group and 344 from the comparison group. At baseline, both groups had similar median CD4 cell counts (279 vs. 265 cells/μL, respectively, p>0.05); 80% and 75%, respectively, had been on ART for >1 year (p>0.05). Over 14 weeks of follow up, we made 2,721 home visits in the intervention group and 2,258 in the comparison group. Intervention group members were less likely than comparison group members to report any illness (13.3% vs. 26.9%, p<0.05) or febrile illness (5.9% vs. 8.9%, p<0.05) in the preceding 48 hours, or to have visited a health facility in the preceding two weeks for any illness (8.5% vs. 14.9%, p<0.05), for diarrhea (0.7% vs. 1.5%, p<0.05), or for respiratory infection (1.0% vs. 2.1%, p<0.05%). The all-cause hospitalization rate (2.2 vs. 6.9 per 1000 home visits, p<0.05) and all-cause mortality rate (0.7% vs. 1.5%, p>0.05) were also lower among intervention than comparison group members. Over the study period, BCP recipients reported fewer illnesses and health facility visits, and had fewer hospitalizations and deaths than PLWHA not receiving BCPs. These data suggest that BCPs can be an effective approach to reducing illness risk in PLWHA. Further research is needed to assess the sustainability of this intervention.

25

LONG-TERM IMPACT OF INTEGRATION OF HOUSEHOLD WATER TREATMENT AND HYGIENE PROMOTION WITH ANTENATAL SERVICES ON MATERNAL HOUSEHOLD HYGIENE PRACTICES IN MALAWI

Anagha Loharikar[1], Elizabeth Russo[1], Anandi Sheth[1], Manoj Menon[1], Amose Kudzala[2], Blessius Tauzie[2], Humphreys Masuku[3], Robert Quick[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]UNICEF, Lilongwe, Malawi, [3]Ministry of Health - Malawi, Lilongwe, Malawi

The risk of diarrhea, a leading cause of childhood mortality, can be reduced by household water treatment and handwashing with soap. A clinic-based program to integrate distribution of hygiene kits - which contained safe water storage containers, water treatment solution (*WaterGuard*), soap, and hygiene education - with antenatal services was implemented in Malawi in 2007. To evaluate program impact on water treatment and handwashing technique, we interviewed mothers (program participants) 9 months after implementation; to assess diffusion of the intervention into the community, we surveyed relatives/friends identified by program participants. The evaluation demonstrated significantly increased *WaterGuard* use and improved handwashing technique among program participants and their relatives/friends. To assess program sustainability, we conducted an evaluation 3 years after program implementation was initiated. We enrolled 389 participants and 386 relatives/friends at baseline; we surveyed 232 program participants and 168 relatives/friends at 3-year follow-up to assess current water treatment practices, test drinking water for residual chlorine, and observe handwashing technique. We compared follow-up results to baseline data. Program participants were more likely to know correct water treatment procedures (68% vs. 29%, p<0.0001), treat drinking water with *WaterGuard* (25% vs. 2%, p<0.0001), purchase and use *WaterGuard* (23% vs. 2%, p<0.0001), and demonstrate correct handwashing technique (37% vs. 22%, p<0.001) at the 3-year follow-up survey than baseline. Relatives/friends were also more likely to know correct water treatment procedures (50% vs. 27%, p<0.0001), treat drinking water with *WaterGuard* (16% vs. 2%, p<0.0001), purchase and use *WaterGuard* (15% vs. 2%, p<0.0001), and demonstrate correct handwashing technique (30% vs. 18%, p<0.005), at 3-year follow-up than baseline. This antenatal-clinic-based program appeared to be effective at promoting sustained water treatment and proper handwashing technique among program participants and selected relatives/friends.

26

THE IMPORTANCE OF HANDWASHING BEFORE PREPARING FOOD: OBSERVED HANDWASHING AND SUBSEQUENT DIARRHEA IN RURAL BANGLADESH

Stephen Luby[1], Amal K. Halder[1], Tarique M. Huda[1], Carole Tronchet[2], Leanne Unicomb[1], Richard B. Johnston[1]

[1]ICDDRB, Dhaka, Bangladesh, [2]UNICEF, Dhaka, Bangladesh

Efforts to improve hand hygiene in communities with high levels of child mortality have focused on encouraging community residents to wash their hands with soap especially after defecation. We analyzed data from the control group enrolled in a large prospective project evaluation to assess the relationship between observed handwashing behavior and subsequent diarrhea. Field workers conducted five hour structured observation and a cross sectional survey in 347 households across 50 villages in rural Bangladesh. Each month for the subsequent two years a trained community resident visited each of the enrolled households and collected information on the occurrence of diarrhea in the preceding 48 hours among all household residents under the age of five years. Field workers observed at least one opportunity to wash hands before preparing food in 281 (81%) and at least one opportunity to wash hands after defecation

in 102 (29%) of the households during structured observation. Compared to children living in households where caregivers prepared food without washing their hands, children living in households where caregivers washed at least one hand with water only (odds ratio [OR]= 0.78; 95% confidence interval [CI] = 0.57, 1.05), washed both hands with water only (OR= 0.67; 95% CI = 0.51, 0.89), or washed at least one hand with soap (OR= 0.30; 95% CI = 0.19, 0.47) before preparing food had less diarrhea. In households where residents washed at least one hand with soap after defecation (OR= 0.45; 95% CI = 0.26, 0.77) children subsequently experienced less diarrhea, but there was no association between handwashing with or without soap before feeding a child, before eating, or after cleaning a child's anus who defecated and subsequent child diarrhea. In conclusion, these observations suggest that before preparing food is a particularly important time to promote handwashing to prevent childhood diarrhea, and that hand rinsing without soap can significantly reduce childhood diarrhea.

27

EFFECT OF DAILY ACTIVITIES ON HAND FECAL CONTAMINATION AMONG TANZANIAN MOTHERS: IMPLICATIONS FOR MEASURING HANDWASHING BEHAVIOR

Amy J. Pickering[1], Timothy R. Julian[1], Simon Mamuya[2], Alexandria B. Boehm[1], Jennifer Davis[1]

[1]Stanford University, Stanford, CA, United States, [2]Muhimbili University of Health and Allied Sciences, Dar es Salaam, United Republic of Tanzania

The lack of reliable handwashing behavior indicators hinders rigorous evaluation of interventions promoting hand hygiene. Fecal indicator bacteria (FIB) levels on hands are used to measure hand cleanliness; however, limited evidence exists regarding how daily activities affect levels of FIB, and whether their occurrence is correlated with the presence of pathogens. A household observational study, combined with hand rinse sampling, was conducted to assess changes in FIB concentrations on hands attributable to typical daily activities among 119 mothers with young children in Dar es Salaam. Twenty-two mothers were observed performing daily activities for an 8-hour period, during which hand rinse samples were obtained every 2 hours. Another 97 mothers were asked to carry out a specific household activity, with hand rinse samples obtained before and after the activity. A "sitting" group was also enrolled as a control. All samples were analyzed for enterococci and *E. coli*, and select samples were analyzed for genetic markers of *Bacteroidales,* enterovirus, and pathogenic *E. coli*. Using the toilet, cleaning up a child's feces, sweeping, washing dishes, food preparation, and bathing were all found to increase FIB on hands, with geometric mean increases ranging from 50 to 6310 colony forming units (CFU) per two hands. Among samples obtained during a mother's daily routine, food preparation, exiting the household premises, and increased time since last handwashing with soap were positively associated with FIB levels. Bathing was negatively associated with FIB. *Bacteroidales,* enterovirus, and pathogenic *E. coli* were all detected on mothers' hands. Given that FIB on hands are influenced by multiple activities commonly performed by mothers throughout the day, single measures of hand FIB should thus be considered highly variable indicators of hand hygiene behavior. This work corroborates hands as vectors of disease; it also highlights the difficulty of maintaining good personal hygiene in an environment characterized by non-networked sanitation and water supply services.

28

LASTING CHANGES IN HAND HYGIENE BEHAVIOR FOLLOWING INTERVENTION AT THE TIME OF ACUTE ILLNESS, KISHOREGONJ, BANGLADESH, 2009-2010

Pavani Kalluri Ram[1], Kimberly A. Rook[1], Margaret A. DiVita[1], Emily Cercone[2], Manoshi Islam[2], Kaniz K-e Jannat[2], Eduardo Azizz-Baumgartner[2], W. Abdullah Brooks[2], Alicia Fry[3], Stephen P. Luby[2]

[1]University of Buffalo, Buffalo, NY, United States, [2]ICDDR,B, Dhaka, Bangladesh, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

In 2009 in Bangladesh, we assessed the impact of handwashing promotion on secondary transmission of respiratory illness in 174 households. The intervention emphasized keeping soap at a handwashing place and washing hands after contact with respiratory secretions and feces. To assess whether intervening at a teachable moment, such as acute illness, results in lasting behavior change, we performed a follow-up study to assess handwashing behavior 4-7 months after enrollment. We observed soap at the handwashing place in intervention and control households. During 90-minute structured observations, we observed handwashing at critical times, such as after contact with respiratory secretions, after toileting, and before cooking. We then prompted one respondent from each household to show how s/he coughs or sneezes (respiratory secretion prompt), and prompted caregivers of children < 5 years old to show how they clean the baby's bottom after defecation (fecal contact prompt). We recorded hygiene behaviors reported or demonstrated by respondents after the prompts. Among 170 enrolled households (89 intervention and 81 control), soap was observed at the handwashing place in 34% of intervention and 19% of control households (OR=2.2, 95% CI=1.1-5.6). During structured observation, soap was used at 6% of all critical times for handwashing in intervention households and 2% in control households (OR=2.4, 95% CI=1.2-4.9). After the respiratory secretion prompt, 61% of intervention and 9% of control respondents said they would wash hands with soap (OR=16.3, 95% CI=6.7-39.4); only 2 intervention and 1 control respondents were observed to wash hands with soap. After the fecal contact prompt, 33% of intervention and 31% of control respondents said they would wash hands with soap (p=.75). Only 3 intervention and 3 control respondents, who actually touched the child's bottom after being prompted, were observed to wash hands with soap. In conclusion, our intervention resulted in lasting increase in maintenance of soap at handwashing places. While awareness of the need for handwashing after respiratory secretion contact was higher in the intervention group, there was no difference in observed handwashing with soap after either respiratory secretion or fecal contact. An intervention to prevent transmission from an acutely ill patient to household members may not result in long-lasting handwashing behavior change.

29

UNDERSTANDING PREGNANT WOMEN'S UPTAKE OF MALARIA PREVENTIVE INTERVENTIONS: A SYSTEMATIC REVIEW OF QUALITATIVE RESEARCH

Helen J. Smith[1], Alexander Kagaha[2]

[1]International Health Group, Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Department of Sociology, Makerere University, Kampala, Uganda

Malaria infection in pregnancy is of particular concern to public health workers in endemic areas because of the serious consequences for mothers and infants. Effective preventive interventions include insecticide treated nets and intermittent presumptive treatment. International donors heavily promote these important interventions to meet progressive coverage targets; but the real challenges lies in understanding what is important to pregnant women, their response to preventive interventions, and the various influences on their preventive health behaviour. Synthesis

of qualitative research is of emerging importance in global health; it can provide evidence of what is important to communities and can help understand what works in terms of implementing prevention programmes. Systematic reviews help draw out useful lessons for policy and programme decisions makers by bringing together a body of evidence in an accessible format. However, there are few examples of qualitative synthesis applied to global health questions; the methods for synthesising qualitative research are still in development, and important methodological questions remain. This research, funded by the MRC, will delineate to policy makers and programme managers what is currently known about social, cultural and behavioural aspects of pregnant women's uptake of malaria preventive interventions, what gaps in knowledge remain, and the agenda for future qualitative research in this area. We will also contribute to the methodological development of the thematic approach to synthesising qualitative research. The review findings have the potential to help various stakeholders in malaria control to better understand implementation in terms of barriers and facilitators to uptake of preventive strategies by pregnant women.We will report our progress in conducting a systematic review of factors influencing pregnant women's uptake of malaria preventive interventions; we will present the methodology and any preliminary findings.

30

CHILDHOOD ROTAVIRUS MORTALITY IN INDIA

Shaun K. Morris[1], Diego G. Bassani[2], Shally Awasthi[3], Rajesh Kumar[4], Prabhat Jha[2], for the MDS Collaborators[5]

[1]The Hospital For Sick Children, Toronto, ON, Canada, [2]Centre for Global Health Research, Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, ON, Canada, [3]Department of Pediatrics, King George's Medical University, Lucknow, India, [4]School of Public Health, Postgraduate Institute of Medical Education and Research, Chandigarh, India, [5]India

India has over 2 million child deaths annually and over 300,000 are due to diarrheal diseases. Rotavirus is a major cause of childhood diarrhea, however, direct estimates of mortality from rotavirus in India are not possible. Our objective is to combine mortality data from a nationally-representative study of deaths in India with the regional, age specific, microbiologic data on etiology of severe diarrhea in children to estimate the number of rotavirus attributable diarrheal deaths in children under 5. This study uses data from the verbal autopsy based Million Death Study (MDS). The MDS surveyed 6.3 million people in 1.1 million nationally representative Indian households for vital status between 2001 and 2003. Diarrheal deaths were those with final ICD-10 codes A00 to A09. The fraction of MDS deaths caused by diarrheal diseases was applied to the gender and age specific number of childhood deaths by region within India. Regional, age-specific proportions of hospitalized children with diarrhea that tested positive for rotavirus by enzyme immunoassay from the Indian Rotavirus Strain Surveillance Network were applied to estimate the number of dirrheal deaths attributable to rotavirus. We estimate that nearly 320 000 children died from diarrhea in India in 2005, of which about 65 000 were due to rotavirus. Approximately 50% of the rotavirus deaths occur in the first year of life and 90% in the first two years of life. The majority of rotavirus deaths are in the Central and East regions. At ages 1-59 months, diarrhea and rotavirus death rates were about 40% and 25% higher respectively in girls than in boys. In conclusion, rotavirus gastroenteritis is a major killer of children in India, particularly those under two years of age and girls. New rotavirus vaccines have been shown to be effective at reducing severe rotavirus disease. If the rotavirus vaccine was introduced into India, then several tens of thousands of annual diarrheal deaths could be prevented and the gender inequalities in childhood mortality would be reduced.

31

PSYCHOGRAPHIC FACTORS RELATED TO FEMALE GENITAL CUTTING IN GUINEA

Sethson Kassegne[1], Lansana Camara[2], Salifou Compaore[2], Aminata Barry[2]

[1]Population Services International, Cotonou, Benin, [2]Population Services International, Conakry, Guinea

Ninety-six percent of Guinean women have undergone female genital mutilation/cutting (FGM/C) (DHS, 2005). In 2000 a law was passed forbidding the practice and specifying that those conducting FGM/C could be punished through forced labor or life imprisonment if the practice led to the girl's death within 40 days. Population Services International/Guinea is conducting a program to reduce the practice of FGM/C, and conducted a baseline study to analyze the factors related to intention to practice FGM/C. A mixed qualitative and quantitative approach was used to collect data on FGM/C in Guinea. In 2008, 16 focus groups were conducted with women and men to collect information that was used to generate scale items for a quantitative survey. The household survey was conducted in 2009 and 4,143 caregivers (women and men aged 18-55) of children aged 4-12 were interviewed nationwide. A descriptive analysis was run on the behaviors of interest and a logistic regression was conducted to identify determinants of the intention not to practice FGM/C. The majority of men and women, 55% and 67%, respectively, intended to practice FGM/C on their child. Roughly half of women and men believed that FGM/C was not a good practice. Those who received the social support of their community environment to stop FGM/C were 2.1 times more likely to not intend to practice FGM/C (P<.001) and those who felt that not practicing FGM/C promotes harmony, equity, non-discrimination, health and wealth in the community were likewise 2.1 times more likely to be non-intenders (P<.001). Social norms and beliefs against FGC/M and locus of control for stopping FGC/M were also associated with non-intention. Intention to practice FGM/C was highest in the "Basse Guinea" district (p<.01). In conclusion, despite the law forbidding the practice, FGM/C intent remains prevalent among caregivers. The determinants identified in this study will be used to develop communications campaigns supporting the reduction of FGM/C among the general population and specifically the residents of Basse Guinea.

32

IMPACT OF A MEDICAL MISSION IN THE HEALTH OF CHILDREN FROM FOUR COMMUNITIES OF THE PERUVIAN AMAZON

Juan F. Sanchez[1], Angela M. Bayer[2], Eric S. Halsey[1], Víctor E. Gonzaga[1], Joel M. Montgomery[1], John W. Sanders[1], Paul C. Graf[1], Andres G. Lescano[1]

[1]United States Naval Medical Research Center Detachment, Lima, Peru, [2]University of California, Los Angeles, CA, United States

Humanitarian assistance (HA) in the form of short-term medical missions (MM) is an important contribution to the immediate needs of populations living in adverse conditions, although there is debate about whether such activities have long-term, population-wide health effects. The US Naval Medical Research Center Detachment (Naval Medical Research Center Detachment) is evaluating the health impact of an annual MM in four riverine communities in the Peruvian rainforest. Two months before and 10 months after the first MM we studied two random samples of children <5y.o. selected after a population census. Caretakers answered a brief survey about the child's health status and trained personnel collected a stool sample and a drop of peripheral blood in children 1-5 years old. Hemoglobin (Hb) was assessed using a digital measurement device, Hemocue®. The reported prevalence of complete immunizations, illness in the last two weeks (fever, cough and diarrhea) and anemia (Hb<11) were compared before and after MM. The study had 80% statistical power to detect differences >10% in any of these markers.The MM provided eight days of medical attention, 3,602 consultations (907 pediatric) and

5,905 prescriptions, including iron supplements and antiparasitic drugs. We studied 453 and 419 children before and after the MM, respectively. Caretakers reported a 6% increase in the frequency of complete immunization coverage after the MM and 6% to 7% increases in the reported prevalence fever, cough and diarrhea in the two weeks before the survey, although none of these differences were statistically significant. However, we observed a significant reduction in the prevalence of anemia (38% vs. 27%, p=0.003) in children 1-5 years old (n=264 and 302, respectively). This reduction was observed separately in all communities but significantly in only one: Saramiriza (27% vs. 25%, p=0.849), Puerto América (33% vs. 19%, p=0.322), Santa Cruz (37% vs. 32%, p=0.642) and Lagunas (43% vs. 27%, p=0.002). Parasite prevalence results will be available at a later date. In conclusion, despite offering extensive medical services this HA activity did not appear to reduce the frequency of childhood infections. However, we observed an 11% reduction in the prevalence of anemia, a trend partially present across all sites. Although other explanations cannot be ruled out, this MM may have increased Hb levels and the possibility of long-term effects of HA deserves further exploration.

33

TREATMENT SEEKING BEHAVIOR OF CAREGIVERS WHO HAVE CHILDREN WITH DIARRHEA IN BOLIVIA

Carter L. Cowden[1], Emily R. Smith[1], Kristen Forney[1], Danielle Wagner[1], Volga Iñiguez[2], Juan Leon[1]

[1]Rollins School of Public Health, Emory University, Atlanta, GA, United States, [2]Instituto de Biología Molecular y Biotecnología, Universidad Mayor de San Andrés, La Paz, Plurinational State of Bolivia

Diarrhea is a major cause of morbidity and mortality worldwide in children under five years of age. In Bolivia, the child mortality rate is 65 deaths per 1,000 children and diarrhea causes 37% of these deaths. The decisions made by caregivers when their child has diarrhea profoundly impacts their child's prognosis. The goal of this study was to determine the relationship between socio-economic status, illness severity, and rotavirus vaccination status with treatment seeking behavior of caregivers who have children with diarrhea. In order to assess these associations, the study conducted caregiver surveys (n= 622) at hospitals, clinics, and emergency rooms in four of the largest cities in Bolivia to collect demographic information, clinical symptoms, and caregiver treatment seeking behaviors. This study found that socioeconomic status, illness severity, and rotavirus vaccination were associated with treatment seeking outcomes. Family income was not associated with treatment seeking behavior, but, interestingly, perceptions of socio-economic status did influence treatment seeking behavior. Self-reported difficulty to pay for treatment was significantly associated with both place of first treatment and type of appointment at time of interview. Hydration status (illness severity indicator) was significantly correlated with treatment seeking behavior. This analysis contributes to the limited literature on treatment seeking behavior for child diarrhea. The results from the analysis of treatment seeking behavior can be used to allocate limited resources by increasing access to care in order to reduce the social and financial costs associated with childhood diarrhea in Bolivia.

34

THE IMPACT OF HOUSEHOLD RELOCATION ON CHILDHOOD IMMUNIZATION AND PHYSICIAN VISITS IN DHAKA, BANGLADESH

Nadira K. Sultana, Stephen P. Luby

International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh

Residential relocation may influence a household's ability to obtain health care. We undertook secondary analysis of a cross-sectional population-based study in an urban area of Dhaka, Bangladesh to examine the relationship of residential mobility with full immunization and healthcare-seeking practices for acute respiratory infection (ARI) among children <5 years of age. We categorized 10742 randomly selected <5-years old children based on the length of time at their current address: < 1 year/ recently relocated, 4515 (42%); > 2 years/residentially stable, 5527 (51%). The remaining 7% of children fell between the two periods. Field workers interviewed the children's primary caregivers using questionnaires soliciting socio-demographic and health care information. Primary study outcomes were full immunization defined according to the WHO definition, among children 9-59 months of age and physician visits. ARI was defined as the most recent episode where a child experienced symptoms of cough or difficulty breathing with any additional danger sign. Compared to residentially stable children, recently relocated children were younger (29 vs. 30 months), had smaller families (4.6 vs. 5.4), less educated parents, an average monthly household income < U.S $73 (24% vs. 18%) and had less knowledge on the location of local children's hospitals (42% vs. 58%). In addition, one quarter of the children from recently relocated households were from the poorest wealth quintile (24% vs. 17%) and the household head was 1.5 times more likely to earn income through daily wages. Controlling for enabling and socio-demographic confounders, recently relocated children were 16% less likely to be fully immunized [OR: 0.84, CI: 0.73-0.97], and 29% [OR: 0.71, CI: 0.54-0.94] less likely to seek care from physicians when suffering from ARI. In conclusion, urban Bangladeshi children from recently relocated households differed from children in residentially stable households on their uptake of full immunization and health care-seeking practices. Connecting relocated households to the existing health care system for both preventive and curative care may be a low-cost intervention to improve child health.

35

INTEGRATING POPULATION, HEALTH AND ENVIRONMENT: CONNECTING REPRODUCTIVE HEALTH CONCERNS WITH HEALTH INTERVENTIONS IN THE CONTEXT OF CONSERVATION

Sigrid Hahn, Lindsey Stephens, Samuel Holzman, Nils Hennig, Natasha Anandaraja

Mount Sinai School of Medicine, New York, NY, United States

Population-health-environment (PHE) is a development approach that recognizes the links between human health, population pressure, and the environment. It is based upon trans-disciplinary collaboration between conservationists and the public health community. We conducted a community-based, general "ecohealth" assessment in the buffer zone of Gorongosa National Park in Mozambique in order to develop interventions that support the park's mission of linking conservation and sustainable human development. As part of the larger ecohealth assessment in the park's buffer zone, we investigated the communities' view of reproductive health and family planning in order to engage the community in developing PHE programs. Trained local moderators conducted twelve focus groups in six communities, segmented by gender, on questions of health and the environment. Focus groups were recorded and then transcribed and translated into English by the trained local translators and moderators. Qualitative analysis software was used to code and organize data. Communities perceived issues of reproductive health to be of importance to their overall health, including the lack of obstetric care and family planning. Lack of information about, and access to, family planning were contributors to disuse of contraception, as was the social desirability of many children and the fear of one's husband. Participants did not explicitly link population growth to local environmental problems or resource scarcity. Community members expressed a desire for family planning and obstetrical services. Providing such services could contribute to improved reproductive health as well as potentially address the impact of local population pressure on the park.

36

INHERITANCE PATTERNS AND RECOMBINATION FREQUENCY IN THE 3D7 X HB3 *PLASMODIUM FALCIPARUM* EXPERIMENTAL CROSS : A NEW GENETIC MAP

Lisa C. Ranford-Cartwright[1], Jonathan M. Mwangi[1], Stacy M. Ricklefs[2], Stephen F. Porcella[2], Xin-Zhuan Su[3]

[1]University of Glasgow, Glasgow, United Kingdom, [2]Rocky Mountain Labs Research Technologies Section, National Institutes of Health, Hamilton, MT, United States, [3]Malaria Functional Genomics Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Three experimental genetic crosses have been performed using the human malaria parasite *Plasmodium falciparum*. Linkage analyses of progeny clones of these crosses has allowed the identification of parasite loci defining phenotypes such as the resistance to antimalarial drugs. Genetic maps produced using markers such as microsatellites and SNPs are available for two of the crosses, but a detailed map has not been available for the first genetic cross between parasites 3D7 and HB3. The frequency of recombination has been investigated in only one cross so far, revealing a map unit size of 17kb/cM. Twenty progeny clones from the 3D7 x HB3 cross have been genotyped using a custom-built Affymetrix molecular inversion probe (MIP) 10K malaria panel array to generate a detailed genetic map. The frequency of recombination in this cross differs from that published for the Dd2 x HB3 cross. Crossing over events in the 3D7 x HB3 cross appear to be more frequent, and non-reciprocal gene conversion events less frequent than in the Dd2 x HB3 cross. The new linkage map and parameters of recombination will be presented and comparisons made with the previous crosses to present a broader picture of the extent of recombination within this species.

37

DEVELOPMENT OF A MALARIA VACCINE CONSISTING OF GENETICALLY ATTENUATED PARASITES

Ivo H. Ploemen[1], Takeshi Annoura[2], Geert-Jan van Gemert[1], Mohammed Sajid[2], Ben van Schaijk[1], Shahid M. Khan[2], Cornelus C. Hermsen[1], Chris J. Janse[2], Robert Sauerwein[1]

[1]NCMLS, Nijmegen, The Netherlands, [2]LUMC, Leiden, The Netherlands

Generating sterile and long lasting protective immunity against malaria parasites with subunit vaccines has been fraught with difficulties. This has renewed an interest in 'whole organism vaccines' consisting of attenuated parasites. Immunization with sporozoites, attenuated by irradiation that invade and arrest during their development inside hepatocytes have been shown to induce strong protective immunity in rodent models of malaria and also, importantly, in humans. Through targeted deletion of genes, or combination of genes, in rodent parasites it has been shown that sporozoites can become similarly as irradiated sporozoites attenuated during liver stage development. Moreover, immunization with these genetically attenuated sporozoites (GAS) results in protective immune responses similar to radiation attenuated sporozoites. This far, two GAS vaccine candidates, Δ*p36&p36p* and Δ*fabb/f*, which can induce full protection against malaria infections in the rodent malaria model, *Plasmodium berghei* are studied in extent. Unexpectedly, we found that not all of these mutants arrested completely, resulting in blood-stage infections subsequent to immunization (i.e. 'breakthrough parasites'). By analyzing liver stage development *in vitro* and *in vivo* with these mutants now generated in fluorescent and bioluminescent backgrounds we demonstrated that these breakthrough parasites originated from small numbers of parasites that developed inside liver cells. These data indicate that, in order for a genetically attenuated human malaria vaccine to be safe a number of genes governing independent biological processes all critical for liver stage development will need to be removed to ensure complete arrest of the parasite.

38

TRANSPORTER GENES OTHER THAN *PMDR1* AND *PFCRT* MAY ALTER THE RESPONSE OF *PLASMODIUM FALCIPARUM* TO LUMEFANTRINE

Leah Mwai[1], Abdi Diriye[1], Victor Masseno[1], Steven Muriithi[1], Theresa Feltwell[2], Jennifer Musyoki[1], Avi Feller[3], Jacob Lemieux[3], Kevin Marsh[1], Chris Newbold[3], Alexis Nzila[1], Celine Carret[4]

[1]Kemri-Wellcome Trust, Kilifi, Kenya, [2]The Welcome Trust Sanger Insitute, Cambridge, United Kingdom, [3]University of Oxford, Oxford, United Kingdom, [4]Instituto de Medicina Molecular, Unidade de Parasitologia, Lisboa, Portugal

The combination of Artemether (ART) and Lumefantrine (LM) is an important malaria treatment regimen in many endemic countries. Artemisinin (of which ART is a derivative) resistance has been reported and recent studies indicate that resistance to ART-LM could evolve quickly, with LM providing the main selective pressure for resistance. Strategies to overcome drug resistance require a detailed understanding of the mechanisms of resistance. For instance, studying the mechanisms of LM resistance may aid in the identification of molecular markers that can be used to monitor ART-LM efficacy. Previous studies have highlighted the important role of transporters in mediating drug resistance. As part of an exploration of the mechanisms of LM resistance, we investigated the change in the expression levels of parasite transporters in *in vitro* selected LM resistant parasites. We cultured the P*lasmodium falciparum* multidrug resistant reference strain V1S for > 1 year under LM pressure. We used the PFsanger affymetrix array to identify genes differentially expressed after LM selection. For a subset of these genes, qPCR was done to confirm microarray results. The initial LM IC50 (inhibitory concentration that kills 50% parasitaemia) of V1S was 24 nM. The resulting resistant strain, V1SLM, could grow steadily in 378nM of LM, 15 times higher than the IC50 of the parent strain V1S. Although this resistant phenotype was unstable, micro array analysis showed that 12 transporters including the multidrug resistance gene (*Pfmdr1*), multidrug resistance associated protein (*Pfmrp*) and the V-type H+ pumping pyrophosphatase 2 (*PVP2*) were differentially expressed; this differential expression was confirmed by qPCR. In addition we observed the over expression of several exportome genes on the left arm of chromosome 2 and 10 in VISLM, suggesting a deletion in the parent line. Further, gene ontology analysis revealed the over-representation of genes with transporter activity in V1SLM. In conclusion, transporters other than *Pfcrt* and *Pfmdr1* may alter *P.falciparum* response to LM. We propose further investigations in field isolates and functional studies to confirm the exact role of the 12 genes and the genes on Chromosome 2 and 10 in mediating resistance to LM and other antimalarials.

39

CCR4-ASSOCIATED FACTOR 1 IS A GLOBAL REGULATOR OF GENE EXPRESSION IN MALARIA PARASITES

Bharath Balu[1], Steven P. Maher[1], Alena Pance[2], Julian C. Rayner[2], John H. Adams[1]

[1]University of South Florida, Tampa, FL, United States, [2]The Wellcome Trust Sanger Institute, Cambridge, United Kingdom

The CCR4-associated factor 1 (CAF1), a component of the major cytoplasmic deadenylase that initiates mRNA decay in eukaryotes, is highly conserved in all *Plasmodium* species. CAF1 is a part of a multi-component, gene regulatory complex in eukaryotes, termed the CCR4-NOT complex. In higher eukaryotes, the CCR4-NOT complex plays a global role in gene regulation with functions in transcription, mRNA decay and protein degradation. Genetic disruption of *P. falciparum* CAF1 results in astounding alterations in the temporal pattern of transcription and increased mRNA half-lives, which lead to mistimed expression of several proteins in the parasite intraerythrocytic stages. Premature expression of proteins controlling parasite egress from host cell, leads to an early release of unsegregated merozoites from the CAF1 disruptant schizonts and

drastically reduces the intraerythrocytic growth rate of the parasite. CAF1 is hence a global regulator of gene expression in malaria parasites essential for optimal intraerythrocytic parasite growth.

40

STRUCTURE-FUNCTION-IMMUNOGENICITY STUDIES OF PFEMP1 DOMAIN DBL2βC2 PF11-0521, A LIGAND FOR ICAM1 AND MALARIA VACCINE CANDIDATE

Andrew V. Oleinikov[1], Emily Amos[1], Valentina Voronkova[1], Tony Getz[1], Patrick E. Duffy[2]

[1]Seattle Biomedical Research Institute, Seattle, WA, United States, [2]National Institutes of Health, Bethesda, MD, United States

We have previously identified a single PfEMP1 domain (DBL2βC2 domain of PF11_0521) from the 3D7 genome with high binding affinity for ICAM1, and showed that its semi-conserved N-terminal sequence is essential for binding. We now have prepared various point mutations in the N-terminal region as well as in Loop 4 (which was previously implicated in ICAM1 binding). We find that binding is impaired by mutations in the N-terminal sequence but not in Loop 4 (Ala to Leu, His, or Tyr), suggesting that existing *in silico* predictions of the ICAM1-PfEMP1 interaction are incomplete. Because functional antibodies that block ICAM1 binding to DBL2βC2PF11-0521 domain protect against hospitalization in our field studies, we studied antibodies raised in animals against this domain. Antibodies against E.coli-expressed truncated variants missing N-terminal sequence recognize the COS cell-expressed full-length domain but lack functional activity, while antibodies raised by DNA vaccination against full-length domain block binding. These data shed light on the requirements for PfEMP1 domain-based vaccines that might prevent severe malaria in young children.

41

A RECOMBINANT *PLASMODIUM FALCIPARUM* MEROZOITE-SPECIFIC THROMBOSPONDIN RELATED ANONYMOUS PROTEIN (MTRAP) IS A HIGHLY EXTENDED FLEXIBLE ROD LIKE PROTEIN THAT BINDS HUMAN ERYTHROCYTES

Onyinyechukwu Uchime[1], Matthew L. Plassmeyer[1], Raul Herrera[1], Karine Reiter[1], Svetlana Kotova[2], Richard L. Shimp, Jr.[1], Jacob Lebowitz[2], Darrell E. Hurt[3], Albert J. Jin[2], Louis H. Miller[4], **David L. Narum**[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 Bioengineering and Physical Science, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, MD, United States, [3]Bioinformatics and Computational Biosciences Branch, Office of Cyber Infrastructure and Computational Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [4]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

The release of a number of *Plasmodium* genome sequences has opened up an extraordinary opportunity to discover new proteins that play crucial roles in development and invasion of red blood cells. The *P. falciparum* merozoite-specific thrombospondin related anonymous protein (MTRAP) is an example of such a novel protein. MTRAP is localized within the micronemes, then released onto the merozoite surface and processed during invasion. MTRAP has previously been shown to interact with aldolase; an actin binding protein for motility. It is hypothesized that MTRAP is released apically during invasion to mediate motility and host cell invasion. MTRAP is a cysteine rich protein with a type I thrombospondin structural homology repeat (TSR) domain. The TSR domain is a conserved domain identified in some cellular signaling proteins such as the *P. falciparum* circumsporozoite protein. We expressed, refolded and purified the full-length extracellular domain of MTRAP protein using an *Escherichia coli* expression system. rMTRAP was fully characterized by a complement of biochemical and biophysical techniques including reverse-phase HPLC analysis, atomic force microscopy, circular dichroism, sedimentation analysis, analytical size exclusion chromatography (SEC) with online multi-angle light scattering and quasi elastic light scattering, and mass spectroscopy. Our results demonstrate that purified rMTRAP appears in solution as a highly extended protein over 1 nm in width x 25 nm in length. An evaluation of whether rMTRAP bound human erythrocytes using a classical erythrocyte binding assay demonstrated that rMTRAP bound human erythrocytes. Taken altogether, MTRAPs role in erythrocyte invasion merits more investigation.

42

PLASMODIUM VIVAX RESURGENCE IN CHILDREN A DECADE AFTER MALARIA ELIMINATION ON ANEITYUM ISLAND

Akira Kaneko[1], Luis F. Chaves[2], George Taleo[3], Renu Wickremasinghe[4], Shin-Ichiro Tachibana[5], Hedvig Perlmann[6], Satoru Takeo[7], Takafumi Tsuboi[7], Chris Drakeley[8], Kazuyuki Tanabe[5], Marita Troye-Blomberg[6]

[1]Karolinska Institutet, Stockholm, Sweden, [2]Emory University, Atlanta, GA, United States, [3]Ministry of Health, Port Vila, Vanuatu, [4]University of Sri Jayewardenepura, Nugegoda, Sri Lanka, [5]Osaka University, Osaka, Japan, [6]Stockholm University, Stockholm, Sweden, [7]Ehime University, Matsuyama, Japan, [8]London School of Hyiene and Tropical Medicine, London, United Kingdom

Islands provide natural ecological experiments with a great potential for intervention studies. Aneityum, the southernmost island in Vanuatu, is located at the southeast edge of the malaria extension in Pacific. The implementation of a combined elimination package led to complete absence of *Plasmodium falciparum* in 1991, and *P vivax* disappeared from 1996 onwards, with the exception of two imported infections in Aneityum. We concluded that malaria can be eliminated on isolated islands with existing tools if there is a high degree of community commitment, as reported previously. One major concern is the possible resurgence due to inter-island human movement. In Aneityum interruption of malaria transmission was sustained until an epidemic of *P. vivax* was reported in early 2002. We investigated age-specific prevalence of malaria parasites during this epidemic in the context of seroepidemiological observations and molecular analysis of parasite diversity. Of *P. vivax* infections (28/1570) detected in two population-wide surveys 26 were found in individuals born after 1991. Positive antibody responses in 1998 to erythrocytic stage antigens and recombinant circumsporozoite proteins of *P. vivax* and/or *P. falciparum* were significantly lower in the population born after 1991 than in those born before 1972 (1 % vs 70% for erythrocytic and 0% vs 15% for sporozoite). Sero-conversion rate (SCR) for both parasite species on Aneityum clearly show a step in sero-prevalence indicative of the change in transmission related to elimination efforts in the past. Current SCR for *P. falciparum* (SCR 0.006, CI 0.003-0.010) and *P. vivax* (SCR 0.002, CI 0.000- 0.040) are 10-20 fold lower than pre-elimination levels (*P. falciparum* SCR 0.04, CI 0.03- 0.06 and *P. vivax* SCR 0.030, CI 0.020- 0.035). Sequence diversity of *Pvmsp1* and *Pvcsp* was very limited in Aneityum (genotype diversity h = 0.15), when compared with that on other islands of Vanuatu (h = 0.89 - 1.0), where SNPs in these antigen genes are stable. Our results suggest recently imported parasites as the probable source of this *P. vivax* resurgence in Aneityum. The persistence of antibody responses to parasites in previously exposed populations and the limited parasite gene pool are likely to have limited the age distribution of parasites to individuals born after elimination in Aneityum.

43

VERY LOW MALARIA PREVALENCE ON ISABEL IN THE CENTRAL SOLOMON ISLANDS

G. Dennis Shanks

Army Malaria Institute, Brisbane, Australia

The Solomon Islands Ministry of Health monitoring data derived from its Vector Borne Disease Control Program indicated very low morbidity and no malaria mortality on the island of Isabel. A comprehensive malaria survey was carried out in October 2009 by the Ministry of Health to confirm this and determine if the island was suitable for malaria elimination activities. One third of the population of Isabel (n=8600) gave their informed consent for a finger stick blood sample from which Giemsa stained blood smears, malaria serology and polymerase chain reaction (PCR) specimens were taken. Forty-nine sites involving 129 villages distributed the survey sampling across the entire island. Only a single positive blood smear for *P. falciparum* was found for a point prevalence rate of 0.012%. Following PCR testing of 2071 samples and review of blood smears from all positive PCR samples, another 2 positive smears were found. When all positive PCR samples were counted, the point prevalence rate increased to 0.193 %. Attempts to use malaria rapid diagnostic tests on febrile persons to identify malaria infections were unsuccessful due to low malaria infection rate in fever patients. Malaria serology testing suggested that transmission of *P. falciparum* markedly decreased 4 years previously and *P. vivax* 8 years previously. Ten percent of participants reported travelling out of Isabel Province from April to October 2009 indicating the potential for reintroduction of exogenous parasites. Isabel has very low malaria prevalence and is a good candidate to advance to malaria elimination. Better parasite detection methods/ algorisms are required in order to facilitate active case finding and thus parasite elimination through directed chemotherapy.

44

PROGRESS ON NATIONWIDE SCALE-UP OF MALARIA CONTROL INTERVENTIONS IN SENEGAL, 2009

Mame Birame Diouf[1], Salif Ndiaye[2], Robert Perry[3], Pape Moussa Thior[1]

[1]Programme National de Lutte contre le Paludisme, Dakar, Senegal, [2]Centre de reserche et developpement humaine, Dakar, Senegal, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

Senegal is a country of 12.5 million people where malaria is endemic with seasonal transmission. The National Malaria Control Program has scaled up nationwide the distribution of long lasting insecticide treated nets (LLINs) through free, subsidized, and market channels, intermittent preventive treatment during pregnancy (IPTp), and case management with rapid diagnostic testing followed by artemisinin-based combination therapy (ACT) of confirmed cases at public health facilities and by community health workers. Proportional morbidity due to parasitologically confirmed malaria was 5.6% in 2008, according to routine health system data. A nationwide two-stage cluster sample household survey (Malaria Indicator Survey) was conducted in October 2008-February 2009 to measure intervention coverage and parasitemia and anemia in children < 5 years. We surveyed 9,291 households in 320 clusters. We found that 60% of all households owned at least one insecticide-treated net (ITN); 23% of the general population, 29% of children < 5 years and 29% of pregnant women slept under an ITN the previous night. Of women who completed a pregnancy in the past two years, 52% had taken two doses of sulfadoxine-pyrimethamine as IPTp. Treatment was sought for one quarter of children < 5 years with fever in the preceding two weeks: 19% at a public health facility, 4% at a private provider, and 2% with a family member or traditional healer. Overall, 4.6% received an ACT, and 2.2% received an ACT within 24 hours. Of children < 5 years, 5.7% had parasitemia and 7.4% had severe anemia. Mortality in children < 5 years fell from 121 per 1000 live births for the period 2000-2005 to 85 for the period 2003-2008. Since this survey, Senegal distributed 2 million LLINs in a free nationwide campaign. Interpreting the proportion of children with fever receiving treatment is difficult as only those with positive RDT results are treated. While we have not yet met our coverage targets, the dramatic decrease in under 5 mortality suggests that the scale-up of interventions may be having a positive impact.

45

PRELIMINARY RESULTS FROM THE FIRST MALARIA INDICATOR SURVEY (MIS) IN UGANDA, 2009

Denis Rubahika[1], Ebony Quinto[1], James Muwonge[2], Stephen Baryahirwa[2], Patrick Okello[3], Sussann Nasr[4], Charles Katureebee[5], Paul Ametepi[6], Manoj Menon[7], Scott Filler[7], Richard Ndyomugyenyi[1]

[1]National Malaria Control Program, Kampala, Uganda, [2]Uganda Bureau of Statistics, Kampala, Uganda, [3]United States Agency for International Development, Kampala, Uganda, [4]Centers for Disease Control and Prevention, Kampala, Uganda, [5]World Health Organization, Kampala, Uganda, [6]ICF MACRO, Calverton, MD, United States, [7]Centers for Disease Control and Prevention, Atlanta, GA, United States

Despite the scale-up of malaria control interventions, malaria remains Uganda's leading cause of morbidity and mortality and is endemic in 95% of the country. In order to obtain national and regional estimates of outcome (e.g. malaria prevention and treatment measures) and impact indicators (i.e. anemia and parasite prevalence) and to monitor progress towards the Roll Back Malaria (RBM) 2010 targets, a malaria indicator survey (MIS) was conducted. The 2009 MIS, used a two-stage cluster sample design and was conducted during peak malaria season. A standardized household and women's (age 15-49 years) questionnaire was administered to 4,421 households in 170 enumeration areas (response rate 97.5%); children, aged 0-59 months, were tested for anemia and parasitemia. Where applicable, results were compared with Uganda's 2006 Demographic Health Survey. The percentage of households owning ≥1 insecticide treated net (ITN) increased from 16% in 2006 to 47% in 2009. The percentage of children <5 who slept under an ITN the night prior to the survey tripled from 10% to 32%. Among ITN owning households, 59% of children slept under an ITN the previous night. Similarly, the percentage of pregnant women who slept under an ITN the previous night nearly doubled from 24% in 2006 to 44% in 2009. Among ITN owning households, 77% of pregnant women slept under an ITN the previous night. The percentage of women receiving two or more doses of SP for IPTp doubled from 16% in 2006 to 32% in 2009. Of the 45% of children < 5 who had a reported fever in the previous 2 weeks, 36% received an antimalarial on either the same or day after presentation. Among children <5 tested, 10% had severe anemia (Hg<8g/dL) and 42% were parasitemic. In conclusion, there have been some significant successes in improving the coverage of lifesaving malaria interventions in Uganda. However, the burden of malaria in the country is still unacceptably high and improving access and usage of malaria control and prevention activities needs to be rapidly scaled-up in order to achieve the RBM targets.

46

CASE DEFINITION FOR MALARIA IN ENDEMIC SETTINGS: ATTRIBUTABLE FRACTION IN THE GAMBIA USING MODELING METHODS

Ebako N. Takem[1], David Ameh[1], David Jeffries[1], Steve Lindsay[2], Margaret Pinder[1]

[1]MRC Laboratories, Fajara, Gambia, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom

A correct case definition for malaria is important for measuring disease burden and an endpoint for trials. In malaria endemic regions, the frequent occurrence of asymptomatic parasitaemia makes the case definition for malaria difficult. A threshold for parasite densities can be used but needs to be defined particularly with the currently changing

levels of infection in many endemic regions. The attributable fraction (AF) i.e. the proportion of fever cases that will be eliminated if individuals were completely cleared of their parasites, can be estimated from classical estimates but this method is not very useful in settings with high proportion of asymptomatic parasitaemia. Modeling methods overcome these pitfalls. We sought to determine the AF for children aged 6 months to 10 years in an area of seasonal transmission and to estimate the sensitivity and specificity of different cutoffs for parasite density. Analysis of data from a study conducted in central Gambia. Cross-sectional surveys were carried out in 2006 and 2007 in the low and high transmission seasons. Febrile children were compared to afebrile children in terms of malaria parasitaemia and the AF was estimated using classical methods. The cumulative probabilities for parasite densities were estimated for febrile and afebrile children. Logistic regression models were compared by varying the parasite density and the best model was chosen based on the fit and plausibility. In the high transmission seasons, 3513 children were examined with a mean age was 5.04 years (SD=2.68) and 52.29% were males. There were 119(3.39%) cases of fever and 512(14.57%) with *P. falciparum* parasitaemia by thick smear (mean parasite density: 1696.54 parasites/µl, SD= 19776.88). The proportion of febrile children with detectable parasites was 40/119(33.61%) compared to 472/3394(13.91%) in the afebrile (OR=3.13, 95% CI=2.11-4.65). The AF, using the classical estimates was 0.2314 while the AF estimated from a logistic model using log of parasite density was 0.9821. A cutoff of 4000 parasites/µl had high enough sensitivity and specificity (about 70%). The models will be presented and the results obtained in the high and low transmission season will be compared.

47

ACTIVE CASE DETECTION TO TARGET RESERVOIRS OF ASYMPTOMATIC MALARIA IN A RURAL DISTRICT IN SOUTHERN PROVINCE, ZAMBIA

Gillian H. Stresman[1], Aniset Kamanga[2], Petros Moono[2], Harry Hamapumbu[2], Sungano Mharakurwa[2], Philip E. Thuma[2], Tamaki Kobayashi[1], William J. Moss[1], Clive J. Shiff[1]

[1]Johns Hopkins School of Public Health, Baltimore, MD, United States, [2]Malaria Institute at Macha, Macha, Zambia

In malaria endemic areas, asymptomatic infections are not uncommon with some areas reporting over half the population carrying malaria parasites. Identifying these individuals is difficult as parasite levels in many asymptomatic infected persons are low and hard to detect using microscopy. If asymptomatic infections are not identified and treated as part of ongoing control strategies, malaria can quickly resurge once control is interrupted. To investigate how best to target asymptomatic individuals, a pilot study was conducted in Choma District, a rural area in the Southern Province of Zambia from June to August 2009. The hypothesis was that in an area where malaria control strategies are implemented and transmission is low, symptomatic cases of malaria do not occur in isolation but arise from a spatially-clustered reservoir of asymptomatic infections. Each week, nurses at participating rural health centres (RHC) communicated the number of rapid diagnostic test (RDT) positive malaria cases to a central research team. During the dry season, when malaria transmission was lowest, the research team followed up each positive case reported by the RHC by a visit to the homestead. The location was obtained by GPS and all consenting residents completed a questionnaire and were screened for malaria using thick blood film, RDT, nested-PCR, and RT-PCR for asexual and sexual stage parasites. Persons who tested positive were treated with artemether/lumefantrine (Coartem®). Data were compared with a community-based study of randomly selected households to assess the prevalence of asymptomatic parasitemia in the same localities collected in September 2009. Preliminary results show that 2.3% of 87 individuals in the household of a symptomatic case were RDT positive whereas, 0.71% of the 141 participants in randomly selected households were RDT positive. Spatial data of homesteads of the index cases suggests that clusters of malaria may be present during the low transmission season. PCR assays and statistical analysis are in progress.

48

A MODEL OF THE EFFECTS OF ARTEMISININ-BASED THERAPY ON MALARIA TRANSMISSION

Geoffrey L. Johnston[1], David L. Smith[2], David A. Fidock[1]

[1]Columbia University, New York, NY, United States, [2]University of Florida, Gainesville, FL, United States

Current efforts to reduce the worldwide malaria burden depend on utilizing a variety of different interventions to either kill the parasite within the human host or interrupt its transmission. In order to determine how individual-level interventions impact parasite spread, mathematical models predicting the effects of drug treatment on infectiousness are needed. We describe here a model that simulates various stages of the *Plasmodium falciparum* lifecycle and predicts how artemisinin-based combination therapy (ACT) affects *P. falciparum* transmission from humans to mosquitoes in low endemicity settings. The model includes a within-host simulation of the asexual and sexual forms of the parasite that was developed from malaria therapy data and predictions of the onset of fever. Transmission to the mosquito is a function of gametocyte densities. ACTs are assumed to rapidly kill asexual parasites and early-stage gametocytes but not affect later-stage gametocytes. Our model predicts that early initiation of ACT treatment would interrupt transmission in low-transmission settings (i.e. where R0 is near unity) if treatment levels are 50% or greater. Active case detection would be required in order to improve effectiveness of ACTs at reducing transmission in areas of higher transmission. However, due to the extremely high R0 values found in some hyper-endemic areas of Sub-Saharan Africa, treatment with ACTs alone would not be predicted to succeed in interrupting transmission there, even with active case detection. Further, we generate the first estimates of the heterogeneities in infectivity found within populations and find a wide variance in infectivity between the 5th and 95th percentiles. These results will be incorporated into a larger transmission model that allows for the simultaneous simulation of a variety of different interventions over various endemic regions.

49

A SIMPLE HAND-HELD TEST FOR RAPID DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN THE FIELD: OPTIMIZATION OF SAMPLE COLLECTION METHODS FROM LESIONS

Raymond Houghton[1], Afif Ben Salah[2], Bel Haj Hamida Nabil[2], Amor Zaatour[2], Adel Gharbi[2], Jean Chen[1], Stan Morkowski[1], Jennifer Moon[1], Yvonne Stevens[1], Syamal Raychaudhuri[1], Max Grogl[3], Mara Kreishman-Deitrick[4], Louis Jasper[5], Alan J. Magill[3]

[1]InBios International Inc., Seattle, WA, United States, [2]Institut Pasteur de Tunis, Tunis, Tunisia, [3]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [4]United States Army Medical Materiel Development Activity, Fort Detrick, MD, United States, [5]United States Army Medical Materiel Development Activity, Silver Spring, MD, United States

Initial studies were performed to identify the optimal sampling method for obtaining parasites from Cutaneous Leishmaniasis (CL) lesions for use in combination with a field usable lateral flow immunochromatographic immunoassay (dipstick) for parasite detection. The test is based on the detection of thiol specific antioxidant protein (TSA, Peroxidoxin) a highly expressed protein present in amastigotes and promastigotes of *Leishmania major* and other *Leishmania* spp. The test uses a capture polyclonal antibody to TSA in combination with a gold conjugated monoclonal antibody directed to *L. maj*or amastigotes but reactive with TSA. Sixty patients (10 per sampling method, randomly selected) ranging from 7-89 years in age were enrolled with written informed consent at a study site endemic for *L. major* infections in Central Tunisia. We compared several methods for collecting samples for the dipstick assay since the sampling

process is critical for FDA clearance. These included dermal scraping (gold standard), swab, dental broach, dermal curette, fine needle aspirate, and plastic pipette aspirate. In each case smears were prepared, stained, and graded by microscopy using the WHO scale and compared to dipstick activity graded on a photographic color scale from 0-15. The most promising method based on this preliminary data set was the dental broach, which showed high sensitivity (100% vs microscopy) identifying 7/7 microscopy positive samples and 0/3 of microscopy negative samples. It also showed a high correlation (R2= 0.86) between dipstick intensity and parasite load. The merits of the other tissue sampling methods will be discussed. Further studies to evaluate the dental broach as the sampling method for the rapid immunoassay are planned. The pairing of a rapid diagnostic assay that can be used far forward in low resource settings and rugged environments with a safe and easy to use topical treatment drug will define the future management of patients with CL.

50

A CLUSTER OF CUTANEOUS LEISHMANIASIS ASSOCIATED WITH HUMAN TRAFFICKING

Anthony P. Cannella, Sanjay Mehta

University of California, San Diego, La Jolla, CA, United States

Cutaneous leishmaniasis is an infectious disease rarely seen in the United States and therefore can be a diagnostic dilemma. Determining the social and geographic context of infection is key to diagnosis and management of this disease. This study was undertaken to study the epidemiology and response to liposomal amphotericin therapy in a cluster of five patients with cutaneous leishmaniasis due to L. panamensis at a University hospital. Patients included Somali and one Ethiopian in US Border Patrol custody. All five patients came to the US by the same human trafficking route: Djibouti ◊Dubai◊Moscow◊Havana◊Quito; from Quito, by ground to the Columbian/Panamanian border where they camped out; finally, by ground to the US/Mexico border where they were detained. After routine medical care failed to adequately treat a variety of skin lesions, all five patients simultaneously presented to our institution. The patients had chronic ulcerative skin lesions at different sites (pinna, thumb, leg, foot and thighs), stages of evolution, and size (range, 1 - 8 cm). Histological examination of punch biopsies from all patients demonstrated chronic inflammatory infiltrates; one demonstrated intracellular amastigotes typical of Leishmania spp. Culture of biopsy specimens in M199 medium grew promastigotes identified as Leishmania panamensis (Viannia group) by isoenzyme analysis and PCR. Patients' lesions responded to liposomal amphotericin B dosed at 3mg/kg on days 1-7, 10, & 14. Three patients had mild, self-resolving renal failure (maximum creatinine: 1.6mg/dL). At one month, all lesions were resolved. In conclusion, we have documented a new human trafficking route associated with importation of new world cutaneous leishmaniasis to the US. Liposomal amphotericin treatment was effective. Clinicians and public health officials should be aware of this emerging infectious disease risk.

51

INACTIVATED, CELL-BASED YELLOW FEVER 17D VACCINE-SAFETY AND IMMUNOGENICITY IN ANIMAL MODELS AND RESULTS OF A PHASE 1 CLINICAL TRIAL

Thomas P. Monath[1], Cynthia K. Lee[1], Justin G. Julander[2], Alicja Brown[1], David W. Beasley[3], Edward Hayman[1], Joseph Crowell[1], Merribeth Morin[1], Elizabeth Fowler[1], Casey Johnson[4], Lin H. Chen[5], Maggie Sisti[6], Dennis W. Trent[1]

[1]Xcellerex Inc., Marlborough, MA, United States, [2]Utah State University, Logan, UT, United States, [3]University of Texas Medical Branch, Galveston, TX, United States, [4]Johnson County Clin-Trials, Lenexa, KS, United States, [5]Mt. Auburn Hospital, Cambridge, MA, United States, [6]Sisti Consulting Inc., Sausalito, CA, United States

The live, attenuated yellow fever (YF) 17D vaccine produced in eggs causes rare but serious adverse events, including viscerotropic disease (case-fatality rate 64%). Moreover, live 17D vaccine is contraindicated in persons allergic to eggs, pregnant and nursing mothers, immune-suppressed persons, and must be used with caution in the elderly. As a safer alternative free from these contraindications and precautions, we have developed an inactivated cell culture-based YF vaccine. The 17D strain was grown in Vero cells grown on microcarrier beads in a single-use XDR bioreactor. Virus was purified, inactivated with β-propiolactone, and adsorbed to aluminum hydroxide adjuvant. The inactivated vaccine (XRX-001) elicited high titers of neutralizing antibody in mice, hamsters, and cynomolgus monkeys. A single IM inoculation of XRX-001 resulted in antibody titers similar to those following live 17D vaccine and two doses of inactivated vaccine induced antibody titers significantly higher than live 17D. Solid protection against challenge with virulent YF virus was demonstrated after one or two doses of XRX-001. A randomized, double-blind Phase 1 clinical trial of the inactivated vaccine was conducted in 60 healthy subjects 18-49 years of age, who received two IM injections of XRX-001 at a dose of 4.4 µg or 0.44 µg or placebo on Day 0 and 21. In a parallel study 30 travelers matched for age received live YF 17D. Subjects in the XRX-001 trial were followed for adverse events, and subjects in both studies were tested for neutralizing antibodies. Results of the trials will be presented.

52

ETIOLOGY OF FEVER IN CHILDREN FROM URBAN AND RURAL TANZANIA

Valerie D'Acremont[1], Mary Kilowoko[2], Ester Kyungu[3], Sister Philipina[3], Willy Sangu[2], Judith Kahama-Maro[4], Christian Lengeler[1], Blaise Genton[1]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Amana Hospital, Dar es Salaam, United Republic of Tanzania, [3]St. Francis Designated District Hospital, Ifakara, United Republic of Tanzania, [4]City Medical Office of Health, Dar es Salaam, United Republic of Tanzania

Several studies have looked at the proportion of either malaria, pneumonia, diarrhea or bacteremia among fever cases in Africa but none of them at the overall spectrum of etiologies. We aimed at investigating the precise cause of fever episodes in children attending an outpatient clinic in an urban and a rural setting in Tanzania. All consenting children aged 2 months - 10 years with a temperature >38°C were recruited. A detailed medical history and clinical examination were done to identify obvious foci of infection. A blood sample was taken to perform rapid tests for malaria and typhoid, blood culture and serological and molecular analyses. All had nasal/throat swabs taken for viral molecular investigation, urine when no obvious cause was found and stools when diarrhea was present. A chest X-ray was performed when IMCI criteria for clinical pneumonia were met. Each diagnosis was assigned a probability level (high, moderate, low) on the basis of pre-defined criteria. 1010 children were recruited, 510 in Dar es Salaam and 500 in Ifakara. Preliminary results on the causes of fever of high probability were: 50% acute respiratory infection (ARI) (31% URTI, 4% bronchiolitis, 12% non-documented pneumonia and 3% pneumonia documented by X-ray), 11% malaria, 9% diarrhoea (3% rotavirus and 6% bacterial or unknown), 6% urine infection, 3% typhoid, 1% skin infection and 20% still unknown at this stage. 4% of the children had significant bacteremia, of which half were occult. 13% had more than one diagnosis (of high probability); 1% only had both malaria and pneumonia (documented or not). 104 children had a severe disease based on WHO criteria: 38% severe ARI, 36% severe malaria, 10% severe sepsis of unknown aetiology, 8% gastroenteritis with severe dehydration, 8% severe sepsis with another infection and 2% meningitis. In conclusion, these results provide for the first time an accurate picture of the respective causes of fever in African children. As expected, ARI contribute to the largest burden of disease, most of them being URTI. There was a sizeable proportion of fevers due to typhoid documented by the rapid test for most of them. Malaria confirmed to be lower than generally thought. Results of molecular analyses and serologies

will be presented and will provide further insight on the respective contribution of bacteria and viruses, a critical issue for appropriate management of fever and rational use of antibiotics.

53

PERIPHERAL BLOOD STEM CELL TRANSPLANT RELATED *PLASMODIUM FALCIPARUM* INFECTION IN A SICKLE CELL ANEMIA PATIENT

Rojelio Mejia[1], Garrett Booth[2], Harvey G. Klein[2], Thomas B. Nutman[1], Matthew M. Hsieh[3], Willy A. Flegel[2], Amy Klion[1]

[1]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [2]Department of Transfusion Medicine, National Institutes of Health, Bethesda, MD, United States, [3]National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, United States

Nosocomial *Plasmodium falciparum* (Pf) infection is rare. Most cases involve a blood transfusion from a donor who traveled to an area endemic for malaria. We report a case of peripheral blood stem cell transplant-related Pf infection in a patient with sickle cell anemia. Both recipient and donor are from Sierra Leone. At the time of the transplant, the patient had been in the United States for two years. The donor arrived in the United States three months prior to donation. The patient developed fever and chills several days post-transplant. A diagnosis of malaria was made incidentally on a peripheral blood smear. Examination of prior blood smears revealed parasitemia beginning two days earlier. Thick and thin smear had been performed on blood from the donor prior to stem cell donation and were negative for malaria parasites. To determine whether the infection represented reactivation of occult malaria in the recipient or was related to infusion of donor stem cells or other blood products, blood samples were analyzed by real-time PCR and Pf-HRP2 antigen ELISA. Pf PCR was positive in the patient one day prior to the first positive blood smear and remained positive until treatment. Similarly, Pf-HRP2 antigen ELISA was positive days prior to the first positive blood smear. ELISA testing of pre-transplant plasma from the recipient was negative. Although PCR of the donor's blood at the time of his initial evaluation was negative, ELISA testing was positive for Pf at this time. Furthermore, repeat testing one month later, on a sample obtained prior to stem cell mobilization, was positive by PCR. The donor was treated for malaria, and follow up PCR was negative. All blood transfusion donors were screened with travel questionnaires and were negative by malaria indirect fluorescent antibody tests. These results are consistent with transmission of malaria by transfusion of peripheral stem cells from an infected asymptomatic donor with a negative blood smear. Not only does this case demonstrate that malaria parasites can survive freezing in the absence of red blood cells under appropriate conditions, but it raises questions as to the most appropriate screening method for donors from malaria-endemic regions. We propose that such donors be screened with both multi-species PCR and Pf-HRP2 antigen ELISA, and that asymptomatic donors with a positive test be treated for malaria prior to donation.

54

MALARIAL RETINOPATHY IN BANGLADESHI ADULTS

Abdullah Abu Sayeed[1], Richard J. Maude[2], Mahtab Uddin Hasan[1], Md. Gofranul Hoque[1], Arjen M. Dondorp[2], Md. Abul Faiz[3]

[1]Chittagong Medical College, Chittagong, Bangladesh, [2]Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [3]Sir Salimullah Medical College, Mitford, Dhaka, Bangladesh

A unique spectrum of retinal signs with important clinical and prognostic significance has been well described in African children with cerebral malaria. It has not been established whether the assessment of malarial retinopathy in adult malaria using simple direct or indirect ophthalmoscopy by non-ophthalmologists has a similar significance. 170 adult Bangladeshi patients with *falciparum* malaria, 20 with vivax malaria and 20 healthy subjects were assessed by both direct and indirect ophthalmoscopy. Healthy subjects and patients with vivax malaria did not show retinal changes, whereas in patients with *falciparum* malaria indirect ophthalmoscopy revealed malarial retinopathy in 18/21 (86%) patients with a fatal course, 31/75(41%) with cerebral malaria, 16/64 (25%) noncerebral but severe malaria, and 1/31 (3%) with uncomplicated malaria. By direct ophthalmoscopy, retinopathy was missed in one patient with cerebral malaria and graded as less severe in 7. More retinal haemorrhages were found by indirect ophthalmoscopy than direct (mean difference (95%CI) 3.09 (1.50-4.68), p<0.0001). In three patients, papilloedema was found by direct ophthalmoscopy but not indirect. For both techniques there was an increase in the severity of retinopathy with increasing severity of disease, from uncomplicated to severe to cerebral malaria (p for trend p<.0001). Renal failure, acidosis and presence of moderate/severe retinopathy were independent predictors of mortality by both indirect and direct ophthalmoscopy. Direct ophthalmoscopy by the non-ophthalmologist to assess malarial retinopathy is an important clinical tool to aid diagnosis and prognosis in adults with severe malaria. Indirect ophthalmoscopy is more sensitive to detect retinal pathology in severe malaria, but provides minimal additional prognostic information in the hands of a non-ophthalmologist.

55

A RETROSPECTIVE STUDY OF SEVERE *PLASMODIUM KNOWLESI* INFECTIONS AT QUEEN ELIZABETH HOSPITAL, SABAH, MALAYSIA

Timothy William[1], Giri S. Rajahram[1], Leslie Chan[1], Gordon Ma[1], Serena Khoo[1], Samantha Donaldson[1], Charlie Fredrick[1], Jayaram Menon[1], Jenarun Jilip[2], Nicholas M. Anstey[3], Tsin Wen Yeo[3]

[1]Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia, [2]Sabah State Health Department, Kota Kinabalu, Sabah, Malaysia, [3]Menzies School of Health Research and Royal Darwin Hospital, Darwin, Australia

The simian parasite *Plasmodium knowlesi* is an important cause of potentially-fatal adult malaria in Malaysia. The spectrum of disease of *P. knowlesi* in tertiary referral centers has not been described. A retrospective clinical chart review of all patients diagnosed with *P. malariae* or *P. knowlesi* by microscopy or PCR from December 2007-November 2009 at Queen Elizabeth Hospital (QEH), a tertiary care hospital in Kota Kinabalu, Sabah, Malaysia. During this period, there were 324 cases of malaria and 78 (24%) were *P. malariae/knowlesi*, with 70 records reviewed. 47 (66%) patients had uncomplicated disease, while 23 (34%) had severe disease resulting in 5 (6.4%) deaths. PCR was performed in all severe cases and confirmed *P. knowlesi* in 20 and mixed *P. vivax*/knowlesi in 1. 41 of the uncomplicated cases had PCR: 34 (85%) had *P. knowlesi* monoinfection and 5 mixed with other species. In severe disease, no cases of coma were reported, 7 patients had one, and 6 had two clinical criteria for severity, with the remainder having ≥3. Those with severe disease were significantly older (56 yrs vs 36 yrs; p<0.001), with lower oxygen saturation (90% vs 97%, p=0.006), and increased thrombocytopenia (45, 0000/µl vs 66,000/µl, p=0.006). Uncomplicated disease was successfully treated with oral chloroquine (n=19), quinine (n=18) or artemether/lumefantrine (n=10), and severe disease with quinine (n=15; 3 deaths) and artesunate (n=8; 1 death). *P. knowlesi* is a major cause of malaria in QEH, Sabah, with a high proportion having severe and fatal disease.

56

ADIPOKINETIC HORMONE (AKH) TRIGGERS LIPID MOVEMENT TO DEVELOPING INTRAUTERINE LARVAE IN FEMALE TSETSE FLIES, *GLOSSINA MORSITANS MORSITANS*

Joshua B. Benoit, Guangxiao Yang, Serap Aksoy, Geoffrey M. Attardo

Yale University, New Haven, CT, United States

Adipokinetic hormone (AKH) is an insect neuropeptide released to mobilize nutrients. In particular, this hormone functions as a trigger

for lipolysis in the fat body. AKH is linked to periods of high activity and starvation, however little is known on the role of its during insect reproduction. Tsetse fly reproduction is unique as larval development occurs entirely within the mother's uterus, and are deposited as 3rd instar larvae that immediately undergo pupariation. This reproductive strategy limits a female to 8-10 offspring during her lifespan. Nutrients are provided to developing larvae through a modified accessory gland (milk gland), and consists specialized milk gland proteins and lipids. We examined the ability of AKH to elicit the mobilization of stored lipids from the maternal fat body to intrauterine progeny *via* the milk gland. Analysis of *G. m. morsitans* genomic databases revealed two distinct AKH sequences that vary by only a single amino acid. One gene coding for the AKH receptor (AKHR) was also identified. Transcript abundance of the *akh* and *akhr* genes increased at the end of oogenesis/beginning of embryogenesis during the first gonotrophic cycle, then subsequently decreased and remained constant. Levels of AKHR remained constitutive throughout larval development, suggesting that protein expression is not transcriptionally regulated. Localization of AKHR utilizing western blotting and immunohistochemistry revealed this receptor is present on the tsetse fat body and larva. *In vitro* analysis of the fat body indicated lipids are released by this tissue following AKH exposure. Injections of AKH into pregnant females increased lipid levels in the hemolymph, and daily injections increased the lipid content within the larva compared to insect saline injections. Based on these results, AKH is a candidate hormone, functioning alone or in combination with other hormones, for signaling lipid mobilization from the fat body to the milk gland to feed the developing larva in pregnant female tsetse.

57

ANALYSIS OF THE PROMOTER AND REGULATORY REGION OF THE TSETSE FLY (*GLOSSINA MORSITANS MORSITANS*) MILK GLAND PROTEIN GENE (GMMMPG): THE SEARCH FOR MECHANISMS REGULATING MILK GLAND AND PREGNANCY SPECIFIC GENE EXPRESSION

Geoffrey M. Attardo, Josh Benoit, Serap Aksoy

Yale University, New Haven, CT, United States

The tsetse Milk Gland Protein (GmmMGP) is an essential component of nourishment during intrauterine larval development. Transcription of gmmmgp is regulated in tight corellation with larval development. The aim of this work is to identify the regulatory sequence responsible for spatial/temporal regulation of gmmmgp expression. Regulatory region localization was accomplished via generation of transgenic Drosophila with promoter/reporter constructs utilizing 2 kB and 0.5 kB fragments of the 5' upstream sequence from the gmmmgp gene transcription start site. Transgenic analysis of the gmmmgp regulatory region in Drosophila demonstrates that the 0.5 kB region of the 5' upstream is capable of tissue and sex specific reporter expression within the Drosophila accessory gland (ortholog to the tsetse milk gland), the paraovaria. In silico identification of transcription factor binding sites was performed using MatInspector. Tsetse transcription factor homologs were identified from cDNA and genomic libraries via BLASTx search. 21 putative transcription factor binding sites were identified within this region representing 11 transcription factor families. Tsetse homologs were identified for 8 families, 4 of which have representative homologs specific to a fat body/milk gland cDNA library. Comparative analysis of the 0.5 kB gmmmgp regulatory region with 0.5 kB upstream sequence of 4 other milk gland/pregnancy specific genes (gmmtsf, gmmmgp2-1, gmmmgp2-2 and gmmmgp3) predicted binding sites for the transcription factor families of caudal, dorsal and paired homoeodomain factor. This work identifies and characterizes the upstream regulatory sequence required for tissue/sex specific expression of the tsetse gmmmgp gene and identifies putative cis-regulatory elements critical for this expression pattern. These results demonstrate the conservation of cis regulatory elements between Drosophila and tsetse and validate the use of a Drosophila model system for genetic analyses of tsetse gene transcription.

58

ABDOMINAL CONTRACTIONS DRIVE EXTRACARDIAC HEMOLYMPH CIRCULATION IN THE MOSQUITO HEMOCOEL

Julian F. Hillyer, Jonathan W. Andereck, Jonas G. King

Vanderbilt University, Nashville, TN, United States

Hemolymph circulation in mosquitoes is primarily controlled by a dorsal vessel that runs underneath the dorsal midline and is subdivided into a thoracic aorta and an abdominal heart. Wave-like peristaltic contractions of the heart alternate in propelling hemolymph in anterograde (toward the head) and retrograde (toward the tip of the abdomen) directions, where it empties into the hemocoel at the terminal ends of the insect. During our analyses of hemolymph propulsion in *Anopheles gambiae*, we observed that the ventral abdomen periodically contracts and hypothesized that these contractions facilitate extracardiac hemolymph propulsion in the abdominal hemocoel. To test this, we devised methods to simultaneously analyze both heart and abdominal contractions, and to measure hemolymph flow in the mosquito hemocoel. Qualitative and quantitative analyses revealed that the ventral diaphragm periodically contracts in a peristaltic manner, initiating each contraction at the thoracico-abdominal junction and propagating them toward the tip of the abdomen. All ventral abdominal contractions occur in the retrograde direction and correlate with anterograde heart contractions. To test whether abdominal contractions potentiate extracardiac hemolymph flow, we intrathoracically injected fluorescent microspheres and tracked their trajectory through the hemocoel. Quantitative measurements of microsphere movement in extracardiac regions of the abdominal cavity showed that, during periods of abdominal contractions, hemolymph flows in dorsal and retrograde directions at a higher velocity and with greater acceleration when compared to periods without abdominal contractions. In summary, these data show that abdominal contractions potentiate extracardiac retrograde hemolymph flow in the abdominal hemocoel during periods of anterograde heart flow. The physiological implications of these findings on immune competence and pathogen migration through the hemocoel will be discussed.

59

LABORATORY STUDIES ON THE COLOR PREFERENCES OF *AEDES POLYNESIENSIS* MOSQUITOES: COLOR SELECTION FOR THE DEVELOPMENT OF AN INSECTICIDE IMPREGNATED LETHAL TARGET

Eric W. Chambers[1], Herve Bossin[2], Scott A. Ritchie[3], Stephen L. Dobson[1], Richard C. Russell[4]

[1]University of Kentucky, Lexington, KY, United States, [2]Instituit Louis Malarde, Papeete-Tahiti, French Polynesia, [3]James Cook University, Cairns, QLD, Australia, [4] University of Sydney Westmead Hospital, NSW, Australia

Long lasting insecticide treated materials are showing great effectiveness as tools for reducing *Anopheles*-transmitted malaria. A modification of this approach may also prove to be a valid intervention in reducing the transmission of lymphatic filariasis (LF) in regions of the South Pacific where the *Aedes polynesiensis* mosquito is the primary vector. In lieu of using insecticide-treated bed nets we propose the use of insecticide impregnated outdoor visual resting targets (lethal targets; LT) better suited to control this exophilic, day-biting mosquito. These targets could be placed in strategic locations throughout communities were LF transmission is endemic and thus serve as an adjunct control along with the current method of disease control in the South Pacific; mass drug administration of DEC and Albendazole. In order to achieve the aim of impacting *Aedes* mosquito populations it is essential that the target color be attractive to *Ae. polynesiensis* mosquitoes. It is also vital that the color and pattern of the target be acceptable to the local community in order to maximize uptake of the resulting product. Here we present results showing comparative mosquito attractiveness for six different potential targets using a novel photographic-based small cage attractiveness assay system

developed in our laboratory. Mosquito landing behavior and resting behavior on targets of interest paired with an adjacent white control target was measured using the ImageJ software program. Comparisons were made between *Ae. polynesiensis* females of different ages as well as between female and male *Ae. polynesiensis* mosquitoes. *Ae. polynesiensis* mosquitoes displayed significant differences in terms of their preference for different colored targets. Future tests will need to evaluate how insecticide impregnation of the targets will affect *Ae. polynesiensis* response in both small cage and semi-field cage conditions. The results of this study demonstrate the promise of this approach for vector control in the South Pacific.

60

SAND FLIES DEFENSIN: EVOLUTIONARY AND PHYSIOLOGY ASPECTS

Andre N. Pitaluga[1], Lucie Lantova[2], Erich Telleria[1], Luisa D. Rona[1], Petr Volf[2], Yara M. Traub-Cseko[1]

[1]FIOCRUZ, Rio de Janeiro, Brazil, [2]Charles University, Prague, Czech Republic

Characterization of insect's antimicrobial peptides (AMP) is important in attempt to find novel molecules that could act against vector-borne diseases. This includes not only the identification of the gene itself but also biochemical features and the expression regulation of such AMP under several different situations naturally faced by the insect vector. Considered by WHO as one major neglected disease, the leishmaniasis is transmitted by its vector, the sand flies. We are presenting the characterization of defensin, the only sand flies AMP identified to date, from several different species from New and Old World, including the full DNA sequence of the *Lutzomyia longipalpis* defensin also the intron, 5' and 3' UTR regions. We established some aspects of this AMP like the molecular evolution in the Phlebotominae subfamily and analyze the putative occurrence positive selection. We identified differences in the expression profile of New and Old world sand flies during the developmental stages and observed a defensin positive modulation in *Leishmania chagasi* infected *L. longipalpis* and a negative regulation after a blood meal. We also analyze the expression profile of male and female challenged *L. longipalpis.* The orally challenges from a Gram + and Gram - bacteria result in little modulation of the defensin expression, but the sugar feeding result in the increase of the AMP transcription. It would be interesting to further analyze the differences from Old and New World sand flies defensin including a putative gene duplication as observed in some mosquitoes, this issue will be more clear after the release of sand flies genomes. As future goals is the evaluation of any leishmanicidal activity of the sand fly defensin. The characterization of the AMP physiology roles in the insect biology would give more tools in the development of new strategies in vector-borne diseases controls.

61

EVIDENCE OF PRE COLUMBIAN TUNGIASIS IN AMERICA

Vicente Maco[1], Manuel E. Tantalean[2], Eduardo Gotuzzo[3]

[1]Beth Israel Medical Center, Albert Einstein College of Medicine, Manhattan, NY, United States, [2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru

Tungiasis is an ancient parasite that originated in America and was later transported to the Eastern Hemisphere on transatlantic voyages in the nineteenth century. Although it was first documented by Spanish chroniclers after the arrival of Christopher Columbus in the New World, little is known about its presence in pre-Hispanic America. The Inca Empire (A.D. 1430-1532), the vast pre-Columbian civilization of South America, was one of the most evolved cultures at the time of the discovery of the Americas. Several diseases, including tungiasis, were represented in clay potteries, jars or ceramics (called huacos) by their predecessors, tribes that occupied the coastal area of modern Peru. In view of the scarcity of documentation of the presence of tungiasis in Peru, we conducted a retrospective search that included the appraisal of written evidence such as ancient manuscripts and later books by chroniclers, travelers, entomologists, naturalists and anthropologists; doctoral theses; ephemeral journals, periodicals, and pamphlets in the original Spanish, French and English; and assessment of earthenware representations during selected visits to storage facilities in museums in Peru and in the US. We used all available local (n=35) and scientific names (n=9) ascribed to *Tunga* spp. over the last four centuries. To date, 4 anthropomorphic figures representing pre-Hispanic tungiasis have been identified: two anthropomorphic globular potteries, and a single-spout bottle, all from Chimu Culture (c. A.D. 1200-1470); and a new, never described or reproduced fragment from Maranga Culture (c.A.D. 150-650), with pathognomonic lesions of tungiasis. Tungiasis is an old disease that has been endemic to indigenous Peruvians for centuries, a fact that can be illustrated by anthropomorphic vessels with pathognomonic lesions in diverse stages of evolution. Our new photographed fragment is the fourth representation of *Tunga* spp. known to date in pre- Columbian American art and the only vessel that depicts different stages of tungiasis, thus representing an explicit evidence of its of its endemicity among Ancient Peruvians. Identification and analytical evaluation of these anthropological pieces dispersed now among numerous museums worldwide are of paramount importance to understand the history and impact of this flea that continues to affect Peruvians as it did in pre-Incan times

62

RNAI SILENCING OF *PHLEBOTOMUS PAPATASI* MIDGUT MOLECULES AND EFFECTS ON *LEISHMANIA MAJOR* DEVELOPMENT

Marcelo Ramalho-Ortigao, Iliano V. Coutinho-Abreu, Maricela Robles-Murguia

Kansas State University, Manhattan, KS, United States

For a successful development within the midgut of the sand fly vector, *Leishmania* must overcome several barriers which are imposed by the vector that include an early proteolytic attack, the need to escape the peritrophic matrix (PM), and attachment to the midgut epithelia to prevent excretion with the remnants of the blood meal. The ability to overcome these barriers has been associated with species specificity, and interference with the sand fly vector-parasite balance can change the outcome of the infection in the the vector. Leishmania lipophosphoglycan (LPG) was shown to be a critical molecule in the midgut attachment process for some sand fly-Leishmania pairs. Further, blockage of a midgut LPG receptor, PpGalec, severely impaired *L. major* development and survival in the midgut of *Phlebotomus papatasi*. These results supported the use of transmission blocking strategies against sand fly-transmitted leishmaniasis (TBV). Following overall analyses of the midgut transcriptome of the sand fly *P. papatasi*, several midgut molecules were selected as potential TBV candidates based on their response to infection with *Leishmania major*. We are investigating some of these targets, which include a midgut-specific, blood induced chitinase (Ppchit1) previously characterized, three novel peritrophins (PpPer1-PpPer3) and several proteases. Analyses of expression profiles of the peritrophins revealed that PpPer1 and PpChit1 are only expressed in midguts whereas PpPer2 and PpPer3 are also expressed in the hindgut and malpighian tubules, respectively. PpChit1 is presumably involved in the formation/maturation of the PM in the gut of the sand fly after a blood meal. As we predicted, knockdown of PpChit1 via RNAi led to a significant reduction of *L. major* within the gut. In contrast, knock down of PpPer1 led to an increase in the parasite load. One possibility for the effect detected for PpPer1 is that its knockdown increases the escape of the parasite from the PM. Another option could be related to an increase in the availability of nutrients to the Leishmania due to greater influx of digestive proteases towards the blood bolus. These results will likely bring new understanding of underlying events involved in the cycle of Leishmania within the sand fly vector. These and other issues related to the RNAi-induced phenotypes detected will be discussed.

63

PREDICTING THE CHANCES OF ELIMINATION IN PARASITE INTERVENTION PROGRAMS

Manoj Gambhir[1], Edwin Michael[1], Daniel Tisch[2], Moses Bockarie[3], James Kazura[2], Robert Spear[4], Justin Remais[5]

[1]Imperial College London, London, United Kingdom, [2]Case Western Reserve University, Cleveland, OH, United States, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [4]University of California Berkeley, Berkeley, CA, United States, [5]Emory University, Atlanta, GA, United States

Large-scale intervention programs to control or eliminate several neglected tropical diseases are underway worldwide, yet stopping points for these programs remain unclear. Recent epidemiological work has highlighted how the heterogeneity inherent in the transmission dynamics of macroparasites can result in elimination thresholds varying between local communities. We examine the empirical evidence for this hypothesis and its implications for the global elimination of the major macroparasitic disease, lymphatic filariasis, by applying a statistical procedure, named Bayesian Melding, to fit a dynamic model of the transmission of this parasitic disease to field data from nine villages with different ecological and geographical characteristics. Baseline lymphatic filariasis microfilarial age-prevalence data from three geographically distinct endemic regions were used to fit the relevant filariasis transmission models. We then examined elimination thresholds implied by each of the datasets to evaluate site-specific heterogeneity in the values of these thresholds and investigate the ecological factors that underlie such variability.

We also applied 5 rounds of simulated mass drug administration (MDA) to the model and compared model predictions of the likelihood of elimination, or infection re-emergence, with longitudinal follow-up data from Papua New Guinea, where the population was monitored during 5 rounds of MDA and then 10 years after the final treatment round. Model parameters relating to immunity, parasite establishment, and parasite aggregation, varied significantly between the nine different settings, contributing to varying parasite elimination thresholds. The probability that the parasite will be eliminated following 5 rounds of MDA decreases markedly but non-linearly as the Annual Biting Rate and parasite reproduction number increases. We also discuss the possibility that reintroduction of infection through immigration may be occurring and thwarting elimination efforts.

64

LONG-TERM IMPACT OF REPEATED MDA ON LYMPHATIC FILARIASIS DISEASE IN PAPUA NEW GUINEA

James W. Kazura[1], Edward Thomsen[1], Manasseh Baea[2], Peter A. Zimmerman[1], Christopher L. King[1], Peter Siba[3], Daniel J. Tisch[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Papua New Guinea Institute for Medical Research, Madang, Papua New Guinea, [3]Papua New Guinea Institute for Medical Research, Goroka, Papua New Guinea

In addition to breaking transmission, a goal of the Global Program to Eliminate Lymphatic Filariasis (GPELF) is to ameliorate clinical pathology due to *Wuchereria bancrofti* infection. Prior observations in a highly endemic area of Papua New Guinea showed that the prevalence of leg lymphedema and hydrocele was reduced during a 5-year mass drug administration trial, as reported previously. However, the long-term impact of MDA on clinically overt LF morbidity has not been established. We investigated the possible resurgence of LF morbidity 10 years after 5 annual rounds of MDA were given from 1994 to 1998. Physical examinations were performed on all persons >1 year old; the presence and severity of hydrocele and lymphedema of the extremities and breasts were graded according to WHO criteria as before. In communities where pre-MDA transmission was moderate, hydrocele prevalence decreased from 12% to 3% after 5 years after MDA began ($p<0.001$) and remained low (1%, $p=0.194$) 10 years after no additional LF interventions. In communities where pre-MDA transmission was high, hydrocele prevalence decreased from 27% to 10% ($p<0.001$) 5 years after MDA began but increased to 20% ($p=0.021$) 10 years later. For leg lymphedema, the most common disease site after hydrocele, 52% (14/27) and 44% (8/18) of residents of moderate and high transmission communities with chronic leg pathology during the MDA trial no longer had disease 10 years later. Less than 2% of 2474 individuals examined had newly diagnosed leg lymphedema. Transmission as measured by landing catches of infective anopheline vectors was ongoing but reduced at all sites 10 years after cessation of MDA. These data support hypotheses that the community-wide disease reversal effect of MDA may be attributable to reduction in both established infections and decreased exposure to mosquito-borne infective larvae.

65

SELECTING THE DIAGNOSTIC TOOL TO DEFINE THE ENDPOINT OF PROGRAMS TO ELIMINATE *WUCHERIA BANCROFTI*

Katherine Gass[1], Eric Ottesen[1], Dominique Kyelem[1], Kimberly Won[2], Patrick Lammie[2]

[1]Task Force for Global Health, Decatur, GA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States

Successful mass drug administration (MDA) campaigns have brought several countries near the point of Lymphatic Filariasis (LF) elimination. A diagnostic tool is needed to determine when the prevalence levels have decreased significantly such that MDA campaigns can be discontinued without the threat of recrudescence. This tool needs to be highly specific, as the proportion of false positive results will increase as the true prevalence of infection declines. Antigen detection by either the ICT Card Test or Og4C3 ELISA assay has been considered the best approach to detecting this low level of *W. bancrofti* infection. The objective of this study was to determine which of these two tests is better suited for detecting the endpoint of the elimination program. An eight-country study was conducted assessing the performance of eight diagnostic tests, including Bm14, PanLF, *Brugia* Rapid, Urine SXP, ICT, Og4C3, Blood Smear, and PCR on a panel of 9,884 patient specimens. ICT and Og4C3 tests detected similar levels of antigen prevalence, 9.7% and 10.8% respectively. The specificities of the two tests were also similar (Og4C3 sp=93%, ICT sp=92%), while the sensitivity of the Og4C3 was significantly better than the ICT (Og4C3 se=87%, ICT se=76%). A closer look at the ICT/Og4C3 discordant pairs suggests that ICT test may be capturing a large number of false positives, relative to Og4C3. Adopting a test-retest strategy with the ICT test can greatly improve the specificity of the ICT test relative to Og4C3. Under such a strategy, an initial positive ICT result would be considered 'indeterminate' until a second confirmatory ICT test is conducted. This test-retest method can reduce the ICT false positivity rate by 92%, increasing the specificity of the ICT test to 99%. Because the Og4C3 test is lab-based, it does not easily lend itself to such a strategy. Therefore, we recommend that countries employ this test-retest strategy with the ICT card test to assess whether or not the threshold for stopping MDA has been met.

66

EPIDEMIOLOGIC ANALYSIS OF LYMPHATIC FILARIASIS DIAGNOSTIC ASSAY CHARACTERISTICS FOR MONITORING ELIMINATION

Daniel J. Tisch[1], Edward Thomsen[1], Zachary Kloos[1], Rajeev Mehlotra[1], Lisa C. Reimer[2], Laurie R. Gray[1], Peter Siba[3], 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]Papua New Guinea Institute for Medical Research, Goroka, Papua New Guinea

Existing tools for lymphatic filariasis (LF) detection have been optimized for mapping and prioritizing areas that are targets for elimination programs using mass drug administration (MDA). However, the value of these tools for monitoring progress towards transmission elimination has not yet been established. This study evaluates human blood samples from a ten year follow-up of five annual MDAs in Papua New Guinea to quantify the population-level operating dynamics of four diagnostic assays: microscopy for blood microfilaremia (MF), parasite DNA, filarial antigen, and IgG4 antibody against BM14 across moderate and low transmission intensities. Sensitivity and specificity of the tools relative to MF were respectively 100% and 66% for antibody against BM14, 91% and 80% for filarial antigen, and 82% and 96% for parasite DNA. Whereas sensitivity and negative predictive value for these tests were high regardless of transmission intensity, filarial antigen and IgG4 antibody tests were 1.6 and 2.4 times more specific in areas of low relative to high transmission. Assays for DNA remained sensitive and specific (>70%) across a range of transmission indices. Considering children under 10 years who were born after completion of MDA revealed a pattern of biomarkers such that positivity by antigen and parasite DNA were 89% and 92% percent lower than IgG4 antibody to BM14. The variations in these assays are likely related to varying levels of transmission, limits of detection of MF, and differing uptake and decay mechanisms of the biomarkers. These results suggest that combinations of various diagnostic tools will be optimal for monitoring and potentially verifying local elimination of LF.

67

A FIELD-TESTED, POST-MDA APPROACH FOR LYMPHATIC FILARIASIS PROGRAMS

Philip J. Budge[1], Ameyo Dorkenoo[2], Felix K. Otogbe[3], Yao Sodahlon[4], Els Mathieu[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]National Program for Elimination of Lymphatic Filariasis, Lomé, Togo, [3]Ministry of Health, Lomé, Togo, [4]Mectizan Donation Program, Decatur, GA, United States

WHO guidelines for elimination of lymphatic filariasis (LF) using mass drug administration (MDA) are well established, and guidelines for stopping MDAs are currently being reviewed. Less defined are the steps to take once MDAs are interrupted. We developed a post-MDA approach that includes (1) a sustainable, nation-wide LF surveillance system, (2) nation-wide re-mapping, and (3) active follow-up of cases identified by these methods. We piloted this approach in Togo with initiation of surveillance in 2006 and national remapping in 2010. The LF surveillance system tests hospitalized patients for microfilaria. During its first two years, 8050 persons were tested and two were positive. Follow-up of these cases found no evidence of ongoing transmission. In 2009, we evaluated this system. Patients tested resided in 1214 distinct villages or cities, and in all 35 districts of the country. Plotting these villages revealed that some remote parts of the country were under-sampled. We therefore adapted the system to include collection of filter-paper blood spots, which are sent to Lomé for Og4C3 ELISA, at health posts in these regions. In considering testing of donated blood as an alternate approach, we plotted the national blood bank's catchment area. This revealed that most blood donors reside near the main urban donation centers. Of the 9438 donors whose blood was screened in Lomé in 2008, over 7800 (83%) live in Lomé or its immediate outskirts. This suggests that screening of donated blood may be an inefficient tool for LF surveillance.

To ensure that no undetected foci of endemic transmission remain in Togo, we repeated national mapping. Because 30-cluster surveys were conducted in previously endemic areas in 2008, re-mapping was only undertaken in districts considered non-endemic during the initial mapping (done in 2000). Villages were selected by randomly placing a 35 km2 grid over the country; additional villages were selected in areas with high LF morbidity and along national borders adjacent to endemic areas of neighboring countries. In total, 7600 persons in 76 villages were tested by rapid ICT test. Data from this mapping will be presented.

Countries nearing elimination of LF cannot afford to lose their investment by allowing re-introduction or recurrence of LF post-MDA. We present here a field-tested approach that can serve as a model for post-MDA activities in developing countries.

68

HAS INTERRUPTION OF *SIMULIUM NEAVEI S.S.* TRANSMITTED ONCHOCERCIASIS BEEN ATTAINED IN THE KASHOYA-KITOMI FOCUS?

Moses N. Katabarwa[1], Tom Lakwo[2], Peace Habomugisha[3], Joseph Wamani[2], Stella Agunyo[3], David Ogutu[2], Edson Byamugisha[3], Edson Byamugisha[3], Rolf Garms[4], Tom Rubaale[5], Richard Ndyomugyenyi[2], D.K.W Lwamafa[2], Frank O. Richards[1]

[1]Emory University/The Carter Center, Atlanta, GA, United States, [2]Ministry of Health, Kampala, Uganda, [3]The Carter Center-Uganda, Kampala, Uganda, [4]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, [5]GTZ, Fort Portal, Fort Portal, Uganda

Uganda announced a new policy of onchocerciasis elimination in early 2007. The strategy for elimination includes increasing ivermectin administration to twice per year, and vector elimination activities through ground laviciding. The effort to eliminate *Onchocerca volvulus* in the Kashoya-Kitomi focus in western Uganda began that same year. The focus covers about 339 km2 with "at risk" population of about 200,000 people. Annual ivermectin treatment has been offered in that focus for 16 years. Some experimental larviding was done in 2003. 1991 and 2005 baseline data were available for *Simulium neavei s.s.* infection, and 1991 and 2003 baseline data for human infection (skin snips). Before beginning larviciding in 2007, *Simulium* adult fly collection was established at 6 sites, and crabs were trapped and analysed for infestation of *S. neavei* larval stages. Abate larviciding began at 63 sites during May, 2007. Mass treatment with an annual dose of ivermectin had started in 1992, and twice yearly treatment launched in 2007. The focus attained at least 90% in every round of treatment every year for the last three years. In 1991, Community microfilarial load (CMFL) was 21 mf/skin snip. In the same year, *Simulium* flies density was 500 fly man hour (FMH), fly infection rate (all larval stages) above 50%, and fly infective rate (L3 larvae) at 10%-12%. In 2005, fly density was 72 FMH (probably decreased due to experimental larviciding), fly infection, 14.2%, fly infective rate, 3.2%. CMFL in 2003 was 6.4. With full implementation of the elimination policy, *Simulium* fly abundance was reduced to less than 10 FMH since December, 2008. Crab infestation with *S. neavei* has decreased from 40.5% in May, 2007 to less than 1% by February 2008, and zero for the last quarter of 2009. Interruption of onchocerciasis transmission may have been attained, but new results from skin snips in the population, and Ov16 antibody in children are required to determine if all interventions can be halted.

69

IMPACT ASSESSMENT OF REPEATED ANNUAL IVERMECTIN ON OCULAR AND CLINICAL ONCHOCERCIASIS 14 YEARS OF ANNUAL MASS DRUG ADMINISTRATION IN EIGHT SENTINEL VILLAGES, SOUTHEAST NIGERIA 2008

E. Emukah[1], J. Nimzing[2], M. Ezeabikwa[3], J. Obiezu[1], NT Okpala[1], E. S. Miri[2], M. Katabarwa[4], D. Hopkins[4], **F.O. Richards**[4]

[1]The Carter Center, Owerri, Nigeria, [2]The Carter Center, Jos, Nigeria, [3]Parasitology Department University of Nigeria, Nsukka, Nigeria, [4]The Carter Center, Atlanta, GA, United States

We conducted a cross sectional survey in 2008 after 14 annual rounds of mass drug administration (MDA) with ivermectin to measure the impact on clinical onchocerciasis. We studied eight sentinel villages in southeast Nigeria, for which we have baseline parasitological data from 1995. During the survey we 'skin snipped' 940 consenting participants to determine microfilaria (mf) prevalence and community microfilaria load (CMFL); we also examined 839 persons by slit lamp for evidence of ocular onchocerciasis. We found a significant (76%) decrease in mf prevalence in all villages (62.43% in 1995 compared to 14.72% in 2008) as well as an 89% decrease in CMFL (2.1mf/gm in 1995 compared to 0.23mf/gm in 2008). Both findings were significant (P<0.001). *Onchocerca*l nodule prevalence also decreased significantly in all the villages. We observed 2% overall punctate keratitis that could have been attributable to onchocerciasis. These observations show that onchocerciasis is no longer a public health problem in the sentinel villages. However, we found 14.7% of 102 children below 10 years had mf in their skin snips, suggestive of continued acquisition of onchocerciasis infection during the 14 year treatment period. Treatment coverage in most of the villages were <80% (eligible population) with occasional omitted rounds, which likely contributed to continued transmission. We conclude that ivermectin treatment needs to continue, and if elimination is contemplated, enhanced programmatic support is needed to increase coverage, and twice per year treatment should be considered.

70

PLANTS USED BY TRADITIONAL HEALTH PRACTITIONERS OF NATORE AND NAOGAON DISTRICTS, BANGLADESH TO TREAT DIABETES MELLITUS

Md. Ariful Haque Mollik[1], Md. Abu Jilani[1], Md. Mahamudul Hasan[1], Md. Omar Faruque[1], Md. Ziaul Haque[1], Dilara Ferdausi[1], Rownak Jahan[1], Mohammed Rahmatullah[1], M. Taufiq-Ur Rahman[2]

[1]University of Development Alternative, Dhaka, Bangladesh, [2]University of Cambridge, Cambridge, United Kingdom

Diabetes mellitus (DM), particularly Type 2 DM is estimated to affect over 05 million people in Bangladesh alone. This ailment is spread world-wide and particularly in the developed countries. Modern allopathic medicines cannot cure the disease but only aids in lowering blood sugar levels, which is a particular characteristic of this ailment. DM is mostly treated in the rural areas of Bangladesh by traditional health practitioners (THPs). The common form of treatment is with plants, the usage of which varies from district to district and even can vary considerably between THPs. As part of our ongoing program to complete an ethnopharmacological survey of Bangladesh, we undertook a survey amongst the THPs of Natore and Naogaon districts, which lie in the central-north region of the country. Interviews were conducted of the THPs and detailed information obtained as to plants, parts of the plant used, formulation, mode of preparation, and dosages. Plant samples were collected and pressed in the field and identified at the Bangladesh National Herbarium. 04 were found to be used by the THPs of Natore district to treat DM. These plants (with plant parts used given in parenthesis) included *Mangifera indica* (fruit), *Coccinia cordifolia* (leaf), *Lawsonia inermis* (leaf), and *Cynodon dactylon* (leaf). The THPs of Naogaon district uses 06 plants to treat DM. These plants included *Catharanthus roseus* (leaf, flower), *Alocasia macrorrhizos* (all-parts), *Coccinia cordifolia* (all-parts), *Kalanchoe pinnata* (leaf), *Mentha spicata* (leaf), and *Scoparia dulcis* (all-parts). *Cynodon dactylon*, and *Catharanthus roseus* are also used in the alternative medicine systems of Mexico and South Africa; respectively, to treat DM. Scientific studies have established the hypoglycemic potential of *Mangifera indica*, *Coccinia cordifolia*, *Lawsonia inermis*, *Cynodon dactylon*, *Catharanthus roseus*, and *Scoparia dulcis*. It is important to conduct more studies towards elucidation of the pharmacological constituent(s) responsible for the hypoglycemic activity and evaluate their potential in the treatment of DM.

71

AN ETHNOPHARMACOLOGICAL SURVEY OF JAMALPUR SADAR AREA, JAMALPUR DISTRICT, BANGLADESH USED FOR TREATMENT OF "HARD TO CURE" DISEASES

Md. Ariful Haque Mollik[1], Md. Abdur Rahman[1], Md. Farid Ahmed[1], Rownak Jahan[1], Mohammed Rahmatullah[1], Majeedul H. Chowdhury[2], M. Taufiq-Ur Rahman[3], Mst. Afsana Khatun[4]

[1]University of Development Alternative, Dhaka, Bangladesh, [2]New York City College of Technology, New York City, NY, United States, [3]University of Cambridge, Cambridge, United Kingdom, [4]Lincoln College, Kuala Lumpur, Malaysia

Jamalpur district is in the north-central region of Bangladesh. The predominantly rural population of the district relies on folk medicinal practitioners, otherwise known as Kavirajes for treatment of their various ailments. We conducted an ethnopharmacological survey among the Kavirajes of Jamalpur Sadar area in this district with the objective of collecting information about the medicinal plants used by the local Kavirajes. A semi-structured questionnaire was used for the interview and Kavirajes pointed out medicinal plants during field-walks and described their uses. Medicinal plant specimens were identified at the Bangladesh National Herbarium. The various medicinal plants used by the Kavirajes (with ailments treated given in parenthesis) included *Cestrum nocturnum* (to stop bleeding), *Lannea grandis* (dog-bite, low sperm count), *Heliotropium indicum* (cataract), *Citrus grandis* (to increase appetite, blood purifier, fever), *Syzygium cumini* (tooth infection, dysentery, diabetes, kidney stones), *Ficus hispida* (dysentery), *Streblus asper* (debility), *Curcuma longa* (allergy), *Typhonium giganteum* (kidney stones, to stop bleeding), *Terminalia belerica* (asthma, allergy, to maintain heart, lungs & liver in good condition), *Parthenocissus quinquefolia* (edema), *Spilanthes acmilla* (infections on the head), *Litsea glutinosa* (low sperm count), *Caesalpinia bonduc* (menstrual problems, infertility in women, to expedite childbirth), *Achyranthes aspera* (jaundice), *Justicia adhatoda* (coughs, asthma, menstrual problems, jaundice, hepatitis B), *Calotropis gigantea* (asthma, pneumonia), *Cassia alata* (scabies), *Averrhoa carambola* (dandruff), *Cucurbita maxima* (gastrointestinal problems, joint pain, colds, constipation, piles), *Costus speciosus* (erectile dysfunction, low sperm count), *Cissus quadrangularis* (bone fracture), and *Terminalia chebula* (bloating, gastrointestinal disorders, stomachache, heart disorders, debility, helminthiasis). The medicinal plants warrant further scientific studies as potential sources of pharmacologically active compounds for treatment of diverse ailments.

72

APPLYING A KNOWLEDGE-TO-ACTION FRAMEWORK FOR PRIMARY PREVENTION OF SPINA BIFIDA IN TROPICAL AFRICA

Masumbuko Claude Kasereka[1], Kwibuka Juvenal Lunjwire[1], Michael Hawkes[2]

[1]HEAL Africa, Goma, The Democratic Republic of the Congo, [2]University of Toronto, Toronto, ON, Canada

The objective of this study was to increase capacity for primary prevention of spina bifida through folate supplementation in the Democratic Republic of Congo (DRC), using a knowledge-to-action methodology. The design was a mixed quantitative and qualitative methods: surgical case series,

survey questionnaires (knowledge, attitudes and practices), focus group discussions (FGDs), video media evaluation (satisfaction and knowledge gain questionnaires). HEAL Africa hospital in eastern DRC, where resource limitations and threats to human security contribute to restricted capacity for the management and prevention of congenital malformations. Participants included women of reproductive age, families affected by spina bifida, and community members. A case series of 27 patients undergoing surgery for spina bifida demonstrated a short tem mortality of 15% and long-term disability in survivors. Qualitative data revealed an additional heavy psychosocial burden of illness. A survey of knowledge, attitudes and practices demonstrated a low level of folate awareness (53%) among women of reproductive age. FGDs revealed exotic etiologic views, significant gender issues, and several barriers to folate use. A culturally tailored radio broadcast and an educational video were designed and produced locally based on qualitative and quantitative findings. Evaluation of the video documented high levels of viewer satisfaction and unequivocal knowledge gain ($p<0.001$). In conclusion, spina bifida poses a significant burden on affected patients and their families in the African context, but folate is under-utilized as a prevention strategy. Patient education through video media results in increased awareness and understanding of spina bifida and folate, a first step in empowering women to reduce the risk of spina bifida in their children in the absence of population-wide food fortification.

73

HOME AND COMMUNITY MANAGEMENT OF MALARIA AND PNEUMONIA IN CHILDREN UNDER FIVE: A CLUSTER RANDOMIZED TRIAL OF AN INTEGRATED APPROACH

Elizeus Rutebemberwa[1], George W. Pariyo[1], Edson A. Mworozi[2]

[1]Makerere University School of Public Health, Kampala, Uganda, [2]Makerere University School of Medicine, Kampala, Uganda

Malaria and pneumonia are major causes of mortality in children under-five in Uganda. Most children receive care first from drug shops which sometimes have untrained providers, expired drugs and give under doses. Distribution of pre-packed antimalarials by community medicine distributors (CMDs) has been shown to reduce under five mortality. Pneumonia was not catered for at CMD level and children with pneumonia were often not taken to health facilities when referred. This study was undertaken to determine the impact on under-five mortality achievable through distribution of pre-packed antimalarials (Coartem) and antibiotics (Amoxicillin) by CMDs for presumptive treatment of malaria and pneumonia in children 4-59 months. Febrile children in nine intervention parishes are given pre-packaged Coartem and pre-packed Amoxicillin when they present with a high respiratory rate. In nine control parishes, febrile children are given pre-packed Coartem and those with high respiratory rate are referred to health facilities. The proportion of febrile children treated by CMDs and adherence to treatment has been assessed. After three months of implementation, 10.7% of those who sought treatment outside the home got it first from the CMDs, 13.4% from government health facilities, 35.7% from drug shops, 35.7% from private clinics and 4.6% from general shops or neighbours. There was no significant difference between intervention and control areas. A total of 208 caretakers who had received treatment from CMDs were assessed, 97.6% (203/208) took all the Coartem given to them and the other five were saving it for later use. Of the 49 children who had taken Amoxicillin, 11/49 (22.4%) did not take all the tablets given and 5/11 stopped because the child got better. Utilization of CMDs is still low but adherence to treatment especially for Coartem is high. More behaviour change communication to improve utilization of CMDs needs to be done and non-completion of doses needs to be strongly discouraged as it brings drug resistance and children may not get cured.

74

BRUCELLA - A GREAT IMITATOR

Vishwanath Sathyanarayanan, Abdul Razak

Kasturba Medical College, Manipal, India

Brucellosis is an important re-emerging bacterial zoonosis and class B bioterrorist agent. The timely diagnosis is a challenge to clinicians because of its wide spectrum of manifestations and slow growth rate in blood cultures. This retrospective study was carried out in a tertiary care center in Southern India. 68 patients with brucellosis in the last five years were studied with reference to symptoms, occupation, epidemiology, treatment, complications and outcome. Of the 68 patients, 46 (67.64%) were males, 22 (32.35%) females in the age group of 9-75 years. 44 (64.70%) had history of contact with domestic animals. 44 (64.70%) presented with fever of <60 days, and 24 (35.3%) >60 days. The symptoms included fever in 68 (100%), arthralgia 23 (33.82%), myalgia 21 (30.88%), headache 16 (23.52%), gastrointestinal symptoms 19 (27.94%) and altered sensorium in 3 (4.41%) patients. HBsAg was +ve in 8 (11.76%), HIV +ve 2 (2.94%), steroid therapy 3 (4.41%), type 2 diabetes mellitus 13 (19.11%) and alcoholism in 10 (14.70%) patients. Labs showed anemia in 39 (57.35%), leucocytosis 10 (14.70%), leucopenia 10 (14.70%), monocytosis 43 (63.23%), thrombocytopenia 23 (33.82%), thrombocytosis 2 (2.94%), high ESR 55 (80.88%), elevated transaminases 26 (38.23%), elevated alkaline phosphatase 16 (23.52%) and ultrasound (hepatospenomegaly) in 24 (35.29%) patients. *Brucella* species (BACTEC) was grown in 50 (73.52%), brucella agglutination test titers >1:320 in 61 (89.70%), widal positive 14 (20.58%), endocarditis 2 (2.94%), bone marrow granuloma 2 (2.94%), bone marrow culture growing brucella 3 (4.41%) and CSF culture with brucella in 2 (2.94%) patients. Complications were meningitis 2 (2.94%), carditis 2 (2.94%), orchitis 2 (2.94%), musculoskeletal 2 (2.94%) and death in 2 (2.94%) patients. Treatment-6 (8.82%) patients received empirical antitubercular therapy, 48 (70.58%) doxycycline and aminoglycosides, 17 (25%) doxycycline and rifampicin and 3 (4.41%) doxycycline, rifampicin and aminoglycosides. There were three conclusions: 1. Brucellosis is misdiagnosed as enteric fever or tuberculosis in endemic areas; Physicians must consider brucellosis in prolonged febrile diseases. 2. Risk factors are diabetes mellitus and immunocompromised states; 3. Early diagnosis and treatment can prevent morbidity and mortality; and 4. Effective control measures should be instituted in developing countries.

75

ASSESSING THE IMPACT OF TOPOGRAPHY ON MALARIA EXPOSURE AND MALARIA EPIDEMIC SENSITIVITY IN THE WESTERN KENYA HIGHLANDS

Christine L. Wanjala[1], Robert S. Shivairo[2], John Waitumbi[1], Andrew K. Githeko[1]

[1]Kenya Medical Research Institute, Kisumu, Kenya, [2]Egerton University, Nakuru, Kenya

The sensitivity of a site to malaria epidemics depends on the level of immunity of human population. This study examined how terrain in the highlands affects the exposure and sensitivity of a site to malaria. The study was conducted in five sites in western Kenya, two U-shaped valleys (Iguhu, Emutete), two V-shaped valleys (Marani, Fort-Ternan) and one plateau (Shikondi) for ten months among 6-15 years old children. Exposure to malaria was tested using circum-sporozoite protein and merozoite surface protein immunochromatographic antibody test; malaria infection was tested by microscopic examination of thick and thin smears. The mean antibody prevalence was 20.5% in Iguhu, 23.6% in Emutete, 12.7% in Shikondi, 9.6% in Fort-Ternan and 10.6% in Marani. The mean malaria infection prevalence was 23.5% in Iguhu, 21.1% in Emutete, 5.1% in Shikondi, 3.1% in Fort-Ternan and 3.6% in Marani. There was a significant difference in the antibodies and malaria infection prevalence among the two valley systems and the plateau ($P<0.05$). There was no significant difference in the antibodies and malaria infection

prevalence within the U-shaped valleys and within the V-shaped valleys (P> 0.05). There was a 5.7 fold and a 2-fold greater parasites and antibody prevalence respectively, in the U-shaped compared to the V-shaped valleys. The plateau antibody and parasite prevalence was similar to that of the V-shaped valleys. U and V-shaped valleys have similar climate therefore the observed differences in parasites and antibody prevalence are likely to be due to their drainage characteristics.

76

SURVIVIN GENE EXPRESSION AND TOTAL P53 IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

Hatim A. El-Baz[1], Manal I. Fouda[2], Raida S. Yahya[3]

[1]National Research Center, Giza, Egypt, [2]Faculty of Medicine, Mansoura University, Mansoura, Egypt, [3]Children Hospital, Mansoura University, Mansoura, Egypt

Treatment and prognosis are still problematic in management of acute leukemia patients. Survivin is one of the most cancer-specific proteins identified to date, being upregulated in almost all human tumors. Biologically, survivin has been shown to inhibit apoptosis, enhance proliferation and promote angiogenesis. The aim of this study is to detect the biologic and/or prognostic significance of survivin (S) expression and total P53 in acute lymphoblastic leukemia and its correlation to patients' outcome. Sixty two patients newly diagnosed acute lymphoblastic leukemia were followed up for 2 years or until death and they were treated with chemotherapy. Survivin protein and total human P53 were measured by quantitative sandwich enzyme immunoassay technique from peripheral blood from those patients at diagnosis and at complete remission. Twenty apparently healthy individuals were used as control group. A highly significant elevation in S protein and total P53 levels in acute lymphoblastic leukemia patients at diagnosis compared to controls. At complete remission no significant difference were found between acute leukemia patients at remission and healthy control group. S protein and total P53 was significantly higher in non-survived compared to survived group. A positive correlation was found between S gene expression level and total human P53 level in children with ALL. In conclusion, S protein related to anti-apoptotic proteins and act as the most widely characterized drug resistance mechanisms leading to unsuccessful treatment of ALL.

77

BEST PRACTICES AND INNOVATIONS WITH POTENTIAL TO INCREASE COVERAGE OF INTEGRATED COMMUNITY CASE MANAGEMENT OF COMMON CHILDHOOD ILLNESS - UGANDA AND MOZAMBIQUE

Karin Källander[1], Agnes Nanyonjo[1], Zelee Hill[2], Guus tenAsbroek[3], Sylvia Meek[4], Betty Kirkwood[3], James Tibenderana[1]

[1]Malaria Consortium Africa, Kampala, Uganda, [2]University College London Centre for International Health and Development, London, United Kingdom, [3]London School of Hygiene and Tropical Medicine, London, United Kingdom, [4]Malaria Consortium, London, United Kingdom

During the last decade child mortality has reduced significantly in a number of African countries. Scale up of appropriate management of diarrhea, pneumonia and malaria was partly the reason behind the success. As a way of increasing access to treatment for sick children, several African countries are investing in community health workers (CHWs) to deliver integrated community case management (iCCM) for diarrhea, pneumonia and malaria. However, CHW programs have been faced with challenges of scale up while maintaining effectiveness, largely due to problems of ruptures in medicine supplies, lack of community involvement, shortfalls in training materials, lack of re-fresher training and supervision, high attrition and low performance of CHWs. The objective of this study was to identify best practices in starting up iCCM in Uganda and Mozambique and identify innovations with potential to increase coverage and improve its quality through better CHW performance and retention. A combination of methods will be used during the first 3 quarters of 2010, including consultative mapping, literature and policy document reviews, stakeholder interviews, focus group discussions and key informant interviews. Main outcomes will be an understanding of the key obstacles for regular and effective supervision; the contextual factors and ways which have an impact on CHW work motivation and satisfaction; the role of psychometric scales in measuring motivation; challenges and innovative solutions for information collection and flow; and improvements in delivery and content of iCCM training package. In conclusion, to reach the overall project goal of demonstrating that government led iCCM programs in 2 African countries can be driven to scale with quality solutions with potential to improve supervision, motivation and data use will be suggested. Innovations that have potential to address the project goal, but lack sufficient evidence of impact, will be formally evaluated through a randomized trial. The Ministries of Health will play major roles throughout the project by giving input into intervention design, participating in dissemination activities, involvement in development of implementation guidelines, supporting resource mobilization, supporting districts with regular medicine supply, and sustaining the program at national scale.

78

RENAL AND BLOOD PRESSURE LOWERING EFFECTS OF THE METHYLENE CHLORIDE-METHANOL EXTRACT OF THE STEM BARK OF *MAMMEA AFRICANA* SABINE (GUTTIFERAE)

Nguelefack-Mbuyo P. Elvine[1], Dimo Théophile[2], Nguelefack Télesphore Benoît[1], Dongmo A. Bertrand[3], Kamtchouing Pierre[2], Kamanyi Albert[1]

[1]University of Dschang, Dschang, Cameroon, [2]University of Yaounde I, Yaounde, Cameroon, [3]University of Douala, Douala, Cameroon

L-NAME induced hypertension has been associated with various renal morphological alterations. Previous studies have demonstrated that the methylene chloride-methanol (CH2Cl2-MeOH) stem bark extract of *Mammea africana* prevents the development of arterial hypertension in L-NAME treated rats. The purpose of the present work was to evaluate the effects of this extract on established hypertension and on renal function and morphology. Normotensive male Wistar rats were randomly assigned to control group (4% DMSO solution, per os for 4 or 6 weeks), L-NAME treated group (40 mg/kg/day, per os for 4 or 6 weeks), captopril group (L-NAME + captopril 20 mg/kg/day, per os) and *M. africana* group (L-NAME + M. africana 200mg/kg/day, per os). Captopril and the plant extract treatment were initiated 2 or 3 weeks after the beginning of L-NAME (40 mg/kg/day) treatment and were administered concomitantly with L-NAME for further 2 or 3 consecutive weeks. Systolic blood pressure (SBP) was recorded at baseline and at the end of every week by tail-cuff plethysmography. At the end of the experiment, urine, blood sample and kidneys were collected for creatinine clairance used as an estimation of glomerular filtration rate, plasma lipids determination and histological analysis. Captopril and M. africana significantly ($p < 0.001$) decreased blood pressure by 31.07 % and 30.59 % respectively in two weeks L-NAME hypertensive rats compared to animals receiving only L-NAME. But in 3 weeks L-NAME hypertensive rats both captopril and the plant extract failed to lower SBP. The administration of L-NAME for 6 weeks resulted in hyperlipidemia, a significant decrease ($p < 0.01$) in glomerular filtration rate, renal vascular thickness and lumen narrowing. These alterations were corrected by the plant extract demonstrating that *M. africana* is effective in managing moderate arterial hypertension and associated renal impairment. Thus it is a potential candidate for new antihypertensive drugs.

79

DENGUE HEMORRHAGIC FEVER: DIRECT COSTS AND CLINICAL FEATURES IN AN AMAZONIAN CITY IN NORTHERN PERU

Stalin F. Vilcarromero

Naval Medical Research Center Detachment, Iquitos Loreto, Peru

Despite the high prevalence of dengue virus (DENV) infection in the Amazonian region of Peru, incidence, clinical features, and economic consequences of dengue hemorrhagic fever (DHF) have not been well characterized. Herein, we describe these characteristics of the first documented cases of DHF identified from Hospital Santa Gema, in Yurimaguas, the second largest city in northeastern jungle region of Peru. Febrile patients were enrolled in our ongoing passive febrile surveillance study at which time an acute-phase blood sample was obtained. Patients with clinical alarm signs of DHF were hospitalized and monitored by medical staff and our research physician. Patient samples were processed for viral culture, RT-PCR, and ELISA IgM for recent DENV infection. A confirmed DHF case was a patient which presented with the four WHO criteria for DHF and had laboratory evidence of DENV infection. From 2005 to 2008, 1,024 febrile patients were enrolled, 37 (3.6%) of whom were suspected DHF cases. A total of 19 cases (51.4%) were confirmed as DENV infections, including 13 by virus isolation and six by IgM ELISA. Of the 13 patients confirmed by virus isolation, DENV-3 was recovered from 11. In addition there was one case of DENV-1 in 2005 and one case of DENV-4 in 2008. The principal alarming clinical sign was marked restlessness or lethargy (extreme prostration). Of the 19 confirmed DHF cases, 16 were grade II and 3 were grade III. All patients required hospitalization, one required intensive care in Lima, but no deaths were reported. The median age was 18 and 52.6% were male. The average duration of hospitalization was six days. The total direct cost for all suspected cases (bed days, laboratory tests, and radiologic tests) was $2,114.5 ($57.15 per person). The cost for confirmed cases was slightly higher ($62.30/case) than unconfirmed cases ($51.60/case), which should be put into the context of the local economy where a typical wage amounts to less than $50-60 per month. Additional costs and implications for the Health Sector will be discussed.

80

I TOLD YOU SO, WORDS OF WISDOM FROM YOUR WIFE CAN SAVE YOUR LIFE

Lhissa N. Santana, Deborah Asnis, Ibtehal Jaralla

Flushing Hospital Medical Center, Flushing, NY, United States

Fifty million people infected with dengue, the most prevalent mosquito borne viral disease each year. Most infections (90%) are asymptomatic. There are 4 related but distinct dengue viruses from the genus flavivirus. One serogroup does not offer cross protection to others. The second most common cause of fever in Western travelers to developing countries, symptoms develop 4-7 days after bite of Aedes egypti mosquito. Classic dengue fever includes headache, eye pain and severe myalgias- "breakbone fever". Exam is nonspecific, occasional macular rash. Leukopenia, thrombocytopenia and liver function abnormalities are seen. Diagnosis by serology. There is no treatment. Prevention is mosquito repellent, not bed netting, as mosquito bites during the day. Aerial spraying not effective, the mosquito breeds indoors. No vaccine is available, must contain all four serotypes. We present a case of two health care workers who returned to Trinidad with different outcomes. The wife used repellant but the husband did not. Unfortunately, he suffered the consequences. The subject was a 60 year old male complaining of severe headache, weakness and subjective fever, starting 4 days pta. Headache was L sided, constant, radiating to the neck and lower back, 8/10, no aggravating factors. Denies prior episode. No other associated symptoms. No significant medical history. Physical exam benign, except temp. of 38.5C and few petechiae on arms and legs. Initial platelet count 33 thousand only lab abnormality. On day 6 platelet count fell to 9 thousand after receiving 6 units of platelets. ALT and AST increased to 448 and 457 on day 9. Antibodies to dengue virus IgM and IgG positive at 1.2 and 7.6 (cutoff < 0.9). Patient given prednisone with improvement of platelet count.

The pathogenesis of thrombocytopenia in dengue includes: bone marrow suppression, platelet destruction, and molecular mimicry between viral protein and coagulation factors like plasminogen. Monoclonal antibodies directed to the virus bind to human fibrinogen, platelets and endothelial cells in mice. Risk factors for severe DHF include serotype 2 and "Asian" genotypes, as well as prior infection, younger age, well nourished and white. Platelet transfusion indicated in severe thrombocytopenia; anti-D immune globulin is still investigational. No adequate prophylaxis, mosquito repellant is mandatory. Our patient now heeds his wife's advice.

81

PRE-TRAVEL COUNSELING PROGRAM IN SAIPEM'S OCCUPATIONAL HEALTH DEPARTMENT FOR THE PREVENTION OF INFECTIOUS DISEASES

Vincenzo Nicosia, Massimo Consentino, Cesare Gialdi, Sabatino De Sanctis

Saipem, San Donato Milanese, Italy

Saipem, with more than 38,000 employees, is one of the biggest contractor company in oil and gas industry. The workplace is in ra emote area with a high risks of infectious diseases. The Occupational Health Department has developed a pre travel counseling program for the prevention of infectious risks. Pre travel Counseling is a fundamental step before that employee leave to worksite. He will stay for few days or for many years in a country where there are infectious risks. Employee receives information regarding the country risk through leaflets (Hepatitis A e B, Malaria, Typhoid fever, Sexual transmitted diseases, etc), health booklet for travelling workers, and all the protective means (vaccination, malaria prophylaxis). The Saipem Medical Department through qualified Occupational Health personnel, gives the employee in a properly manner, details information regarding infectious disease and the possibility to be submitted to a vaccination program before he leaves. All vaccinations are noted in his vaccination booklet and he has the possibility to receive the booster in our medical Unit in the country where he will work. In case of Malaria he will receive information for avoid mosquito bites and the correct manner to take the chemoprophylaxis with the correct drugs (Atovaquone + Proguanil, Mefloquine, Cloroquine). During his stay in the workplace, employee has the possibility to continue the counseling program. An example is our two major courses that are mandatory: sexual transmitted diseases and the malaria control program. Full implementation of prevention programs like counseling brings an added value to both the employee and the company. Statistics show a decrease of infectious diseases in our workforce since the counseling program was established and implemented.

82

EVALUATION OF THE EXTRACTS OF THE INDIAN MEDICINAL HERB *PHYSALIS MINIMA* L. FOR ANTIOXIDANT ACTIVITY AND INHIBITORY POTENTIAL AGAINST KEY ENZYMES RELEVANT TO ALZHEIMER'S DISEASE AND HYPERGLYCEMIA

Manian Sellamuthu, Loganayaki Nataraj

[1]*Bharathiar University, Coimbatore, India*

Physalis minima L. (Solanaceae) is well known in the Indian traditional medicinal system as a remedy for spleen disorder and as a tonic, diuretic and purgative. The plant is also extensively used for cancer, inflammation and diabetes. In the present investigation, the aerial part of *P. minima* was successively extracted with petroleum ether, chloroform, ethyl acetate, acetone and methanol. The extracts were investigated for *in vitro* antioxidant activities and inhibition of the key enzymes acetylcholinesterase involved in Alzheimer's disease and α- glucosidase and

α-amylase relevant to type 2 diabetes. The plant extracts had substantial concentration of total phenolics, tannins and flavonoids. In antioxidant activity assays, the acetone and methanol extracts showed the maximum reducing power and DPPH and ABTS + scavenging activities, which were highly correlated with the total phenolic contents (R2=0.9822, R2=0.8801 and R2=0.8840, respectively). In contrast, the low polar extracts such as chloroform and ethyl acetate exhibited higher levels of Fe2+ chelating ability. All the extracts were found to have a dose dependant activity in DPPH, superoxide, hydroxyl and nitric oxide scavenging, and lipid peroxidation inhibition assays. Further, the methanol and acetone extracts showed marked inhibition on the activities of acetycholinesterase and α- glucosidase whereas the ethyl acetate extract significantly inhibited the activity of α- amylase over other extracts. The results of the study will lead in favour of the use of this plant as a potential additive for the replacement of synthetic antioxidant compounds. Further, the inhibitory activity on acetylcholinesterase, α- glucosidase and α-amylase highlights its medicinal property. Isolation and characterization of the bioactive constituents from the active fractions are in progress in our laboratory.

83

WHAT WOULD PCR ASSESSMENT CHANGE IN THE MANAGEMENT OF FEVERS IN A MALARIA ENDEMIC AREA? A SCHOOL-BASED STUDY IN BENIN IN CHILDREN WITH AND WITHOUT FEVER

Jean-François Faucher[1], Agnes Aubouy[1], Patrick Makoutode[2], Grace Abiou[3], Todoégnon Béhéton[1], Justin Doritchamou[1], Pascal Houzé[4], Edgard Ouendo[2], Philippe Deloron[1], Michel Cot[1]

[1]IRD, Paris, France, [2]IRSP, Ouidah, Benin, [3]Parasitology, Cotonou, Benin, [4]Saint-Louis Hospital Biochemistry Laboratory, Paris, France

We recently showed in a school-based study in Benin, that applying a policy of anti-malarial prescriptions restricted to parasitologically-confirmed cases on the management of fever is safe and feasible. Additional PCR data were analyzed in order to touch patho-physiological issues, such as the triggering of a malaria attack or the usefulness of PCR in the management of malaria in an endemic area. PCR data were prospectively collected in the setting of an exposed (with fever) / non exposed (without fever) study design. All children had a negative RDT at baseline, were followed up to day 14 and did not receive drugs with anti-malarial activity. The index group was defined by children with fever at baseline and the control group by children without fever at baseline. Children at high risk for malaria in these two groups were defined by a positive PCR at baseline. PCR was positive in 66 (27%) children of the index group and in 104 (44%) children of the control group respectively. The only significant factor positively related to PCR positivity at baseline was the clinical status (control group). When definition of malaria attacks included PCR results, no difference of malaria incidence was observed between the index and control groups, neither in the whole cohort, nor in children at high risk of malaria. The rate of undiagnosed malaria at baseline was estimated to 3.7% at baseline in the index group. In conclusion, non malarial fevers do not or do not frequently trigger malaria attacks in children at high risk for malaria. Treating all children with fever and a positive PCR would have led to a significant increase of antimalarial consumption, with few benefits in terms of clinical events.

84

DELAYED TREATMENT IN TYPHOID PATIENTS WITH PERFORATED BOWEL IN NIGERIA: WHAT ARE THE CAUSES AND EFFECTS?

Brian S. Barnett[1], Margaret Tarpley[1], Mario Davidson[2], Daniel Gbadero[3]

[1]Institute for Global Health, Vanderbilt University School of Medicine, Nashville, TN, United States, [2]Department of Biostatistics, Vanderbilt University School of Medicine, Nashville, TN, United States, [3]Department of Pediatrics, Baptist Medical Centre, Ogbomoso, Oyo State, Nigeria

The early nonspecific features of typhoid often result in patients mistaking their illness for malaria or something less serious. This fact, along with a poorly regulated healthcare system and lack of patient knowledge concerning where and when to seek healthcare, may lead patients in Nigeria to take actions that delay appropriate treatment. It is unknown whether these actions and the ensuing delays, along with delays encountered post-presentation, impact mortality. The objectives of this study was to identify factors that cause treatment delay and determine their impact on mortality in typhoid patients with perforated bowel at Baptist Medical Centre in Ogbomoso, Nigeria (BMCO). We reviewed all charts of typhoid patients admitted to BMCO for surgical repair of perforated bowel from January 2004 to March 2009. There were 173 patients treated during that period; however, adequate records were obtained for 144 patients. These were analyzed for relationships between various treatments/factors and delayed presentation/mortality. Most patients (88%) received treatment before presenting to BMCO for surgical repair of perforated bowel. Patients received treatment from private clinics (67%) and traditional healers (8%) and also self administered pharmaceuticals (23%) and herbal remedies (5%). Eleven percent of patients reported having been treated for malaria. Associations between delayed presentation were found with receiving any pre-presentation treatment (p=0.005) and treatment at a private clinic (p=0.009). Treatment delays following presentation were due to difficulties paying the required surgical fee (19%) and obtaining blood for transfusion pre-operatively (11%) and post-operatively (5%). Having a delay in securing blood pre-operatively was associated with increased mortality (p=0.028). Increased mortality rates were also found for longer durations of that delay (p=0.037) and the presentation-surgery time interval (p=0.025). In conclusion, several factors delay treatment and impact mortality of typhoid patients with perforated bowel. Though financial hardship plays a prominent role in treatment delay, a multifaceted approach that includes education of patients and community healthcare providers; elimination of required surgical fees; and efforts to increase blood donation can ensure that patients with typhoid present for and receive proper treatment as quickly as possible.

85

CHEMOKINES AND CYTOKINES INDUCED BY MONOCYTES EXPRESSING DENGUE VIRUS NONSTRUCTURAL PROTEINS NS4B AND NS5 STIMULATE MICROVASCULAR ENDOTHELIAL CELL ADHESION MOLECULES

James F. Kelley, Pakieli Kaufusi, Esther Volper, Vivek R. Nerurkar

John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI, United States

Dengue virus (DENV) continues to spread worldwide and the incidence of dengue hemorrhagic fever (DHF) is on the rise. DHF immunopathology involves elevated levels of circulating chemokines and cytokines which stimulate the expression of adhesion molecules on vascular endothelial cells during acute infection. DENV has a plus-sense RNA genome encoding for three structural and seven nonstructural proteins (NS). Previous data demonstrated that NS5 can induce interleukin-8 (IL-8) but whether NS5 or other NS induce host immunomediators involved in endothelial cell activation remains unclear. We cloned each nonstructural gene of

the DENV type 2 New Guinea C (NGC) strain and either infected or transfected the monocytic cell line, THP-1, with the wild-type (wt) virus or DENV nonstructural plasmids, respectively. Further, we analyzed the culture supernatant for secreted immunomediators using the Luminex technology followed by incubation of UV-treated THP-1 culture supernatant with human microvascular endothelial cells (HMVEC). Changes in the expression of ICAM-1, VCAM-1 and E-selectin were measured using quantitative real-time RT-PCR (qRT-PCR) and Western blot. Our plaque assay, qRT-PCR and Luminex data demonstrated that maximum DENV titers and RNA copies in THP-1 infected or transfected cells correlated with significant secretion of IL-6, IL-8, IP-10, TNFα or INFγ. Subsequent incubation of UV-treated THP-1-infected culture supernatant with HMVEC significantly stimulated the expression of VCAM-1 and E-selectin, but not ICAM-1. Furthermore, when expressed in THP-1 cells, NS4B and NS5 induced IL-6, IL-8, IP-10 and IFNγ, which also stimulated HMVEC VCAM-1 and E-selectin production. In conclusion, we present here for the first time an *in-vitro* model consisting of a monocytic cell line that supports both wt-DENV infection and DENV NS expression as well as primary HMVEC that show variable modulation of adhesion molecules. Our results indicate that DENV NS4B and NS5 induce chemokines and cytokines that stimulate the expression of HMVEC adhesion molecules similar to that of wt-DENV infection. These findings provide insight into viral-host interactions and responses that may be exploited by therapeutic interventions to mitigate DHF.

86

INVESTIGATIONS OF DENGUE VIRUS ENTRY IN MEGAKARYOCYTE CELL LINES

Kristina B. Clark, Sansanee Noisakran, Hui-Mien Hsiao, Guey C. Perng

Emory University, Atlanta, GA, United States

Dengue virus (DV) is endemic in virtually every country in the tropics and subtropics and causes frequent outbreaks, making it one of the most important vector-borne pathogens today. It is estimated that two-fifths of the world's population is at risk of infection and about 50 million dengue infections occur every year. Though dengue normally causes a self-limiting infection, some patients may develop a life-threatening illness, dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS). Viremia and thrombocytopenia are the dominant clinical features and are well-correlated with disease severity. Despite the amount of research conducted on dengue disease, the host cell(s) accounting for viremia and cell receptor(s) for viral entry remain elusive. Previous research utilizing the rhesus macaque model indicated that DV antigen can be found in megakaryocytes. To investigate their role in the dengue life cycle, we used CHRF-288-11, a megakaryoblastic cell line, which can be stimulated into megakaryocytes and produce platelets, and K562, a progenitor erythroleukemia cell line and close relative of megakaryoblasts. The kinetics of dengue viral RNA amplification within these cell lines were determined with DV2 by quantitative real-time RT-PCR. K562 is capable of supporting dengue virus replication, whereas CHRF-288-11 is less permissive for dengue virus replication. Our hypothesis is that dengue virus may enter progenitor cells and differentiate them into their preferred cell type. Future investigations will focus on the binding and entry into these cell lines using reporter DV VLPs (virus like particles). These will be constructed by transfecting a plasmid containing structural genes of dengue 16881 into K562 cells, which stably express EGFP-DV replicon. These EGFP-DV VLPs will allow us not only to distinguish which cells could be infected but also to dissect which receptor(s) are responsible for binding and entry.

87

DENGUE IN RURAL NORTHERN COASTAL ECUADOR

Meghan Milbrath

University of Michigan School of Public Health, Ann Arbor, MI, United States

Dengue is increasingly prevalent in rural areas, but the factors that drive transmission in these settings remain largely unknown. An unpublished pilot study completed in 2007 demonstrated high prevalence of dengue antibodies in the population living in remote villages in north coastal Ecuador, and confirmed that *Aedes aegypti* is present in these villages. In the last two years, the first two cases of DHF in the area were recorded at the local hospital, indicating that dengue is a serious concern in this region. Construction of a new highway may affect dengue transmission in this area through increased travel to coastal urban areas (known centers of dengue transmission), or through environmental change, creating a vector population sufficient to sustain local transmission. We examine the effect of this highway on dengue in eight communities with varying levels of road access, all part of a larger epidemiologic study. The objective of this project is to isolate and sequence dengue virus from mosquitoes and from whole blood and serum samples from suspected and laboratory confirmed clinical dengue cases in the area, providing data on circulating dengue strains. Viral presence in mosquitoes and homology to virues isolated from clinical specimens confirms local transmission in the villages. Conversely, low sequence homology or lack of mosquito infection supports the hypothesis that infections are acquired elsewhere, linking current incidence to human movement patterns. Sequence data are also compared to reported sequences circulating elsewhere in South America. Molecular data complement the migration, epidemiological, and serological investigations in this region, providing a clearer picture of the sources and risk factors of dengue infection. This information can be used to develop effective and cost-efficient interventions that can reduce disease associated morbidity and mortality and preserve the health of the people in this region.

88

INVESTIGATING DENGUE VIRUS SEROTYPE-SPECIFIC BIOMARKERS VIA MALDI-TOF/TOF

B. K. Poole-Smith[1], Momar Ndao[2], Brian Ward[2], Elizabeth Hunsperger[1]

[1]CCID/Centers for Disease Control and Prevention/DZVED/DVBID/DB, San Juan, PR, United States, [2]McGill University, Montreal, QC, Canada

Dengue is the most important viral vector-borne disease and more than 2.5 billion people are at risk for dengue infection, with 50 million infections occurring annually in over 100 tropical and sub-tropical countries. At least 500,000 people are hospitalized annually for dengue hemorrhagic fever (DHF), a more severe form of the disease, with fatality rates exceeding 20% in the absence of appropriate treatment. The onset of DHF occurs late in course of disease after the patient's fever subsides and the patient is sent home from the hospital and away from vital supportive therapies. Current diagnostic methods cannot predict which cases develop into DHF. Early identification of cases at risk for DHF at point of care (POC) could reduce mortality. Matrix assisted laser desorption/ionization time of flight time of flight (MALDI-TOF/TOF) is a technique which can be used to analyze the role of proteins in healthy and disease states and discover biomarkers which can be used to develop POC diagnostics. MALDI-TOF/TOF has been successfully used in the discovery of host biomarkers for cardiac disease and cancer and we have already used this technique to identify serum biomarkers which distinguish between DF and DHF. To aid development of a POC diagnostic test, we investigated dengue serotype-specific variation in biomarkers by screening serum from laboratory-confirmed cases of dengue virus serotypes 1-4 via MALDI-TOF/TOF. Preliminary results suggest no serotype-specific variation in DF biomarkers which may be important to incorporate in our previous studies

for severity of disease and aid the development of serotype specific DHF diagnostic tests. These results can be used to further develop diagnostic assays for point-of-care tests for clinicians.

89

VACCINATION AND HOMOTYPIC IMMUNITY RESTRAINS EMERGENCE POTENTIAL OF SYLVATIC DENGUE VIRUS TYPE 4 IN THE URBAN TRANSMISSION CYCLE

Nikos Vasilakis[1], Anna P. Durbin[2], Sandra V. Mayer[1], Shannan L. Rossi[1], Josefina Duran-Bedolla[3], Jose Ramos-Castaneda[3], Eng E. Ooi[4], Jane M. Cardosa[5], Jorge L. Munoz-Jordan[6], Robert B. Tesh[1], Scott C. Weaver[1], Mark D. Estes[1]

[1]University of Texas Medical Branch, Galveston, TX, United States, [2]John Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]Instituto Nacional de Salud Publica, Cuernavaca, Mexico, [4]NUS/Duke, Singapore, Singapore, [5]Universiti Malaysia Sarawak, Kota Samarahan, Malaysia, [6]Centers for Disease Control and Prevention, San Juan, PR, United States

Sylvatic dengue viruses (DENV) are both evolutionary and ecologically distinct from urban DENV. Sylvatic DENV are maintained in an enzootic transmission cycle most likely between non-human primates and arboreal *Aedes* spp. mosquitoes. Recent evidence from West Africa and Southeast Asia suggest that sylvatic DENV come into regular contact with humans, where *Ae. furcifer* and *Ae. albopictus* respectively, may act as bridge vectors between forest and peridomestic habitats. The ability of sylvatic DENV to emerge into an urban transmission cycle may limit the potential for eradicating dengue with vaccines currently in late stages of development. Here we assessed the likelihood of sylvatic DENV-4 emergence in the face of natural immunity to current endemic strains and to two vaccine candidates. Our data, based on the capacity of primary DENV-4 infection sera from convalescent patients and vaccinees to neutralize both endemic and sylvatic DENV-4 strains, indicate homotypic cross-immunity but limited heterotypic immunity. Therefore, emergence of sylvatic strains into an urban cycle would appear to be limited by homotypic immunity.

90

SEROPREVALENCE OF DENGUE FEVER IN U.S. ARMY SPECIAL OPERATIONS FORCES - INITIAL RESULTS AND THE WAY FORWARD

Jennifer B. Caci[1], Arthur Lyons[2], Danielle M. Tack[3]

[1]United States Army Special Operations Command, Fort Bragg, NC, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

Worldwide endemicity of dengue is increasing, making it a threat of particular concern to our United States Army Special Operations (USASOC) soldiers. These personnel routinely deploy to areas with multiple dengue serotypes, potentially increasing the risk of more severe disease manifestations and loss or degradation of operational capability. Since October 2008, at least nine USASOC soldiers have contracted dengue; four with multiple serotypes. We hypothesized that many more had been exposed. To characterize the risk, we initiated a seroprevalence study using approximately 500 samples from the DoD Serum Repository. The first stage of the study focused on personnel who served in the dengue endemic areas for at least 30 days from 2006 through 2008. An 11**%** seroprevalence rate, (determined using a sandwich ELISA procedure) confirmed our hypothesis, provides us with an epidemiologic baseline in our population, helps guide medical threat planning, and drives development of countermeasures while highlighting concerns about the introduction of dengue into non-endemic areas.

91

EVALUATION OF A RAPID ASSAY FOR DETECTION OF DENGUE EARLY MARKER NONSTRUCTURAL PROTEIN 1 (NS1)

Scott Fry[1], Matthew Semple[1], Michelle Meyer[1], Catriona McElnea[1], Paul Young[2], Andrea Valks[1], **Chang-Yi (Charlie) Huang**[1]

[1]Alere, Sinnamon Park, Brisbane, Australia, [2]The University of Queensland, St. Lucia, Brisbane, Australia

Dengue is a flavivirus found largely in areas of the tropics and sub-tropics, placing over half of the world's population at risk. Traditional methods for diagnosis of dengue include detection of serological markers such as IgM and IgG antibodies to dengue. Dengue NS1 is an early marker which can be detected in serum on day 1 after onset of fever and up to day 9. In comparison, an IgM response is not detectable until days 3 to 5. Rapid detection of dengue NS1 antigen is a valuable procedure, as it allows detection of infection prior to seroconversion. Early diagnosis of dengue allows promote implementation of supportive therapy and monitoring which reduces risk of severe complications such as Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS), especially in dengue endemic countries. The Panbio Dengue Early Rapid detects dengue NS1 in sera. The dipstick test was evaluated at two reference laboratories in South-East Asia. The studies used samples from acute fever patients (days 1 to 9 post onset of illness) characterised for dengue by a combination of PCR, virus culture, hemagglutination inhibition (HAI) and IgM and IgG ELISA. Positive samples were representative of primary or secondary infections with different serotypes. The results reported from the two sites were 71.1%, 68.9% sensitivity and 96.0%, 96.7% specificity respectively. Importantly, when combined with the Panbio Dengue Duo Cassette (IgM and IgG), the overall sensitivity increased to 93.5%. These results demonstrate that the Panbio Dengue Early Rapid is a valuable tool in the early diagnosis of dengue.

92

MOLECULAR EPIDEMIOLOGY OF DENGUE 2 VIRUS IN PERU

Cristhopher D. Cruz[1], Vidal Felices[1], Patricia V. Aguilar[1], Carolina Guevara[1], Manuel Cespedes[2], Victor Suarez[2], Luis Suarez[3], Tadeusz J. Kochel[1], Eric S. Halsey[1]

[1]Naval Medical Research Center Detachment, Lima, Peru, [2]National Institute of Health, Lima, Peru, [3]Directorate of Epidemiology, Lima, Peru

Dengue (DEN) virus is a member of the family *Flaviviridae*, genus *Flavivirus*, and is responsible for more than 50-100 million cases annually throughout the world. Dengue infection is caused by four different dengue serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) and the clinical spectrum of disease ranges from dengue fever, dengue hemorrhagic fever (DHF), dengue shock syndrome, and death. Epidemiological information has linked the development of DHF with secondary dengue infections and has also suggested that certain DEN strains are more virulent than others. In 1995, the first cases of DEN-2 were detected in Iquitos and later spread to other areas in Peru; however, despite intensive surveillance efforts, no DHF cases were recognized. In 2001, the first DHF cases associated with DEN-2 virus were recognized in the coastal region of Peru. To investigate the genetic diversity of DEN-2 strains circulating in Peru before, during, and after the appearance of DHF cases, RNAs extracted from 58 viruses isolated in C6/36 (*Aedes albopictus*) cells were performed by RT-PCR. The envelope (E) and E/NS1 gene junction sequences were determined and used in a phylogenetic comparison with a global sample of DEN-2 viruses. Phylogenetic analysis revealed the circulation of two genotypes in Perú: American and American/Asian. Interestingly, the DEN-2 strains circulating prior to the reports of DHF only belong to the American genotypes whereas the DEN-2 strains isolated during and after 2000 belong primarily to the American/Asian genotype. Although clinical information is lacking for some of the cases included in the study, our results support previous findings of association of DHF with DEN-2 American/Asian genotype and highlight the need for continuous monitoring for the emergence of new DEN genotypes that may be associated with severe disease.

93

FOR WHAT IS A DENGUE VIRUS FIT?

Nahid Choudhury, **John Aaskov**

Queensland University of Technology, Brisbane, Australia

While phylogenetic analyses have demonstrated significant variation between the consensus sequences of dengue virus (DENV) populations, there is much less information about the, perhaps more relevant, variation within populations of DENV in natural hosts - humans and mosquitoes. The limited genetic data which is available for intra-DENV population variation has shown most genomes to contain changes/defects which would be expected to render the genome non-infectious. While these population studies also have identified recombinant DENV genomes, it is unclear whether these genomes are infectious or whether they are fit enough to enter natural cycles of transmission. The fitness of a DENV might be considered to have two major components, the ability to infect cells in an host and the yield of virus from infected cells. We have found that the C6-36 mosquito cell line is orders of magnitude more sensitive to infection with DENV 1 than human cell lines which are used commonly for the study of DENV (e.g. HuH7, HepG2, K562) such that some of the human cell lines could not be infected with these viruses. All cultures of C6-36 cells which could be infected with one infectious dose of DENV 1 released virions into the culture supernate which could be detected either in direct or virus-capture ELISAs. While all cultures achieved peak virus production by 8-10 days after infection, the amount of virus produced by each culture varied. Fewer than 10 percent of cultures infected with one infectious dose of DENV 1 produced more virus than cultures infected with undiluted (10^3 - 10^5 TCID) parental virus. This is interpreted as showing that most members of DENV 1 populations are less fit than the population as a whole. This could be interpreted as evidence for extensive complementation between members of DENV populations in host cells. It also suggests strategies that might be employed to enhance the effectiveness of the dengue vaccines currently under trial.

94

PAN-SEROTYPE SEQUENCE-SPECIFIC DETECTION OF DENGUE VIRUS USING LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

Allison Dauner, Sajeewane Ekanayake, Subhamoy Pal, Tadeusz Kochel, Shuenn-Jue Wu

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

Dengue fever is one of the world's most common infectious diseases. Traditional laboratory diagnostic methods for dengue virus (DENV) such as virus isolation are labor-intensive and time-consuming. Polymerase Chain Reaction (PCR)-based diagnostic assays have proven to be very useful for accurate diagnosis of acute infectious diseases in a laboratory setting. Functioning similarly to PCR, loop-mediated isothermal amplification (LAMP) is a promising new technology that allows for rapid amplification of RNA or DNA. Unlike PCR, this technology does not require costly thermocyclers, and the results can be visualized using the naked eye. In this study, we investigated the feasibility of detecting all four serotypes of DENV utilizing a single set of degenerate primers. We successfully developed a reverse transcription (RT)-LAMP assay capable of detecting all serotypes of DENV in less than 60 minutes. We have also evaluated our assay using a panel of clinical samples obtained from either DENV-positive febrile patients or normal human sera and observed 86% sensitivity and 94% specificity. We have occasionally observed non-specific amplification in our assay, and have successfully implemented a novel technique designed to eliminate this problem. Using the re-designed assay, we have compared the limit of detection of our RT-LAMP to that of a popular DENV RT-PCR assay, using DEN3 as a model. Both assays were capable of detecting virus down to 10-100 copies/rxn. The ease and relative cost-effectiveness of this RT-LAMP assay makes it a promising candidate for point-of-care use, thereby reducing the time between symptom presentation and accurate diagnosis.

95

DENGUE VIRUS CROSS-PROTECTIVE IMMUNITY IN SHAPING HETEROGENEOUS SEROTYPE EPIDEMIC CIRCULATION PATTERNS, A STUDY OF DENGUE VIRUS TYPE 1 AND 4

Chonticha Klungthong, Ananda Nisalak, Butsaya Thaisomboonsuk, Napaporn Latthiwongsakorn, Yongyuth Poolpanichupatam, Taweewun Hunsawong, Robert V. Gibbons, Richard G. Jarman

United States Army Medical Component-Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Mathematical modeling has postulated that the presence of moderate cross-protective antibodies from dengue virus type 4 (DENV-4) infected people may shape DENV-1 circulation resulting in alternating epidemic patterns of these two DENV serotypes circulating in Bangkok between 1973-2002. Previous phylogenetic analyses linked to the epidemic cycle also indicated that the DENV-1 clade extinction and replacement was the consequence of the DENV-4 cross-immunity selective pressure. In this study, we used the plaque reduction neutralization test to investigate if late convalescent (6 and 12 months) serum from 5 people infected with DENV-4 between 1994-1995 could cross neutralize 23 DENV-1 isolates representing both the modern existing (1990-2002) and the older extinct (1980-1994) DENV-1 clades. The results revealed that the antibody from known past DENV-4 infections exhibited varying levels of cross-reactivity against DENV-1 isolates compared with late convalescent DENV-1 infected control serum. Serum from one DENV-4 sample showed a marked increase in neutralization while the remaining 4 were reduced compared with the DENV-1 control serum. There was no measurable neutralization difference between the modern and extinct DENV-1 isolates. The small number DENV-4 infected people during this time frame and these results suggest that cross-immunity from DENV-4 infections have less effect on DENV-1 prevalence and/or its clade extinction and replacement. In addition, the replication rate of each serotype might be a factor effected to the epidemic pattern. Our examination on the replication rate of the wild type isolates from this period in *Aedes aegypti* mosquitoes showed a low DENV-4 replication rate in single, dual, and multiple infections.

96

UPDATE ON THE PHASE III STAGE SANOFI PASTEUR RECOMBINANT TETRAVALENT DENGUE VACCINE

Jean Lang, Melanie Saville, Bruno Guy

sanofi pasteur, Marcy l'Etoile, France

The development of the Dengue tetravalent (TV) recombinant YF17D-based chimeric vaccine has been continuously driven by a benefit/risk evaluation and mitigation strategy. Earlier pre-clinical studies have demonstrated that the sanofi pasteur dengue vaccine is genetically and phenotypically stable, non-hepatotropic, less neurovirulent than YF17D and does not infect mosquitoes by the oral route. *In vitro* and *in vivo* preclinical studies also show that the TV dengue vaccine induces controlled stimulation in human dendritic cells, and has significant immunogenicity in monkeys. Results of Phase II trials in the USA, the Philippines and Mexico show that the majority of adverse events are mild to moderate and transient in nature. Observed Viraemia is transient and low, and does not increase after the initial dengue TV administration, even in the case of incomplete responses. PRNT50 seroconversion ranges between 80 and 100% for all four serotypes in subjects injected with 2 to 3 doses of TV dengue vaccine. Responses have been monitored at the cellular level in humans: Th1 and CD8 responses are induced with an IFN-γ/TNF-α ratio favoring IFN-γ. A worldwide clinical development program for dengue TV is underway including completion of the enrollment of 4000 school-age children (4-11 years) in an efficacy trial in Thailand and the planned evaluation of industrial scale clinical lots in Phase III by year end. Assuming continued successful outcomes, initial submission to national regulatory authorities can now be considered within a 5-year period.

97

HIGH INCIDENCE OF PERIPHERAL BLOOD PLASMACYTOSIS IN PATIENTS WITH DENGUE VIRUS INFECTION: A PROSPECTIVE STUDY

Khoa T. Thai, Josta A. Wismeijer, Catrien Zumpolle, Menno D. de Jong, Marie José Kersten, Peter J. de Vries

Academic Medical Center, Amsterdam, The Netherlands

One of the characteristic features of dengue virus (DENV) infection is the occurrence of leucopenia and thrombocytopenia, probably resulting from virus induced bone marrow suppression. Despite the general bone marrow suppression, polyclonal peripheral blood plasmacytosis has occasionally been described in DENV infected patients. The frequency of blood plasmacytosis in patients with dengue infection, the origin of these plasma cells (PCs) and the mechanisms by which they appear in the blood are not known. We initiated this prospective observational study to quantify and describe the kinetics and phenotype of peripheral blood plasma cells (PCs) in these patients. Morphological examination and flow cytometric (FC) analysis for the characterization and immunophenotyping of lymphocyte subsets and PCs were performed in 35 and 31 patients suspected of DENV infection, respectively. Our results show that blood plasmacytosis is a very common hematological finding. Depending on the days of illness at presentation, blood plasmacytosis was observed in 64% to 73% of patients. Blood plasmacytosis was the most pronounced before 7 days of illness and declined rapidly thereafter, to completely disappear after 14 days of illness. Blood plasmacytosis was higher in secondary DENV infection. The majority of $CD138^+$ PCs (89%) had a shared immunophenotype ($CD45^+/CD19^-/CD56^-$) and in all cases the PCs were polyclonal. In conclusion, blood plasmacytosis is an unusual hematological finding that is most commonly seen in plasma cell leukemia or advanced stage multiple myeloma. However, blood plasmacytosis, characterized by a transient presence of polyclonal PCs in the circulation, is a common event in DENV infection. Blood PCs may play a role in the humoral immune response to and pathogenesis of dengue.

98

DENGUE DYNAMICS IN BINH THUAN PROVINCE, SOUTHERN VIETNAM: PERIODICITY, SYNCHRONICITY AND CLIMATE VARIABILITY

Khoa T. Thai[1], Bernard Cazelles[2], Nam V. Nguyen[3], Long T. Vo[4], Maciej F. Boni[4], Jeremy Farrar[4], Cameron P. Simmons[4], H. Rogier van Doorn[4], Peter J. de Vries[1]

[1]Academic Medical Center, Amsterdam, The Netherlands, [2]UMR 7625, UPMC-CNRS-ENS, Ecole Normale Supérieure, Paris, France, [3]Binh Thuan Medical College, Phan Thiet City, Vietnam, [4]Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam

Dengue is a major global public health problem with increasing incidence and geographic spread. The epidemiology is complex with long inter-epidemic intervals and endemic with seasonal fluctuations. This study was initiated to investigate dengue transmission dynamics in Binh Thuan province, southern Vietnam. Wavelet analyses were performed on time series of monthly notified dengue cases from January 1994 to June 2009 (i) to detect and quantify dengue periodicity, (ii) to describe synchrony patterns in both time and space, (iii) to investigate the spatio-temporal waves and (iv) to associate the relationship between dengue incidence and El Niño-Southern Oscillation (ENSO) indices in Binh Thuan province, southern Vietnam. We demonstrate a continuous annual mode of oscillation and a multi-annual cycle of around 2-3-years was solely observed from 1996-2001. Synchrony in time and between districts was detected for both the annual and 2-3-year cycle. Phase differences used to describe the spatio-temporal patterns suggested that the seasonal wave of infection was either synchronous among all districts or moving away from Phan Thiet district. The 2-3-year periodic wave was moving towards, rather than away from Phan Thiet district. A strong non-stationary association between ENSO indices and climate variables with dengue incidence in the 2-3-year periodic band was found. In conclusion, multi-annual mode of oscillation was observed and these 2-3-year waves of infection probably started outside Binh Thuan province. Associations with climatic variables were observed with dengue incidence. Here, we have provided insight in dengue population transmission dynamics over the past 14.5 years. Further studies on an extensive time series dataset are needed to test the hypothesis that epidemics emanate from larger cities in southern Vietnam.

99

DEFINING DETERMINANTS OF ANTIBODY PROTECTION AND ENHANCEMENT IN A NOVEL MOUSE MODEL OF DENGUE VIRUS INFECTION AND DISEASE

Katherine L. Williams[1], Ruben Lachica[1], Wahala Wahala[2], Soila Sukupolvi-Petty[3], Martina Beltramello[4], Antonio Lanzavecchia[4], Federica Sallusto[4], Aravinda de Silva[2], Michael S. Diamond[3], P. Robert Beatty[1], 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 Microbiology and Immunology, University of North Carolina, Chapel Hill, Chapel Hill, NC, United States, [3]Departments of Medicine, Pathology and Immunology, and Molecular Microbiology, Washington University in St. Louis, St. Louis, MO, United States, [4]Institute for Research in Biomedicine, Bellinzona, Switzerland

The development of a mouse model to study dengue virus (DENV) infection and disease has provided the opportunity to pursue investigations of antibody determinants of protection and enhancement. Using an AG129 mouse model that reproduces lethal antibody-dependent enhancement (ADE) as well as antibody-mediated protection, we first showed that the interaction of the Fc portion of the antibody-virus complex with Fcγ receptors on target cells is required for ADE *in vitro* and *in vivo*. Now we are dissecting the role of DENV antibodies at the envelope (E) protein domain- and epitope-specific levels using both human and mouse sera and monoclonal Abs (MAbs). To address the contribution of specific antibodies to protection and enhancement of DENV infection, we have depleted murine anti-DENV serum of Domain (D) III-specific Abs. *In vitro* analysis indicates a 75% reduction in functional neutralization capacity upon EDIII-antibody depletion. The effect of transferring to mice intact, control depleted, and EDIII-depleted sera adjusted to equal neutralizing titer followed by DENV challenge will be reported. To further dissect the role of E-specific antibodies, MAbs of different epitope specificities and neutralization capacity were selected to identify the relative importance of each component in modulating disease enhancement or protection. Initial characterization of both murine and human MAbs indicates that MAbs with higher neutralization titer are more effective at neutralizing a sub-lethal DENV infection and less efficient at enhancement than less neutralizing MAbs with comparable epitope specificity, thus indicating an important role for neutralization titer. We have also shown that genetically engineered mouse and human MAbs incapable of interacting with the Fcγ receptor can be therapeutic in mice and prevent ADE-mediated disease when administered after lethal challenge. Analysis of different types of MAbs in this assay has revealed that modified serotype-cross-reactive mAbs directed to the EDII fusion loop are protective ($p<0.05$) and significantly reduce viral load, and that therapeutic neutralizing potential may be predicated upon epitope specificity. These studies are elucidating information about how specific E protein domains and epitopes contribute to protection or enhancement of dengue disease that should be useful for development of safe and effective dengue vaccines and antibody-based therapeutics.

100

UNUSUAL DENGUE VIRUS 3 EPIDEMIC IN NICARAGUA, 2009-2010

Gamaliel Gutierrez[1], Oscar Ortega[1], Federico Narvaez[2], Maria Angeles Pérez[2], Katherine Standish[1], Douglas Elizondo[1], Andrea Nuñez[3], Guillermina Kuan[4], Angel Balmaseda[3], **Eva Harris**[5]

[1]Sustainable Sciences Institute, Managua, Nicaragua, [2]Hospital Infantil Manuel Jesús de Rivera, Managua, Nicaragua, [3]Departamento de Virologia, Centro Nacional de Diagnostico y Referencia, Ministerio de Salud, Managua, Nicaragua, [4]Centro de Salud Socrates Flores Vivas, Managua, Nicaragua, [5]University of California, Berkeley, Berkeley, CA, United States

Dengue has increased in incidence and severity in the Americas over the past 30 years. In 2009-10, Nicaragua experienced the largest dengue epidemic in over a decade, marked by unusual clinical presentation, as observed in two prospective studies of pediatric dengue in Managua. Between August 2009 and January 2010, 220 dengue cases were confirmed among 396 study participants at the National Pediatric Reference Hospital; overall, 5-10 times more suspected dengue was seen there than in prior years. In our pediatric cohort study, an incidence of 4.4% was observed (168 dengue cases) in the 2009-10 dengue season, compared to 0.4-1.9% in 2004-8 (13-65 cases). DENV-3, the predominant serotype in Managua since 2008, caused 89.4% (hospital) and 83.2% (cohort) of cases. In the hospital study, 24% of subjects were transferred to intensive care compared to 5-11% in 2005-8. Although fewer hospital cases in 2009-10 were classified as DHF/DSS (13.6% vs 21-61% in 2005-8), "compensated shock" (≥2 of: cold extremities, poor capillary refill, tachycardia, tachypnea, rapid pulse) was observed in more cases (37.3% vs 9-14.6%). Signs of poor perfusion presented 1-2 days earlier in 2009-10 than 2005-8, but generally did not progress to hypovolemic shock, possibly due to early IV fluid therapy. In Kaplan-Meier survival analysis, "compensated shock", slow capillary refill, and cold extremities presented significantly earlier in 2009 after adjusting for day of presentation to the hospital ($p<0.005$). Similar results were obtained in the cohort. Several hypotheses are now being tested to explain the high incidence and distinct clinical presentation of dengue in 2009-10. Preliminary results of full-length sequencing of DENV-3 do not reveal a major shift in clade between 2008 and 2009, though further sequencing is underway. Due to a pandemic influenza A H1N1 epidemic just before the dengue epidemic, levels of antibodies to H1N1pdm antigen in cohort dengue cases are being tested to determine whether recent influenza infection modulated subsequent dengue cases. The effects of case management and prior DENV infection on disease progression are also under analysis. Finally, DENV antibodies in paired annual blood samples from cohort subjects will be analyzed to determine the incidence of overall DENV transmission in 2009-10 compared to previous years. These studies should improve our understanding of determinants of the varied disease manifestations of dengue.

101

RESULTS OF BASELINE ASSESSMENTS OF COMMUNITY CASE MANAGEMENT SUPPLY CHAINS IN MALAWI AND ETHIOPIA

Tim Williams, Sarah Andersson, Yasmin Chandani, Barbara Felling, Gregory Roche, Michelle Pahl, Alexis Heaton, Megan Noel

JSI, Arlington, VA, United States

Slow progress in effectively treating childhood diseases has influenced governments, NGOs, and donors to adopt new approaches to delivering life-saving health services for young children. Community case management (CCM) is one promising approach which aims to reach children and reduce childhood mortality in underdeveloped areas by training community health workers (CHWs) to treat common childhood illnesses at the community level. Evidence suggests, however, that inconsistent supplies pose important constraints to CCM programs, often adversely affecting outcomes. The Improving Supply Chains for Community Case Management (SC4CCM) Project aims to demonstrate that supply chain constraints at the community level can be overcome, potentially yielding significant improvements in CCM effectiveness and impact. SC4CCM will introduce innovative approaches to strengthen supply chains and effectively deliver key drugs to CHWs, but there is little knowledge in most countries regarding current supply chain practices at the community level. In Malawi and Ethiopia, the first two SC4CCM focus countries, baseline assessments will be carried out during early 2010 to determine current conditions and provide evidence for effective interventions. Methodology will include physical counts of CCM products at CHWs and their resupply points, and structured interviews with CHWs and other stock managers. Experimental and control districts will be selected purposefully, with health facilities sampled randomly within selected districts, and 2-3 CHWs selected randomly per selected facility. The presentation will describe baseline findings, focusing on supply chain strengths and weaknesses, how findings inform program planning and innovations, how interventions will be tested, and potential applications for other settings.

102

USING A THEORY OF CHANGE MODEL TO IMPROVE SUPPLY CHAINS FOR COMMUNITY CASE MANAGEMENT IN RESOURCE LIMITED SETTINGS

Sarah Andersson, Yasmin Chandani, Barbara Felling, Alexis Heaton, Megan Noel, Michelle Pahl, Gregory Roche, Timothy Williams

JSI, Arlington, VA, United States

The Improving Supply Chains for Community Case Management of Pneumonia and Other Common Diseases of Childhood (SC4CCM) project has designed a theory of change (TOC) model that clearly maps out the project's goals and objectives and the steps it will take to achieve these. Community case management (CCM) is an approach designed to reduce childhood mortality by reaching more children in resource limited settings, however evidence shows that CCM is hampered by inconsistent availability of appropriate, quality and affordable medicines. The SC4CCM project aims to demonstrate in four sub-Saharan African countries that supply chain constraints at the community level can be overcome. The SC4CCM TOC model identifies the distinct building blocks that, in combination, comprise an effective supply chain for reaching community health workers in remote and isolated areas. It links interventions, assumptions and indicators to final outcomes and provides a roadmap for measuring and institutionalizing change. While the building blocks are relevant for all supply chains for CCM commodities, the individual interventions and targets for each indicator will be country-specific. The TOC framework will be used to develop country specific implementation and evaluation plans. The SC4CCM TOC will provide an iterative framework that will guide progress while being continuously refined over the course of the project. The final outcome will be a TOC model that can be scaled up and applied to other CCM projects or programs to improve availability of the appropriate quality, affordable commodities at the community level and other levels of the health system.

103

LEAD IS UNIFORMLY DETECTED IN A SAMPLE OF CHILDREN ATTENDING OUTPATIENT PEDIATRIC CARE IN ASMARA

Fitsum G. Debretsion

Post Graduate Medical Education Programme, Asmara, Eritrea

The objective of this study was to determine the prevalence of blood lead levels in Eritrean children. Cross sectional study of 120 children aged 12-71 months oldstaken from a convenience sample of non-seriously ill children presenting to the Orotta Children's Hospital outpatient department during August 2009. After obtaining informed consent from a parent, whole blood was collected from a peripheral vein for blood lead level (BLL) and complete blood count (CBC) determination. The BLL was

performed at the Mayo Clinic sponsored by Washington University in St. Louis, Missouri, while the CBC analysis was performed at the National Health Laboratory of Eritrea in Asmara, Eritrea. Lead was detected in all blood specimens. Mean BLL was 5.0 + 2.9 mcg/dL, median 4.0 mcg/dL, with a range of 0.5 to 16 mcg/dL. There was a significant and direct relationship between BLL and hemoglobin level, with higher levels of hemoglobin found at higher levels of BLL (95% CI 0.01 to 0.62; p=0.043). Conversely there was a significant negative relationship between erythrocyte mean corpuscular volume (MCV) and BLL (CI -0.12 to -0.002 p=0.042), with higher BLL associated with lower MCV. In conclusion, lead was uniformly detected in blood of Eritrean children aged 1 to 5 years old. Further study of environmental causes for BLL is warranted. These findings suggest the need to make the public, government organizations, and health professionals aware of the risk factors for and prevention strategies to mitigate lead exposure.

104

REASONS FOR BREAST FEEDING TERMINATION IN A POOR PERIURBAN COMMUNITY IN THE DOMINICAN REPUBLIC

Danielle L. Cyr, John D. McLennan

University of Calgary, Calgary, AB, Canada

Longer duration of breastfeeding plays an important role in improving child health and reducing mortality. However, breastfeeding length is often suboptimal. Further information is needed on factors impeding longer duration of breastfeeding to inform health promotion efforts aimed at improving the length of breastfeeding. The aim of this study was to determine reasons for breastfeeding termination of mothers in a poor periurban community in the Dominican Republic using a mixed questioning approach. A structured questionnaire was administered through an interview with all mothers or alternative caregivers of children under the age of five who participated in a growth-monitoring program in a Haitian Batey in the Dominican Republic. Reasons for breastfeeding termination were obtained through a combination of an open-ended question and a series of close-ended questions, which had been formulated from previous research on breastfeeding in the Dominican Republic. Of 132 participants, 79 (60%) had terminated breastfeeding prior to the interview. Of this subgroup, 19% terminated breastfeeding at less than six months and 65% terminated breastfeeding at less than two years. A new pregnancy (22%) was the most common reason for breast feeding termination in response to the open-ended question. Of the ten closed-ended questions, "it was time" to stop breastfeeding was the most frequently endorsed (46%). Of those who terminated at less than six months, the most commonly endorsed reason was that the child did not want the breast (53%). In conclusion, breastfeeding duration in this community is suboptimal. Use of different styles of questions identified different sets of breastfeeding termination reasons, which may better inform health promotion efforts aimed at increasing breastfeeding duration.

105

INHERITED BLOOD DISORDERS: AN ONLINE DATABASE OF ALLELE FREQUENCIES TO REFINE HEALTH METRICS

Fred B. Piel[1], Rosalind E. Howes[1], Oscar A. Nyangiri[2], Anand P. Patil[1], Peter W. Gething[1], Thomas N. Williams[3], David J. Weatherall[4], J. Kevin Baird[5], Simon I. Hay[1]

[1]University of Oxford, Oxford, United Kingdom, [2]KEMRI Wellcome Trust, Kilifi, Kenya, [3]KEMRI Wellcome Trust, Kilifi, United Kingdom, [4]Weatherall Institute, Oxford, United Kingdom, [5]Eijkman Institute, Jakarta, Indonesia

A good knowledge of the spatial distribution of diseases is crucial for guiding efficient public health policy. Inherited blood disorders include both the commonest monogenic diseases (haemoglobin structural variants and thalassaemias) and the most common human enzyme defect (G6PD deficiency) worldwide. Their impact on public health is growing both in developed countries due to human migrations and in developing countries due to epidemiological transitions. Nevertheless, our knowledge about their current distribution and burden remains very poor, even in developed countries like the U.S. In order to improve the current situation, inherited blood disorders have recently been included in the Global Burden of Disease Program. The public health significance of sickle cell disease was also acknowledged last year, with the United Nations declaring a World Sickle Cell Day. Using a similar approach to the one used for *Plasmodium falciparum* and *P. vivax*, the Malaria Atlas Project has started creating a global open-access comprehensive database of inherited blood disorder allele frequencies. This database focuses on the most prevalent disorders selected for their malaria protective mechanism: haemoglobin S (HbS), haemoglobin C (HbC), glucose-6-phosphate dehydrogenase (G6PD) deficiency, South-East Asian ovalocytosis and Duffy negativity. The database will also ultimately include the thalassaemias and haemoglobin E (HbE). In an effort to help improve health metrics, especially in the developing world, this database, combined with model-based geostatistics and high resolution population data, allows the development of global contemporary maps for each of these disorders, and the refining of burden estimates. The development of such a resource highlights the difficulties in accessing some of the existing data and also flags up those areas from which data are really lacking.

106

SOCIOECONOMIC INEQUALITY IN UNDER FIVE MORTALITY: EVIDENCE FROM NAVRONGO DSS

Mahamadou M. Ndiath

UCAD, Dakar, Senegal

Improving the health of the poor and reducing health inequalities between the poor and non-poor has become central goals of certain international organizations like World Bank and WHO, including national governments in the contexts of their domestic policies and development assistance programmes.

There are also unquantified and poorly understood inequalities in access to health services within and between various population groups. Little is known about the factors that determine these inequalities and the mechanisms through which they operate in various sub-groups. The aim of the study was to first to describe under-five mortality trend according to wealth index, second to describe risk factors for under five mortality and finally to investigate the relationship between socio-economic and demographic factors and under five mortality during the period 2001 2006. The study involved all children born in 2001-2006. A total of 22,422 children younger than 5 years were found in 21,494 households yielding to 49275.13 Person-Years Observed (PYOs) up to 31st December 2006. Household wealth index was constructed by use of Principal Component Analysis (PCA), as a proxy measure of each household SES. From this index households were categorized into five quintiles (i.e., poorest, poorer, poor, less poor and least poor). Life table estimates were used to estimate mortality rates per 1000 PYO for infants (0-1), childhood (1-5) and under-fives children. Health inequality was measured by poorest to least poor mortality rate ratio and by computing mortality concentration indices. Trend test chi-square was used to determine significance in gradient of mortality rates across wealth index quintiles. Risk factors of child mortality were assessed by the use of Cox proportional hazard regression taking into account potential confounders. The results indicates unexpected low mortality rate for infant (33.4 per 1,000 PYO, 95% CI (30.4 - 35.6)) and childhood (34.7 per 1,000 PYO, 95% CI (29.9 - 40.3)). Under-five mortality rate was 90.7 per 1,000 PYO (95% CI (75.6 - 108.0)). The poorest to least poor ratio were 1.1, 1.9 and 1.5 for infants, childhood, and under-five year olds respectively, indicating that children in the poorest quintile were more likely to die as compared to those in the least poor household. Computed values for concentration indices were negative (infant C= -0.02, children C= -0.09 and under-five C= -0.04) indicating a disproportionate concentration of under-five mortality among the poor. The mortality rates trend test chi-square across wealth index quintiles were significant for both childhood (P=0.004) and under-five year old children

(P<0.005) but not for infants (P=0.134). In univariate Cox proportional hazard regression, children in the least poor households were shown to have a 35% reduced risks of dying as compared to children in the poorest category [crude H.R =0.65, P=0.001, 95% C.I (0.50 - 0.84)]. The results shown that for under five children, a boy is 1.15 times more likely to die as compared to a girl [crude H.R =1.14, P=0.038, 95% C.I (1.00 - 1.31)]. Second born had a 18% reduced risk of dying as compared to first born [crude H.R =0.82, P=0.048, 95% C.I (0.67 - 0.99)]. After controlling for potential confounders, the adjusted hazard ratio for wealth index decreased slightly. The estimated hazard for wealth index in the univariate was 0.65 while in the multivariate modeling the estimated hazard ratio is 0.60 in the first model. In conclusion, the study shows that household socio-economic inequality is associated with under-five mortality in Navrongo DSS. The findings suggest that reductions in infant, childhood, and under five mortalities are mainly conditional in health and education interventions as well as socioeconomic position of households. The findings further call for more pragmatic strategies or approaches for reducing health inequalities. These could include reforms in the health sector to provide more equitable resource allocation. Improvement in the quality of the health services offered to the poor and redesigning interventions and their delivery to ensure they are more inclined to the poor.

107

COMMUNITY PERMISSION AND INDIVIDUAL CONSENT PROCESS IN A LOW LITERACY SETTING

Fatoumata Diallo[1], Fadima Cheick Haidara[1], Moussa Doumbia[1], Samba O. Sow[1], Rokia Diakité[1], Milagritos Tapia[2], Karen Kotloff[2], Myron M. Levine[2]

[1]CVD-Mali, Bamako, Mali, [2]Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States

Clinical trials have begun to be conducted in Mali only recently. In keeping with local traditions and with ethical requirements, community permission and then individual consent are necessary. Given the low literacy rate in Mali, it is important to adapt the methods of obtaining consent so that all participants may understand. As an attempt to ensure an accurate understanding of the necessary information, our investigators have the consent form translated into the local language, Bambara, and recorded on audiotape by the Malian National Center for Resources for Non-formal Education. This recording is then used during the meetings to obtain community permission as well as individual consent. Potential participants are encouraged to ask any questions they may have. Finally, community permission and individual consent are documented on a printed form in the official language, French. From 2006 to 2009, we completed 2 clinical trials of a novel conjugate vaccine and included 600 study participants. In total, 10 community meetings were attended by 359 community members and a total of 56 questions were asked. Questions concerned the risks, benefits, compensation and study design. Over the course of the studies, 33 persons withdrew consent; 28 did not provide a reason and 5 cited the blood draws. In conclusion, audiotaped translation of the consent form has been a useful tool during the informed consent process. The success of a study depends on the degree of understanding by the community. In the future, we plan to more formally evaluate the role of the audiotaped consent form.

108

DUST-OFF: PREDICTORS OF AMERICAN AIRAMBULANCE LOSSES DURING THE VIETNAM WAR

David P. Adams[1], Bryan Riemann[1], Joseph Roarty[2], Melissa Becker[1]

[1]Armstrong Atlantic State University, Savannah, GA, United States, [2]Savannah, GA, United States

The United States and its SEATO allies were involved respectively from 1960 - 1975 and 1962-1972. By the end of these periods, the United States committed approximately X number of troops and lost approximately 58,740. The primary SEATO ally, Australia, committed almost 500,000 troops, of whom almost 500 were lost. An important technological innovation, the helicopter, played a significant role in the mortality rate which the troops supplied. The Bell UH-IH, broadly known as the "Huey," became the "Dust off" for the medical evacuation of troops wounded in the field. Indeed, "dust off" informally entered the vernacular as the need - or act of- evacuation of personnel from an L-Z [Landing Zone] often designated as "hot," which meant that enemy troops were in close proximity. The symbol of the International Red Cross, worn by non-combatant medical personnel - not to mention emblazoned on the Huey its self - theoretically protected medics and their assistant as much as possible. The rules of war, however, seemed seldom applied to medics, Corpsmen, enew, and pilots who flew into the "hot" landing zones. As this paper will suggest, despite extraordinary measures to identify as clearly as possible the non-combat "Medevac Hueys" from the more ominous Hueys, equipped with a door gunner, firming an M-60 automatic machine gun. As Vietlong consistently refused to respect the Red Cross, American Medevac defended their aerocraft on both doors with MO Machines and in addition to the Red Cross Painted their Hueys white rather than green. Ironically, as the data suggest, specific factors - not least the bright white Hueys - because predictor of loss of crew members, patients, if not the helicopter itself.

109

THE IMPACT OF GLOBAL MIGRATION OF PEOPLE ON THE MORBIDITY OF CHRONIC PARASITIC INFECTIOUS DISEASES IN JAPAN

Atsuhiro Kanayama[1], Nobuaki Akao[2], Koki Kaku[1], Kenji Ohnishi[3], Takeshi Ono[1], Fumiko Suhara[1], Masaya Takamoto[4], Eiji Takayama[1], Tsutomu Takeuchi[5], Saori Umemoto[1], Yoko Yamaguchi[1], **Yasushi Miyahira**[1]

[1]Department of Global Infectious Disease Tropical Medicine., National Def. Medical College, Saitama, Japan, [2]Section of Environmental Parasitologhy, Graduate School of Tokyo Medical and Dental University, Tokyo, Japan, [3]Department of Infectious Disease, Tokyo Metropolitan Bokutoh General Hospital, Tokyo, Japan, [4]Department of Infectious Host Def., Shinshu University Graduate School Medicine, Nagano, Japan, [5]Department of Tropical Medicine Parasitology, Keio University School Medicine, Tokyo, Japan

As globalization proceeds, people migrate in a borderless manner worldwide. Japan is not exceptional, since much more foreigners have stayed here as residents who have originally come from countries, where chronic parasitic diseases are endemic. Some of the diseases were already eradicated from or have never existed in Japan. As there is little information regarding how many chronically-infected people reside in Japan, there is an urgent need to establish an appropriate screening system to detect such individuals to prevent unexpected accidents to occur. In this study, we conducted field surveillance to clarify the epidemiological status quo regarding how many foreign residents in Japan are actually being infected with chronic parasitic infectious diseases. We conducted medical examinations for foreign residents at 5 cities (Yamato, Fujisawa, Hiratsuka in Kanagawa, Ota in Gunma and Joso in Ibaraki) between 2007 and 2009. The medical check-up includes clinical interviews, blood tests for the detection of antibodies against parasitic diseases, stool examinations and ultrasonography. Among 473 people who accepted to provide with blood samples, 41 were determined as positive for antibodies (positive rate: 8.7 %) against parasites; 2 for malaria, 2 for visceral leishmaniasis, 1 for Chagas' disease, 19 for toxocariasis, 10 for gnathostomiasis, 5 for amoebiasis, 1 for trichinosis and 3 for schistosomiasis. 1 for echinococcosis. Stool examinations detected 16 positive cases among 263 people (positive rate: 6.1 %) who had provided with stool specimens. The positive cases include amoebiasis, giardiasis, cryptosporidiasis and Hookworm infection. As expected, there were no overt clinical symptoms among examinees. We conclude that the chronic parasitic infectious diseases are quite common among the foreign

residents in Japan when compared to the morbidity among indigenous Japanese people. Considering the well-established sanitary infrastructures in the Japanese Society, the immigration of chronically-infected people will not trigger the outbreak of parasitic diseases in Japan; however, transfusion-related infectious diseases such as Chagas' disease will have to be carefully monitored due to the lack of systematic screening system for the diseases in Japan.

110

HOW, WHERE AND WHY DO WE EVACUATE THOSE INFECTED WITH VIRAL HEMORRHAGIC FEVERS?

Robert L. Quigley

International SOS Assistance, Inc., Trevose, PA, United States

The globalization of commerce is manifested by the migration of the workforce to third world environments. Such migration, particularly to the African continent, increases exposure to the traditionally neglected tropical diseases; viral hemorrhagic fevers. Although local, regional and national Departments of Health have attempted to implement educational and protective protocols, the ultimate treatment facilities for these lethal, highly contagious diseases, are usually not locally available. Beyond universal precautions transport guidelines/protocols are non-existent. Consequently a dismal prognosis is the rule. Our experience, in global medical assistance, reveals that the medical evacuation/transport of patients with Ebola, Lassa fever, Marburg etc. requires complete cooperation, as well as authorization, by all government agencies responsible for Public Health in the country of origin as well as the destination country. To address the transport of this unique and growing population of infected patients we have developed and used innovative safety measures to protect our medical teams/flight crews from contamination during medical evacuation/transport. Those measures include the design, in accordance with International Health Authority Guidelines (WHO, CDC), and implementation, of a compact, portable isolation unit (PIU), ideal for regional ground/air travel. More recently, we have incorporated a disposable biological containment unit (BCU) into our comprehensive protocols which is designed for a Gulfstream III, ideal for trans-ocean/continental travel. Both the PIU and BCU enhance our ability to medically transport infected patients. We have demonstrated that: (1) efficient movement of the sick/infected patients has a positive impact on their outcome and (2) the creation and credentialing of a global network of preferred providers willing and able to accept such patients facilitates the transfer to the nearest center of medical excellence rather than repatriations which may not always be practical.

111

THE USE OF SUPPORTIVE SUPERVISION TO STRENGTHEN IMMUNIZATION IN MALI

Fanta Niare[1], Julie Milstien[2], Samba O. Sow[1], Nouhoum Koné[3], Bani Diaby[3], Karen Kotloff[2]

[1]CVD-MALI, Bamako, Mali, [2]University of Maryland School of Medicine, Baltimore, MD, United States, [3]DNS/SI MALI, Bamako, Mali

In collaboration between the Malian Ministry of Health and the University of Maryland School of Medicine, training focusing on introduction of a new vaccine against *Hemophilus influenzae* type b was conducted. At training completion, evaluation showed that immunization information was not passed efficiently from district to health center level. To address this we employed a supportive supervision approach. Four supervisory tools, each focused on a specific category of immunization (injection safety, techniques and communications for immunization, program management, and surveillance) taking approximately 90 minutes to administer, were used. These supervisions were done at three month intervals for a year, with district level staff as part of the team. Before the year-long intervention started, evaluations were done in the four health centers in two regions where the interventions took place, and in four health centers in the same regions but in different districts, which served as controls. These evaluations covered injection safety, surveillance and program management, but did not completely correspond to the areas covered in the supervisory tools. The results of the evaluations showed that any intervention, even just performing an evaluation, had a positive outcome. For both the injection safety and surveillance evaluations, all eight centers improved performance, although those which had the supervisory visits were better performing overall in both regions. For vaccine management, all but one center in each region improved performance. In all centers where the quarterly intervention was done, we found an increased enthusiasm among staff to improve their performance and to ask questions about procedures as the year went on. The study will continue for another year with a crossover design, using the original tools in the control districts and four new tools in the original intervention areas.

112

METEOROLOGICAL SUPPORT THE PUBLIC HEALTH COMMUNITY

Wendy Marie Thomas

American Meteorological Society, Washington, DC, United States

There is a nexus point between public health, tropical medicine, and the environment. Reaching this point, however, requires strategic planning, trans-discipline partnerships, and resources to support new hybrid sciences (e.g., biometeorology). The American Meteorological Society (AMS) is the oldest professional society for the hydromet/climate/ocean sector in the U.S. and recognizes the importance that quality environmental data could bear in bolstering preparedness, improving surveillance, and expediting response in the public health community on monthly to seasonal timescales. We highlight that the impacts from a climate in transition make the union between health, medical, and environmental sciences even more pressing. Because of this, the AMS would like to report to the ASTMH community on our activities to inform our researchers and operational meteorologists, in the U.S. and overseas, about reaching out to your community. In addition, we would also like to hear from ASTMH members on how we could improve the delivery and quality of environmental data and strengthen partnerships in order to support your research and medical care regarding current and emerging diseases.

113

POVERTY, DIARRHEA, AND TREATMENT COSTS: UNRAVELING THE RELATIONSHIP

Emily R. Smith[1], Volga Iniguez[2], Jennifer P. King[1], Juan S. Leon[1]

[1]Emory University, Rollins School of Public Health, Atlanta, GA, United States, [2]Instituto Mayor de San Andres, La Paz, Plurinational State of Bolivia

In Bolivia, the under-five mortality rate is 65 per 1,000 children, and diarrhea is responsible for 37% of these deaths. Pediatric diarrhea causes a substantial economic burden to households and healthcare systems. The goal of this study was to identify demographic, etiologic, and behavioral factors associated with catastrophic costs resulting from treatment of gastroenteritis. We interviewed 384 caregivers of children with diarrhea from hospitals and outpatient clinics in 7 facilities across 4 Bolivian cities. Multivariate linear regression was used to identify predictors of increased treatment cost. A logistic regression model predicting the probability that a household's expenditure for treatment exceeded 1% of annual income (catastrophic cost) was constructed to identify determinants of catastrophic costs. Caregiver feelings about treatment costs and related coping mechanisms were also assessed. We identified demographic characteristics, treatment seeking behavior, and disease severity to be significant predictors of increasing cost burden and significant determinants catastrophic cost. Demographic characteristics, including male gender of child and city where treatment was sought, were correlated with increased treatment expenditures, suggesting a social bias in the seeking treatment for male children and overall higher treatment

costs in the southern lowlands. Treatment seeking behavior (increases in the number of places sought for treatment) predicted a significant increase in the cost burden. The majority of caregivers indicated that they had to find extra money to pay for treatment. More than 20% said that they had withheld treatment for their child because of high treatment costs. Coping mechanisms for high costs were significantly different for families who spent more than 1% of their income on treatment. Despite universal health insurance in Bolivia, pervasive use of health services contributes to caregiver costs. Effectively removing real and perceived barriers to healthcare are needed to achieve further reductions in diarrheal mortality in Bolivia.

114

A COMPARISON BETWEEN CAREGIVER KNOWLEDGE OF SIGNS OF DEHYDRATION AND PRIORITIZED HEALTH EDUCATION RECOMMENDATIONS IN PERI-URBAN DOMINICAN REPUBLIC

Daniela Navia, John D. McLennan

University of Calgary, Calgary, AB, Canada

Dehydration during diarrhoeal episodes is an important contributor to child mortality in the global south. Caregiver recognition of signs of dehydration may be a critical factor in prompting health restoring behaviours. Understanding existing caregiver knowledge relative to signs prioritized by health education recommendations may inform refinement of health education campaigns. This study aimed to determine current dehydration knowledge of caregivers of young children at risk for diarrheal illness. Three samples of caregivers of young children in a peri-urban community on the outskirts of Santo Domingo, Dominican Republic were recruited: a community sample (n=251), attendees of a nutrition rehabilitation program (n=223), and attendees of a general paediatric clinic (n=67). Caregivers participated in a structured interview. Responses were contrasted with commonly promoted prioritized dehydration signs. Thinness (42%), dry lips and/or mouth (33%) and sunken eyes (31%) were the most commonly reported signs across groups. The latter two correspond to prioritized dehydration signs. Various signs suggestive of under-nutrition were frequently mentioned as manifestations of dehydration (49%). Findings suggest some overlap in current caregiver knowledge and prioritized dehydration signs. Further research, including a qualitative approach, is needed to further explore caregivers' perceptions of dehydration in relation to under-nutrition.

115

EMCOUNTER: A NOVEL, DYNAMIC EPIDEMIOLOGIC SURVEILLANCE TOOL FOR THE DEVELOPING WORLD

David R. Anthony, Satchit Balsari

New York-Presbyterian Hospital, New York, NY, United States

In recent years, the specialty of emergency medicine has gained tremendous interest in the developing world. Despite this, it remains a globally nascent specialty. Few countries have formally recognized the field, and even fewer have developed the training programs, infrastructure, legislation, and public education necessary to provide for a comprehensive approach to emergency care. Furthermore, there is little knowledge of what actually comprises medical emergencies in these settings. Current developmental and educational models use largely Western paradigms, which almost certainly differ from the unique needs and local variability of the rest of the globe. To address this knowledge gap, we developed Project EMcounter, a web-based tool capable of analyzing variations in the epidemiology and management patterns of medical emergencies in the developing world. The project makes novel use of an online data entry tool, functioning as a receiving database for the real-time collection of information from the paper charts of select emergency departments across the developing world. EMcounter was piloted at Sundaram Medical Foundation in Chennai, India from 2006 to 2008, during which data was collected on 13,214 separate patients, revealing important variations in disease patterns and practice constraints. However, far more than a static data collection tool, EMcounter has enormous potential as a dynamic real-time epidemic surveillance system. Because data is collected at hospital receiving rooms and emergency departments (often the first points of presentation for emerging epidemiologic trends), and given the real-time nature of the tool's data entry system, the tool is ideally suited to map epidemics and other current medical trends in select locations in the developing world. We are currently exploring this potential by developing an open-access web-based data visualization interface that allows users to modify data sets in order to graphically represent trends of interest. Next steps for the project include interfacing with electronic medical records to fully streamline the data entry process.

116

FETAL HEART RATE DURING ACUTE MALARIA

Stephen Rulisa[1], Steven Agaba[2], Nadine Kaligirwa[2], Placide Kalangayire[2], Petra F. Mens[3], Peter J. De Vries[4], Joseph Vyankandondera[5]

[1]Kigali University Teaching Hospital, Kigali, Rwanda, [2]Centre for Treatment and Research on AIDS, Malaria and TB (Trac-Plus), Kigali, Rwanda, [3]Royal Tropical Institute/KIT Biomedical Research, Amsterdam, The Netherlands, [4]Academic Medical Center, Division of Infectious Diseases, Tropical Medicine and AIDS, Amsterdam, The Netherlands, [5]Kigali University Teaching Hospital, National University of Rwanda, Kigali, Rwanda

To study the time course of the maternal and fetal heart rate (FHR) during recovery from acute malaria, we examined 40 pregnant women with acute malaria and 40 healthy pregnant women. Malaria patients were hospitalized until recovery with a minimum of 3 days. Healthy subjects were measured only once.

FHR during malaria was measured every 4hrs on the first day after initiating Arthemether lumefantrine (AL) treatment, and every 8 h for another two days. Maternal vitals were measured every 8 h for 3 days. The table shows the measurement on T0, of malaria patients and of healthy subjects (1 healthy Baseline measurements of malaria patients (n=40) compared to healthy women (n=39) were respectively: Gest. age (wks) 28.8 and 24.6 (p-value 0.006); upper FHR (bpm) 165 and 158 (p-value 0.054); lower FHR (bpm) 137.5 and 128.7 (p-value 0.016); mean blood pressure (mm Hg) 75 and 81 (p-value 0.001); pulse pressure (mm Hg) 40 and 42 (p-value 0.2); pulse rate (bpm) 109 and 81 (p-value <0.001); and Geometric mean. parasite/µl 13795. Complete time series were collected from 33 malaria patients. During recovery FHR normalized on average within 20 h. Maternal fever clearance was also 20 hrs but maternal heart rate normalized much later, after approx. 32 h. Maternal blood pressure was low and pulse rate was high in malaria patients whereas fetal heart rate(FHR) was elevated. In conclusion, the circulatory effects of acute malaria during pregnancy are compatible with decreased circulating maternal blood volume. In contrast, the FHR normalises at the same rate as the maternal body temperature.

117

IMPLICATING THE S1P PATHWAY IN CEREBRAL MALARIA PATHOLOGY

Constance A. Finney[1], Charles Musoke[2], Aggrey Dhabangi[2], Tamas Oravecz[3], Walter Dzik[4], Christine Cserti-Gazdewich[5], Conrad Liles[1], Kevin Kain[1]

[1]University of Toronto, Toronto, ON, Canada, [2]Mulago Hospital, Kampala, Uganda, [3]Lexicon Pharmaceuticals, The Woodlands, TX, United States, [4]Massachusetts General Hospital, Boston, MA, United States, [5]Toronto General Hospital, Toronto, ON, Canada

Excess inflammatory responses as well as loss of vascular integrity have been implicated in cerebral malaria pathology. Sphingosine-1-phosphate is a tightly regulated signaling sphingolipid whose functions include

regulating endothelium homeostasis and inflammation. We hypothesized that S1P signaling is altered during infection and that this may contribute to endothelial activation and inflammation thought to play an important role in the pathogenesis of cerebral malaria. We found that extracellular plasma S1P levels were significantly decreased in Ugandan children with cerebral, but not uncomplicated, malaria. Using a murine model of experimental cerebral malaria, we also demonstrated that mice with reduced S1P lyase (an S1P degrading enzyme) activity, had higher survival rates compared to their wildtype littermates when infected with *Plasmodium berghei* ANKA (PbA). To further investigate the role played by S1P during infection, we treated mice infected with PbA with compounds that interfere with the S1P pathway and currently in human trials for other conditions (FTY720 or LX3305). Prophylactic treatment with either compound improved survival to PbA infection. However, with therapeutic administration, only FTY720 treatment proved beneficial. In animals having received prophylactic treatment with FTY720, we observed a decrease in IFNg and TNF levels both in plasma (protein) as well as the brain (mRNA). Vascular integrity was also improved in animals treated with FTY720 compared to untreated mice: (1) plasma protein levels of endothelial cell activation markers such as sICAM, and brain mRNA levels of ICAM were decreased, (2) Ang1 (a regulator of endothelial quiescence) plasma levels, as well as brain mRNA levels were increased, (3) Evans blue staining of the brain was also reduced. In summary, we present the first data implicating the S1P pathway in the pathogenesis of human and murine ECM and suggest that therapeutic manipulation of this pathway may represent a new adjunctive treatment strategy for severe or cerebral malaria.

118

ROLE OF CD47 AND SIRPA IN MALARIAL PATHOGENESIS

Kodjo Ayi[1], W. Conrad Liles[2], Kevin C. Kain[2]

[1]Tropical Disease Unit, MRC, Centre for Global Health, TGH-UHN, Toronto, ON, Canada, [2]Tropical Disease Unit, MRC, Centre for Global Health, TGH-UHN; McLaughlin Centre for Molecular Medicine; Institute of Medical Sciences, Department of Medicine, University of Toronto, Toronto, ON, Canada

CD47 engagement by macrophage SIRPα inhibits phagocytic activity and protects red blood cells (RBCs) from erythrophagocytosis. Conversely, decreased levels of CD47 expression are associated with an "eat me" signal and increased macrophage clearance of RBCs. Non-opsonized *Plasmodium falciparum*-parasitized RBCs are phagocytosed by macrophages but, CD47 expression on malaria infected RBCs has not been well studied. Based on the hypothesis that *P. falciparum* may modify RBC expression of phagocytic signals such as CD47, here we report, using enzyme immunoassay and flow cytometric assay, that CD47 expression is decreased on *Plasmodium*-parasitized RBCs at ring-stages ($P < 0.001$) and mature-stages ($P < 0.001$). We further show, that macrophages from SHP-1 knock-out mouse and macrophages treated with anti-SIRPα antibody (anti-CD172) enhance phagocytosis of ring-stage *falciparum*-parasitized RBCs. Finally, mice lacking CD47 and congenic controls were intrapenitonially inoculated with *P berghei* ANKA. At day 6 after inoculation the CD47-/- mice display significantly lower parasitemia ($P < 0.0001$) and survive longer ($P < 0.0001$) compared with their CD47-expressing littermates. These results support a potential role of CD47/SIRPα in protection against severe malaria.

119

MALARIA AND NON-TYPHOIDAL SALMONELLA: UNDERSTANDING THE UNDERLYING MECHANISMS OF HUMAN CO-INFECTION USING AN ANIMAL MODEL

Jennifer Y. Chau, Brian P. Butler, Michael D. George, Renee M. Tsolis, Shirley Luckhart

University of California Davis, Davis, CA, United States

Co-infection of malaria and non-typhoidal Salmonella (NTS) is prevalent in endemic areas of sub-Saharan Africa. In children, co-infection results in severe disease. In particular, bacteremia resulting from NTS intestinal escape is much more frequent in malaria-infected children. We have recapitulated this phenomenon in a murine model to investigate the nature and location of the barrier defect in containment of NTS. In human malaria infection, intestinal permeability is increased, suggesting that bacteremia results from intestinal escape of NTS. Two factors may contribute to malaria-associated intestinal permeability: parasite sequestration in the intestinal vasculature and parasite-induced L-arginine deficiency. Hypoargininenemia is a hallmark of malaria infection, reducing the synthesis of anti-parasite nitric oxide (NO) and exacerbating restricted blood flow by parasite sequestration. Further, L-arginine-related genes are induced in the gut during the early stages of NTS infection, and we can infer that L-Arginine depletion may be occurring in the intestinal response to NTS infection in a non-human primate model. Based on these observations and the knowledge that oral L-arginine supplementation can restore intestinal barrier function, we hypothesized that L-arginine deficiency in co-infected mice contributes to NTS escape from the intestine. To test this hypothesis, we supplemented *Plasmodium* yoelii nigeriensis-infected mice with oral L-arginine and monitored bacterial translocation from the intestine and peripheral parasitemia. As shown previously, parasite infection resulted in increased bacterial translocation in the liver, spleen and Peyer's patches over uninfected controls. However, parasite-infected mice supplemented with oral L-arginine showed significantly decreased bacterial translocation in the mesenteric lymph node when compared to non-supplemented infected mice. We found higher levels of L-citrulline in the serum of supplemented mice, suggesting that oral L-arginine is metabolized by NO synthase as opposed to arginase. These data suggest that oral L-arginine may have restorative or reparative effects on malaria-induced pathology. Our murine studies should help to characterize the mechanisms of co-infection pathology, to aid in the identification of new drug targets, and to enhance currently available therapeutics.

120

EFFECT OF CHRONIC SCHISTOSOMIASIS ON SEVERE MALARIA IN A PRIMATE MODEL

Ruth K. Nyakundi

Institute of Primate Research, Nairobi, Kenya

Malaria and helminth infections are the two most prevalent parasitic diseases globally with annual mortality of about 500 million in the former and approximately 2 billion people infected with helminths. Since the epidemiological distribution of both diseases overlap, concomitant infections are a common occurrence. Studies on the effect of one disease on the other in mouse models and humans, show conflicting results due to differences in mouse strain, intensity of helminth infection, age of population and differing study designs. In order to provide proof of principle, this study hypothesized that chronic schistosomiasis results in delay in the onset and severity of malaria.We used the Olive baboon (Papio anubis) as a study model since they are natural hosts of *S. mansoni* with the capacity of harboring a substantial schistosome infection that is long-term, unlike mice. The baboon also presents a model of *Plasmodium knowlesi* infection that is useful in studying uncomplicated and severe malaria with cerebral involvement, and which is now recognized as the fifth malaria parasite of humans. Four groups of baboons were used. Groups A, B (n=8) and D (n=3) were infected with 500 *S. mansoni* cercarie, and the disease was left to progress to the chronic phase. To determine the effect of treatment on co-infection, group A was treated with praziquantel at week 14 and 15 post infection. Four weeks later, groups A, B and C (n=8) were inoculated with 1x105 *P. knowlesi* parasites. Baboons were monitored daily and clinical parameters recorded. Sera and PBMCs were collected at baseline, before and after treatment and at end-point to determine humoral and cellular immunological responses. Results showed that animals infected with *P. knowlesi* had an early onset of parasitemia and succumbed to severe malaria unlike majority of baboons with co-infection. Comparative assessment of immunological and clinical

parameters will be presented in detail. This study shows that presence of schistosomiasis in malaria infected animals' results in delay in the onset and severity of acute malaria

121

THE IMPORTANCE OF HEMOGLOBIN LEVEL AT ENROLLMENT ON SUBSEQUENT MALARIA RISK: RESULTS FROM A PEDIATRIC COHORT IN MALI, WEST AFRICA

Saibou Doumbia[1], Tatiana M. Lopera-Mesa[2], Seidina A. S. Diakité[1], Drissa Konaté[1], Karim Traoré[1], Mory Doumbia[1], Michael A. Krause[1], Greg Tullo[2], Jennifer M. Anderson[2], Michael P. Fay[3], Mahamadou Diakite[1], Carole A. Long[2], Rick M. Fairhurst[2]

[1]University of Bamako, Bamako, Mali, [2]Laboratory of Malaria and Vector Research/National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States, [3]Biostatistics Research Branch, National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States

WHO estimates around 60% of people (especially children <5 years old) living in malaria-endemic areas of Africa have iron-deficiency anemia. Studies to evaluate the effect of iron supplementation on malaria risk have produced controversial results. Further, the impact of baseline hemoglobin (Hb) level on subsequent risk of malaria has not been well documented or routinely assessed. We initiated a 5-year longitudinal cohort study in three villages in rural Mali. From June 2008 to December 2009, we enrolled 1419 children aged 6 months to 17 years. We enrolled children just prior to the 2008 malaria transmission season (N=1258) or at 6 months of age (N=161). Each child's age, ethnicity, village, Hb level, and ABO blood type were recorded and the presence of sickle HbS, HbC, alpha-thalassemia, and G6PD deficiency determined. In 1356 children with complete data, we diagnosed 1933 episodes of malaria (90% uncomplicated) during 2 consecutive annual transmission seasons. The relative risk (RR) for each factor was calculated by a Poisson regression model. In the model, we divided children into three groups based on Hb level (Hb<8.5 g/dL, N=82; 8.5-12 g/dL, N=877; >12 g/dL, N=397). As expected, older children had lower risk of malaria than younger children. Taking into account all covariates, we found that children with Hb >12 g/dL had significantly lower risk (RR 0.81, 95%CI 0.69-0.95, p =0.008) of malaria than those with Hb 8.5-12 g/dL. Interestingly, children with Hb <8.5 g/dL and Hb 8.5-12 g/dL did not differ in malaria risk. HbS was the only other factor associated with significant change in RR. The reduced malaria risk in children with Hb >12 g/dL was not associated with reduced parasite densities (adjusted geometric mean of the Hb >12 g/dL group was 0.92 times that of the Hb 8.5-12 g/dL group, 95%CI 0.63-1.34, p=0.660). Our data indicate that baseline Hb level may be an important host factor (and thus a major confounder) in natural history and interventional studies which measure malaria incidence as a primary outcome. Further work is needed to determine if relatively high Hb levels are directly involved in the mechanism of protection or whether an unmeasured factor that correlates with high Hb levels confers protection from malaria.

122

PLASMODIUM CHABAUDI INFECTION FOLLOWED BY *P. BERGHEI* INFECTION IN C57BL/6 MICE PROVIDES NOVEL MURINE MODEL FOR STUDYING SEVERE MALARIAL ANEMIA

Juliana V. Harris[1], Jose A. Stoute[2]

[1]Uniformed Services University, Bethesda, MD, United States, [2]Pennsylvania State University College of Medicine, Hershey, PA, United States

Severe malarial anemia from *Plasmodium falciparum* claims the lives of thousands of children in sub-Saharan Africa every day. The pathogenesis of this anemia is not well understood and progress has been hampered by the lack of an inexpensive and reproducible animal model. We aimed to develop a relevant model of severe malarial anemia using C57BL/6 mice. Infection of these mice with *Plasmodium berghei* ANKA is uniformly fatal whereas infection with *P. chabaudi* AS leads to severe anemia with high parasitemia followed by full recovery. We determined that infection with *Plasmodium berghei* ANKA following recovery from a *Plasmodium chabaudi* AS infection resulted in anemia with a low level parasitemia (<10%). Mice developed splenomegaly and hepatomegaly with histological evidence of erythrophagocytosis in the liver. Inflammatory cytokines IL-12 and TNF-α were significantly elevated over those in naïve mice infected with *P. berghei*. This new mouse model provides a highly reproducible and relevant platform for studying host and parasite factors that contribute to the pathogenesis of severe malarial anemia.

123

MODULATION OF MEMBRANE AND SOLUBLE TREM-1 IN MALARIA INFECTION

Laura Erdman[1], Aggrey Dhabangi[2], Charles Musoke[2], Christine Cserti-Gazdewich[3], Kevin C. Kain[1]

[1]McLaughlin-Rotman Centre for Global Health, University of Toronto, Toronto, ON, Canada, [2]Makerere University College of Health Sciences, Kampala, Uganda, [3]University Health Network, Toronto General Hospital, Toronto, ON, Canada

Excessive or dysregulated host pro-inflammatory responses to malaria infection have been implicated in the pathogenesis of severe disease. A number of innate immune components have been shown to contribute to these responses, including Toll-like receptors (TLR), although the full complement of host inflammatory pathways remains to be characterized. Triggering receptor expressed on myeloid cells-1 (TREM-1) is a germline receptor on monocytes and neutrophils that is upregulated upon TLR stimulation. TREM-1 synergizes with TLRs to induce inflammation, and has been found to play a role in sepsis pathophysiology. We hypothesized that TREM-1 expression is modulated during malaria infection and that TREM-1 may contribute to disease severity. We exposed human peripheral blood mononuclear cells (PBMCs) to *Plasmodium falciparum*-infected red blood cells (RBCs) or uninfected RBCs *in vitro*. Incubation of PBMCs with malaria-infected RBCs for 24 hours resulted in a significant decrease in TREM-1 surface levels on monocytes (p=0.018), and induced release of soluble TREM-1 (sTREM-1), which is thought to be generated by cleavage of membrane TREM-1. We next examined TREM-1 expression in the *P. berghei* ANKA model of experimental cerebral malaria. TREM-1 mRNA expression in the brain was elevated in mice on Day 6 of infection compared to uninfected mice (p<0.05). Finally, we measured sTREM-1 in the plasma of pediatric malaria patients in a case-control study in Uganda. Plasma sTREM-1 levels at admission were significantly elevated in severe malaria patients compared to uncomplicated cases (median (range) in pg/mL: uncomplicated 154.9 (44,1519) vs severe 371.5 (72.7,2428); p<0.0001), and were higher in fatal cases of severe malaria compared to survivors (survivors 324.6 (72.7,1321) vs fatal 528.7 (244.3,2428); p=0.0021). In summary, we show that TREM-1 is modulated during malaria infection. We are currently investigating whether TREM-1 signaling contributes to the pathogenesis of severe malaria.

124

THROMBOCYTOPENIA IN PATIENTS WITH *PLASMODIUM VIVAX* IN A COLOMBIAN ENDEMIC AREA

Maria F. Yasnot, Rossana Villegas, Agustina Noble

Grupo de Investigaciones Microbiológicas y Biomédicas de Córdoba, Universidad de Cordoba, Monteria, Colombia

Malaria remains an important health problem in tropical countries like Colombia. The malaria has clinical manifestations that can change from short duration fever episodes, if the diagnosis is opportune and the treatment is effective, up to systemic severe complications and death. The hematic changes associated with malaria are well recognized, but the specific changes can to vary according to the endemicity levels of the malaria, history of hemoglobin disease, nutritional condition, demographic factors and malaria immunity. The proposal of this study was to determine the behavior of the platelets in patients with *Plasmodium vivax*. Venous

blood was collected in K2EDTA Vacutainer® tubes for automatized platelet count; additionally carry out thin and thick smear from peripheral blood from 200 individuals (100 with *Plasmodium vivax* and 100 control individuals of the same area). Thrombocytopenia was defined as platelet count under 150,000/µL. Results show that 67% of patients with *P. vivax* had thrombocytopenia. The platelets average in healthy population was 278.000/uL and 125.000/uL for the patients. Platelets average was 84.500/uL in patients with thrombocytopenia, in these patients the age average was 33 years old and they had a normal Body Mass Index. Male (64%) shows more frequency of thrombocytopenia that female (36%). Mean of parasitemia in patients with *P. vivax* and thrombocytopenia was 4.040 parasite/uL. Did not observe relationship between parasitemia and thrombocytopenia. Neither is clear the relationship between previous episodes of malaria and thrombocytopenia. This study suggests that in this population the thrombocytopenia is a frequently finding in patients with *Plasmodium vivax*. The clinician should consider malaria in patients with fever syndrome and thrombocytopenia when thinking in dengue. Is important to direct future studies to the virulence of the parasite and the immune response in this individuals, for understand the mechanism involved in the decrease of platelets overall in patients with low parasitemia.

125

WHAT IS A HYPNOZOITE?

Miles B. Markus

Imperial College London, London, United Kingdom

In 1980, Krotoski and his colleagues reported their important discovery of a uninucleate, apparently dormant stage in the life cycle of *Plasmodium*. Two years earlier, the suggested use of the term "hypnozoite" had been set out by me in a key paper (reprints of which are still available) that has remained obscure because of the journal in which it appeared. In relation to the prevailing hypnozoite concept, the information to be presented at this meeting is, therefore, dramatically new. Following non-malarial work carried out in the 1970s while I was a PhD student at Imperial College London, the implications of the results of my research if extrapolated to the incompletely elucidated plasmodial life cycle, were pointed out; and I named the dormant, sporozoite-like apicomplexan (sporozoan) form. At that time, the concept of the occurrence of dormant malarial parasites in the liver and/or elsewhere was still a hypothetical idea. Very few people are aware that the "hypnozoite" has been "defined". This was done at some length in the abovementioned publication. It was proposed that the word "hypnozoite" be used for dormant stages that might in the future be found in the life cycle of *Plasmodium* (which has since happened). Moreover, it was explained (*inter alia*) that "hypnozoite" would also describe post-divisional, dormant, sporozoite-like apicomplexan forms that are not biologically or ultrastructurally typical merozoites (leaving aside a possible good example); as well as dormant sporozoites in the life cycle of *Isospora* (*Cystoisospora*), for instance. Although the paper concerned was published more than three decades ago, the analysis is still valid today. In summary, just as "merozoite" and "sporozoite" are not exclusive to *Plasmodium*, the descriptive name "hypnozoite" is not only applicable to dormant liver stages of *Plasmodium*, but (contrary to current general understanding of the use of the term) to some other dormant apicomplexan forms as well.

126

MINIMALLY INVASIVE POST MORTEM TISSUE SAMPLING: A PROTOCOL TO ELUCIDATE HOST-PARASITE INTERACTIONS IN TROPICAL AREAS

Atis Muehlenbachs[1], Cate Speake[2], Patrick E. Duffy[3], Corinne L. Fligner[1]

[1]University of Washington, Seattle, WA, United States, [2]Seattle Biomed, Seattle, WA, United States, [3]National Institute of Allergy and Infectious Diseases, Besthesda, MD, United States

Parasitic diseases are a major cause of death in tropical areas; however, pre-mortem clinical diagnoses are challenging and the in vivo mechanisms are incompletely understood. For example, respiratory distress due to malaria is difficult to distinguish from viral or bacterial causes and the role of parasite sequestration is of uncertain significance. We are developing a minimally invasive, culturally acceptable, easily implemented, safe and rapid autopsy procedure that will allow extensive tissue sampling after death due to infection in resource poor communities such as rural Africa. Using an autopsy cohort at the University of Washington, we utilize a combination of in house manufactured and commercially available laparoscopic surgical tools. We have optimized sampling of lung, liver, spleen, brain and bone marrow. In this cohort, respiratory diseases have been well-represented, including cytomegalovirus pneumonitis, H1N1 influenza, and mycobacterium infection. Messenger RNA levels of vascular endothelial growth factor (VEGF) and other potential biomarkers tied to clinical syndromes are being assessed by quantitative PCR. In future field studies of malaria, we anticipate that the post mortem recovery of parasite and host material will assist in the identification of novel diagnostic markers and further elucidate host-parasite interactions related to severe disease.

127

ELEVATED SFLT-1 AND DECREASED VEGF LEVELS ARE ASSOCIATED WITH MALARIA-RELATED RESPIRATORY DISTRESS IN TANZANIAN CHILDREN

Atis Muehlenbachs[1], Jeff Lachowitzer[2], Whitney E. Harrington[1], Michal Fried[2], Patrick E. Duffy[3]

[1]University of Washington, Seattle, WA, United States, [2]Seattle Biomed, Seattle, WA, United States, [3]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States

Soluble fms-like tyrosine kinase 1 (sFlt-1), an inhibitor of vascular endothelial growth factor (VEGF), is elevated during placental malaria and has been implicated in the pathogenesis of acute respiratory distress syndrome. Prior studies have found that during malaria in non-immune adults, levels of VEGF are elevated and sFlt-1 is unchanged, whereas during pediatric malaria in endemic areas, levels of VEGF are decreased and correlate with severity of disease. We hypothesize that sFlt-1 may contribute to angiogenic imbalance during malaria-associated respiratory distress in endemic areas. Here we report that for children living in Muheza, Tanzania, a high transmission area, uncomplicated malaria was associated with decreased plasma VEGF levels by ELISA, and that malaria-associated respiratory distress was associated with further decreased VEGF level. Plasma sFlt-1 was inversely correlated with VEGF, and sFlt-1 levels were significantly increased during malaria-associated respiratory distress. Paradoxically VEGF expression *in vitro* was stimulated ~20-fold by co-culture of *P. falciparum*-infected erythrocytes with peripheral blood mononuclear cells. This suggests that systemic VEGF levels in the host may be derived from non-hematopoietic tissue and that malaria may have opposing local versus systemic effects on VEGF expression, which may explain some of the differences seen between adults and children with malaria. We previously characterized a microsatellite polymorphism in the 3′ untranslated region of FLT1 that was associated with outcome during placental malaria. We are currently assessing this genotype in relation to outcome during pediatric malaria. These data suggest that sFlt-1 is

associated with decreased circulating free VEGF levels and contributes to the pathogenesis of malaria-associated respiratory distress in endemic areas.

128

MALARIA PARASITE *PLASMODIUM FALCIPARUM* DD2 SPONTANEOUSLY SWITCHES FROM SIALIC ACID-DEPENDENT TO SIALIC-ACID INDEPENDENT ERYTHROCYTE INVASION IN SUSPENSION CULTURE

Gordon A. Awandare[1], Lubin Jiang[2], J. Kathleen Moch[1], Christian F. Ockenhouse[1], J. David Haynes[1], Louis H. Miller[2], José A. Stoute[3]

[1]Division of Malaria Vaccine Development, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Malaria Cell Biology Section, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States, [3]Department of Medicine, Division of Infectious Diseases, Pennsylvania State University College of Medicine, Hershey, PA, United States

Plasmodium falciparum parasites can broadly be classified as sialic acid (SA)-dependent or SA-independent based on their ability to invade neuraminidase-treated erythrocytes. *P. falciparum* Dd2 is classified as SA-dependent since it usually invades neuraminidase-treated erythrocytes at <10% of the rate of invasion of untreated erythrocytes. We compared the rates of SA-independent invasion in two parallel Dd2 cultures, one incubated with gentle shaking (*Suspended*) and another without shaking (*Static*). While the ability of Dd2 *Static* to invade neuraminidase-treated cells remained below 10% relative to invasion of untreated erythrocytes, SA-independent invasion increased steadily in Dd2 *Suspended* over time, reaching about 50% after 12 weeks in culture. Interestingly, this switch in invasion phenotype was reversible, such that when Dd2 suspended was returned to static conditions, there was a gradual loss in its ability to invade neuraminidase-treated erythrocytes. These observations appear to be unique to Dd2 since two other *P. falciparum* strains, 7G8 and FVO, did not significantly alter their invasion patterns when cultivated in suspension for a similar length of time. Targeted gene expression analyses revealed that some known *P. falciparum* proteins were upregulated several hundred-fold in Dd2 *Suspended* compared to Dd2 *Static*, including PfRh4, which has been shown to be involved in SA-independent invasion. Additional genome-wide microarray experiments are currently being performed to further investigate the molecular changes that are responsible for the changes in Dd2 invasion patterns in suspension cultures. Our observations in suspension cultures are similar to those obtained when Dd2 switches to Dd2NM after continuous culture in neuraminidase-treated erythrocytes. Thus, our investigations offer new opportunities for examining the mechanisms of PfRh4 regulation, and for identifying the elusive parasite ligands that mediate SA-independent invasion which remain critical for the design of any successful invasion-blocking vaccine strategies.

129

PCR-BASED POOLING OF DRIED BLOOD SPOTS FOR DETECTION OF MALARIA PARASITES: OPTIMIZATION AND APPLICATION TO A COHORT OF UGANDAN CHILDREN

Michelle S. Hsiang[1], Michael Lin[1], Christian Dokomajilar[1], Jordan Kemere[2], Christopher D. Pilcher[1], Grant Dorsey[1], Bryan Greenhouse[1]

[1]University of California, San Francisco, San Francisco, CA, United States, [2]University of North Carolina, Chapel Hill, Chapel Hill, NC, United States

Sensitive, high-throughput methods to detect malaria parasites in low transmission settings are needed. PCR-based pooling strategies may offer a solution. We first used laboratory prepared samples to compare 2 DNA extraction and 4 PCR detection methods across a range of pool sizes and parasite densities. Pooled Chelex extraction of DNA followed by nested PCR of cytochrome b was the optimal strategy, allowing reliable detection of a single low parasitemic sample (100 parasites/μL) in pool sizes up to 50. This PCR-based pooling strategy was then compared with microscopy using 891 dried blood spots from a cohort of 77 Ugandan children followed for 2 years in a low endemic urban setting. Among 419 febrile episodes, 35 cases of malaria were detected using the PCR-based pooling strategy and 40 cases using microscopy. All five cases of malaria not detected by PCR were from samples stored >2 years with parasitemia < 6000/μL, highlighting the issue of possible DNA degradation with long-term storage of samples. Among 472 samples collected in asymptomatic children as part of routine surveillance, 15 (3.2%) were positive by PCR-based pooling compared to 4 (0.8%) by microscopy (p=0.01). Thus, this PCR-based pooling strategy for detection of malaria parasites using dried blood spots offers a sensitive and efficient approach for malaria surveillance in low transmission settings, enabling improved detection of asymptomatic submicroscopic infections and dramatic savings in labor and costs.

130

THE PREDICTIVE VALUE OF RAPID DIAGNOSTIC TESTS FOR GAMETOCYTEMIA IDENTIFIED BY RT-PCR

Masiliso Phiri[1], Tamaki Kobayashi[2], Sandra Chishimba[1], Gillian Stresman[2], Sungano Mharakurwa[1], Phil Thuma[1], William J. Moss[2]

[1]Malaria Institute at Macha, Choma, Zambia, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Accurate diagnostic tools for malaria are essential to ensure that infections are not missed. In Zambia, rapid diagnostic tests (RDTs) are the main method used to diagnose malaria. The use of RDTs is important for clinical purposes, but the presence of gametocytes are the critical parasite stage for sustaining malaria transmission. Identification of gametocyte carriers will be important for malaria elimination. Our objective was to assess the association between the presence of gametocytes detected by RT-PCR and RDT positivity to provide insight on the proportion of gametocyte carriers identified by RDTs. A cross-sectional survey of individuals residing in randomly selected households was conducted in Mapanza, Choma District, in southern Zambia throughout 2008. In total, 309 blood samples were collected and tested using both RDT and RT-PCR, the latter on samples stored as dried blood spots. The RDT was an ICT Malaria P. f cassette, based on an antibody to the histidine-rich protein 2 antigen of *Plasmodium falciparum* (pfHRP-2) expressed by asexual stages. The RT-PCR detected the pfs25 mRNA expressed in *P. falciparum* gametocytes. Of the 309 individuals, 31 (10%) were RDT positive, 14 (4.5%) were RT-PCR positive for gametocytes and 9 (2.9%) were positive by both methods. Almost half (45%) of the RDT positive individuals also tested positive for gametocytes. Of the gametocyte positive individuals, 64% were RDT positive. As the first line treatment for malaria in Zambia is artemether-lumefantrine (Coartem®), which has activity against gametocytes, treatment of RDT positive, asymptomatic persons may impact malaria transmission by reducing gametocytemia. However, one third of gametocyte carriers were not detected using RDTs, highlighting the need to identify these carriers through alternative methods to achieve malaria elimination.

131

EVALUATION OF A RAPID DIAGNOSTIC TEST OF MALARIA (HISTIDIN RICH PROTEIN 2) IN A PAEDIATRIC HOSPITAL IN DAKAR, SENEGAL

Doudou Sow, Yémou Dieng, Thérèse Dieng, Abdourahmane Sow, Babacar Faye, Jean L. Ndiaye, Daouda Ndiaye, Roger C. Tine, Issa B. Bah, Oumar Faye, Oumar Gaye

Parasitology-Mycology/UCAD, Dakar, Senegal

HRP2- based Rapid diagnostic test is one of the malaria RDTs which can ensure a rational use of ACTs. The HRP2 antigen is a hydro-soluble

protein which can go through the spinal fluid. This study aimed to test the sensitivity and the specificity of these tests in paediatric hospital areas in peripheral blood and to search the HRP2 in the spinal fluid. An exploratory study was conducted into Albert Royer paediatric hospital in Dakar from November 2006 to May 2007. All patients less than fifteen years presenting clinical symptoms of malaria were included. A thick drop and commercial RDT Paracheck*PF was performed and for patients with neurological symptoms, RDT was performed on spinal fluid. Of the 1223 screened patients, 137 were found positive by the RDTs and 136 by the thick drop. The sensitivity and specificity are respectively 98,5% and 99,7%. The positive and negative predictive values were respectively 97,8% and 99,8%. Antigen HRP2 was never detected in the spinal fluid on the 107 patients with clinical severe cases. In conclusion, the paracheck applied to blood in the children is thus very sensitive and specific to *Plasmodium falciparum*. The HRP2 RDT is more sensitive among patients presenting severe malaria than those presenting uncomplicated malaria. Antigen HRP2 not found in the spinal fluid was probably either due to its molecular weight, or because the RDTs unable to detect it. The paracheck*pf is a useful tool for malaria diagnosis but microscopic examination remains the standard method.

132

QUANTITATIVE MAGNETIC FRACTIONATION FOR *PLASMODIUM FALCIPARUM* GAMETOCYTE DETECTION

Stephan Karl, Timothy M. Davis, Timothy G. St.Pierre

The University of Western Australia, Perth, Australia

A method for quantitative analysis of the gametocyte density in human blood samples is presented. This method is based on magnetic fractionation using commercially available magnetic fractionation columns and exploiting the magnetic susceptibility of mature *Plasmodium falciparum* gametocytes. The quantitative approach utilizes magnetic microspheres as a calibration standard, which are added to each blood sample at a known concentration. Gametocytes and magnetic microspheres are captured simultaneously inside the magnetic fractionation columns. The magnetically captured material can be eluted from the columns, placed on a microscope slide and stained according to standard protocol. By counting gametocytes and the magnetic microspheres after magnetic fractionation, the original gametocyte density in the blood samples can be determined from their ratio. The limits of quantification for the presented method were determined from serial dilutions with known gametocyte density. The upper limit of quantification of this method is above 103 gametocytes per mL, where quantitative analysis of the slides became impossible due to an overabundance of observed gametocytes. The lower limit of quantification was determined to be less than 1 gametocyte per mL of blood and was characterized by a departure of the standard curve from linearity. The lower limit of detection for *P. falciparum* gametocytes using this method lay in the range of 0.01-0.1 per mL.

133

VARIATION IN THE EXPRESSION OF DIAGNOSTIC BIOMARKER HISTIDINE RICH PROTEIN II (HRP2) IN COLOMBIAN *PLASMODIUM FALCIPARUM* ISOLATES

Zuleima Pava

The International Center for Medical Training and Research (CIDEIM), Cali, Colombia

More than half of the Colombian population is at risk of contracting malaria. Most of the fatal cases occur because of complications due to delays in diagnosis and treatment. At present, the gold standard diagnosis method for malaria is based on microscopy; but its use in remote endemic areas is restricted by lack of qualified personal and basic infrastructure. Such drawbacks have led to the development of simpler diagnostic strategies, including Rapid Diagnostic Tests RDTs. Most RDTs available use HRP2 as a target; nevertheless, it has been reported that sequence variations of HRP2 affect its sensitivity. At present there is not enough evidence about HRP2 variability in Latin American isolates and its relationship with RDT performance. The aim of this study was to evaluate the amount of HRP2 present in *Plasmodium falciparum* isolates from two endemic cities in the Colombian Pacific coast, in order to determine possible differences between locations and their effects on RDT performance. Twenty-three blood samples from patients with malaria-*falciparum* from Buenaventura and Tumaco, cities located in the Colombian Pacific coast, were assessed by measurement of HRP2 concentration using ELISA-HRP2, RDTs and thick smear. Statistical analysis revealed association between RDT performance and HRP2 concentrations. A slight variation, although without statistical significance, was found in HRP2 antigen levels between study sites, as well as a large variation in antigen concentrations of samples with the same parasitaemia. In contrast to previous reports, there was no correlation between initial parasitaemia and HRP2 concentration, suggesting difference in HRP2 production between parasites. Our results indicate that not only the pfHRP2 antigen sequences, but also the antigen expression levels should be studied more carefully in various endemic areas of the country, as variations in both could have significant consequences on the performance of malaria RDTs.

134

DETECTION OF *FALCIPARUM* GAMETOCYTES USING A REVERSE TRANSCRIPTION-LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP)

Sureemas Buates

Mahidol University, Bangkok, Thailand

Plasmodium falciparum gametocytes are usually present in peripheral blood at a very low level. Therefore, a sensitive assay is needed for gametocyte detection. In this study, loop-mediated isothermal amplification (LAMP) was developed for clinical detection of *P. falciparum* gametocytes. The transcripts of Pfs16 for sexually committed rings and Pfs25 for mature gametocytes were detected by reverse transcription (RT)-LAMP using 82 clinical blood samples. To evaluate an RT-LAMP assay, nested reverse transcription (RT)-PCR was used as a gold standard. RT-LAMP demonstrated a detection limit of 1 parasitized red blood cell (RBC)/500 µl of blood for both Pfs16 and Pfs25. RT-LAMP detected Pfs16 in 30 of 30 samples positive by nested RT-PCR (100% sensitivity) and 1 in 52 samples negative by nested RT-PCR (98.1% specificity). For Pfs25, RT-LAMP detected 15 of 15 samples positive by nested RT-PCR (100% sensitivity) and none of 67 samples negative by nested RT-PCR (100% specificity). The negative predictive value (NPV) and positive predictive value (PPV) of RT-LAMP for the detection of Pfs16 were 100% and 96.8%, respectively. The NPV and PPV for Pfs25 were 100%. Collectively, compared to nested RT-PCR, RT-LAMP had a higher sensitivity and a similar specificity with a shorter assay time. Since RT-LAMP requires solely basic instruments and the result inspection can be done by visualization, RT-LAMP developed here enable a simple and reliable test for analysis in molecular epidemiological study in malaria transmission and gametocyte-targeted control.

135

EVALUATION OF REVERSE-TRANSCRIPTION LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP) FOR *PLASMODIUM FALCIPARUM* GAMETOCYTE DETECTION IN ENDEMIC AREA OF THAILAND

Sirasate Bantuchai

Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Gametocytes are usually present in peripheral blood at a low level. Therefore, a sensitive assay is needed for gametocyte detection. In this study,reverse-transcription loop-mediated isothermal amplification (RT-LAMP) was developed for clinical detection of *Plasmodium falciparum* gametocytes. The transcripts of *P. falciparum* surface antigen 16 (*Pfs*16)

for sexually committed rings and *Pfs*25 for mature gametocytes were detected by RT-LAMP using 30 microscopically *falciparum*-positive blood samples collected from Mae Sot and Mae Kasa, Tak, the North western of Thailand. To evaluate RT-LAMP assay, these samples were tested by reverse transcription-polymerase chain reaction (RT-PCR). The sensitivities of RT-LAMP for *Pfs*16 and *Pfs*25 detection were 105 and 10 times higher than those of RT-PCR and nested RT-PCR, respectively. Among 30 samples, 3.3% was RT-PCR-positive for *Pfs*16 and *Pfs*25. Contrastingly, 56.7% and 40% were RT-LAMP-positive for *Pfs*16 and *Pfs*25, respectively. RT-LAMP provided similar specificity but higher sensitivity as compared to those of RT-PCR and nested RT-PCR with shorter assay time and does not required DNA purification. Collectively, this study indicates that RT-LAMP is a highly sensitive, reliable, and user-friendly method in gametocyte detection applications. RT-LAMP developed here can be useful for the epidemiological study in malaria transmission and gametocyte-targeted control.

136

IDENTIFICATION OF SENSITIVE MALARIA RDTS SUITABLE FOR THE DETECTION OF PFHRP2 NEGATIVE *PLASMODIUM FALCIPARUM* INFECTIONS IN THE PERUVIAN AMAZON

Elizabeth Villasis[1], Jorge Bendezu[1], Katherine Torres[1], Sandra Incardona[2], David Bell[3], Dionicia Gamboa[1]

[1]Instituto de Medicina Tropical, Lima, Peru, [2]Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland, [3]World Health Organization/GMP, Geneva, Switzerland

Malaria RDTs are playing an increasing role in malaria control, especially in remote settings where good microscopy is difficult to maintain or unavailable. Recent findings showed a high proportion of *Plasmodium falciparum* parasites lacking the HRP2 gene in Peru, pointing to the need of sensitive RDT that can detect them. We compared the performance of 2 RDTs detecting pfHRP2/panLDH (First Response Malaria Antigen Combo pLDH/HRP2, Premier Medical Corporation) and pfLDH/panLDH (Advantage Mal Card, J Mitra & Co), for *P. falciparum* diagnosis in the Peruvian Amazon. From November 2009 to March 2010, symptomatic patients with microscopically confirmed *P. falciparum* infections were enrolled in two study arms: i) Active Case Detection (ACD) in 5 remote sites of the Loreto Region, and ii) Passive Case Detection (PCD) in 2 Health Centers nearby Iquitos and Yurimaguas. Each patient was tested with the two above mentioned RDTs. 31 and 44 patients were enrolled by PCD and ACD, respectively, with a significant proportion of HRP2 negative *P. falciparum* infections (PCD: 29.0%, ACD: 33.0%) being detected by microscopy and by the pfLDH line of Advantage Mal Card but not by the pfHRP2 line of First Response RDT. The sensitivities of First Response and Advantage Mal Card RDTs were 71.0% vs 97.1% for samples collected by PCD, and 65.0% vs 100% for samples collected by ACD, but raised up to 100% vs 97.05% (PCD), and 100% vs 100% (ACD), respectively, when excluding HRP2 negative samples. In conclusion, the main cause of the observed differences in sensitivity was the high prevalence of *P. falciparum* HRP2 negative parasites, being detected by First Response RDT as non-*P. falciparum* infections. In the Peruvian context, this leads to a misdiagnosis as *P. vivax* and to dispensing of drugs with poor efficacy against *P. falciparum*. These results corroborate previous reports indicating that HRP2-based RDTs may not be appropriate for *P. falciparum* diagnosis in the Peruvian Amazon, and should therefore be considered if RDTs are to be used in the country.

137

DETECTION OF SINGLE RING STAGE *PLASMODIUM FALCIPARUM* IN HUMAN THIN FILM BLOOD SMEARS USING FTIR MICROSPECTROSCOPY AND DIFFERENTIATION OF PLASMODIUM POSITIVE FROM PLASMODIUM NEGATIVE RED BLOOD CELLS

Kenneth A. Puzey, Bryan Holmes, Adam Hunt, **McKale Santin**

Quantaspec, Inc., Burlington, VT, United States

Currently, rapid diagnostic tests for malaria infection perform poorly at low parasite loads, are degraded by severe temperatures, and contain reagents, which contribute to their costs. The overall objective of this study was to perform a preliminary evaluation of the utility of FTIR microspectroscopy for *in vitro* diagnosis of thin film blood smears for malaria infection. FTIR microspectroscopy has potential advantages in detecting low parasite loads, is not affected by temperature, and does not require any reagents. Geimsa-stained thin film blood smear slides were analyzed in this study. 240 slides with ring stage *Plasmodium falciparum* infected human blood were prepared from culture. *P. falciparum* negative controls included 80 clinical *P. vivax* slides (collected and verified by expert microscopy (EM) and Polymerase Chain Reaction (PCR)), 40 slides with *Salmonella*- infected human blood (prepared from culture), and 40 uninfected human blood slides. Infrared spectra were measured from a small area of each slide (~12 microns x 12microns) usually containing only one red blood cell. Algorithms were written to differentiate *Plasmodium* positive spectra from *Plasmodia* negative spectra and tested by cross-validation. The sensitivity was 98.8% to 100% and the specificity was 95.4% to 100% for *Plasmodia* positive samples with a 95% confidence interval. These results suggest that further study of FTIR spectroscopy as an automated reagent-less diagnostic method with potential for detection of single parasites is warranted. Infrared spectroscopy could radically lower marginal test costs by eliminating the need for expensive consumables.

138

RAPID PARASITEMIA DETERMINATION BY FLOW CYTOMETRY USING A DNA-BINDING FLUORESCENT DYE

Gyo Jun, Yun-Jae Jung, **Jae-Won Park**

Graduate School of Medicine, Gachon University of Medicine and Science, Incheon, Republic of Korea

Although the determination of parasitemia by light microscopy with Giemsa staining still remains as the golden standard in malaria diagnosis, this method becomes very laborious when the number of samples increases. To set up a high-throughput assay system of *Plasmodium falciparum* malaria, we tried to use flow cytometry (FACS) using PicoGreen that binds to double-strand DNA, and compared the results obtained by light microscopy and FACS. The two methods yielded fairly concordant parasitemias, the latter being more reproducible and faster than the former. For two samples or less, the microscopic method was faster, but FACS was faster for more than three samples. With the latter method, parasitemias of 200 samples could be determined by one person in about 6 hours including sample preparation steps when 50,000 cells per sample were read. Because the parasitemia values by the FACS method included some background noises of 0.2-0.5%, however, samples had to be treated with RNase prior to PicoGreen staining. Although this step was helpful in reducing the background to some extent, approximately one third of it persisted presumably due to mitochondrial DNA of reticulocytes. Under our assay condition, the dilution factor of PicoGreen over the range of 1/2,000 and 1/200,000 resulted in almost similar parasitemias.

139

PREVALENCE AND TRANSMISSION PATTERN OF *PLASMODIUM FALCIPARUM* INFECTION IN OSOGBO METROPOLIS, SOUTHWEST, NIGERIA

Adekunle O. Olowe, O. T. Ogungbamigbe, O. O. Ojurongbe, P. S. Ogunro, P. S. Elemile

Ladoke Akintola University of Technology, Osogbo, Osun State, Nigeria

Plasmodium falciparum malaria is an endemic disease especially in tropical areas with heavy rainfall that spread round the year. We therefore sought to investigate the prevalence pattern and clinical presentation of *falciparum* malaria in Osogbo and level of degree of prevalence were assessed and screened for *Plasmodium falciparum* infection by clinical assessment and microscopy using both thick and thin blood smears over a period of 12 months- August 2004 and July 2005. The prevalence of *Plasmodium falciparum* infection was found to be 52.8% with 341/646 of the patients been positive for *P. falciparum* parasite based on microscopy. Three hundred and five (47.2%) were aparasitaemic of which 162 (25.1%) had bronchopneumonia, 99 (15.3%) had upper respiratory tract infection, 32 (5.0%) had gastroenteritis and 12 (1.9%) had Otitis media. Between August and November 2004, 250 patients were screened and 160 (57.6%) of these patients were positive, while 180 patients were screened between December 2004 and March 2005 and 51 (28.3%) were positive. Between April 2005 and July 2005, 216 patients were screened and 130 (60.2%) of the patients were positive. When compared, the differences in the percentage of patients with positive microscopy in December to March with April to July and August to November were found to be significant ($P < 0.0001$), whereas the percentage difference in patients with positive microscopy in August to November and April to July was not significant ($P = 0.442$). The result of this study clearly shows that there are two distinct peaks of malaria transmission pattern in consonance with the rainfall pattern in the area.

140

QUANTIFYING AND MODELLING CROSS-BORDER HUMAN POPULATION MOVEMENTS INTO KENYA IN RELATION TO MALARIA INFECTION MOVEMENTS

Deepa Pindolia

KEMRI-University of Oxford-Wellcome Trust Collaborative Programme, Nairobi, Kenya

High levels of *Plasmodium falciparum* malaria transmission are found in certain areas of Kenya, principally areas bordering Uganda around Lake Victoria and bordering Tanzania at the coast. Cross-border movement is hypothesized to play a part in maintaining pockets of high transmission and human movement from such areas to regions of lower or zero transmission are likely to make malaria control and elimination challenging. These difficulties justify quantitative investigation of patterns and rates of population movement and infections they carry in and out of high transmission areas. This research draws on a wide range of data sources to investigate the role of human movement in driving transmission in these areas. Micro-census data, travel history surveys, air passenger flows, settlement location and sizes, cross-border crossings and road traffic datasets have been assembled to explore the range of population movements seen across Kenya and how they may vary by malaria season, region and demographic characteristics of the population. These will then be integrated with existing spatial datasets for Kenya, including demographic data, malaria endemicity maps and detailed transport networks to build network-based meta-population models of human and parasite movements, and to explore the likely effects of these movements on differing control policy scenarios. To understand population movement patterns, meta-population gravity models over a range of spatiotemporal scales have been developed, which will be fitted to the movement data and be used to predict movement patterns in parts of the country where data is less readily available. Linking these models with recently developed malaria transmission maps and simulation models allows assessment of malaria dispersal across Kenya and surrounding regions. The malaria transmission model used is an individual-based simulator, developed by the Malaria Atlas Project. The framework developed permits human movement to be incorporated and its implications of these to be assessed. The models will form evidence-based tools for malaria control planning in Kenya and whilst focus will remain on Kenya, methodologies developed will ultimately have strong relevance and application to other malaria endemic areas across the globe and for the study of other infectious diseases.

141

COMPLICATED *VIVAX* MALARIA AT A REFERENCE CENTRE IN THE BRAZILIAN AMAZON

André M. Siqueira[1], Belisa L. Magalhaes[1], Marcia A. Alexandre[1], Priscila C. Ballut[1], Quique Bassat[2], Marcus V. Lacerda[1]

[1]Tropical Medicine Foundation of Amazonas, Manaus - AM, Brazil, [2]Barcelona Center for International Health Research, CRESIB, Barcelona, Spain

In South America, around 80% of Malaria episodes are caused by *Plasmodium vivax*. Although formerly seen as a benign condition, an increasing number of reports have changed this perception, pointing to a wide-array of complications associated with infection by this parasite. The objective of this study was to to describe the clinical manifestation of complicated *P. vivax* infection at a tertiary-reference centre in the Brazilian Amazon. All the inpatients with *P. vivax* infection evidence admitted at the Tropical Medicine Foundation of Amazonas at Manaus underwent a thorough clinical evaluation and additional complementary exams (several blood analysis,urine, feces and radiological tests) for clinical characterization of their malaria episodes, and accurate diagnosis of concomitant conditions. PCR diagnosis was performed in all patients to confirm *Plasmodium* species and exclude coinfections with *P. falciparum*. During a nine-month period, 154 patients were admitted at our hospital with an associated diagnosis of *P. vivax* infection. Of these, 32 were children younger than 12 years of age and 81 fulfilled one or more of the WHO severity criteria defined for *P. falciparum*. Previous co-morbidities were present in 82% of these inpatients. The most common severe criterion was hyperbilirrubinemia (bilirubin> 3.0 mg/dL), occurring in 77% of the patients (62/81), followed by severe anemia (Hemoglobin < 7.0 g/dL in adults and < 5.0 in children), which was present in 30% of the patients (25/81). Other complications that occurred include respiratory distress, acute kidney failure and splenic rupture. G6PD deficiency was diagnosed in 26 patients, who presented with hemolytic anemia following use of primaquine. Two patients died during follow-up, one of them with extensive subdural hematoma (a 76-year-old patient with previous arterial hypertension) and the other one, a patient with chronic liver failure, due to severe gastrointestinal bleeding. *P. vivax* infection may present with a wide-array of clinical complications both in children and adults. The use of primaquine for radical cure of *P. vivax* hypnozoites increases the risk of hemolytic complications among people with G6PD deficiency. More detailed analysis and case control studies being undertaken at our centre will certainly help to identify risk factors for complications associated with *P. vivax* infection, and validate WHO definitions currently based only on *P. falciparum* cases.

142

THE EFFECT OF CHANGES IN RAINFALL ON THE BURDEN OF MALARIA IN AREAS OF HIGH AND LOW TRANSMISSION SETTINGS

Ruth K. Nassali[1], Hasifa Bukirwa[1], Stella Kakeeto[1], Fred Kizito[1], Asadu Sserwanga[1], Grant Dorsey[2], Moses Kamya[3], Ann Gasasira[1]

[1]Uganda Malaria Surveillance Project, Kampala, Uganda, [2]University of California, San Francisco, CA, United States, [3]College of Health Sciences, Makerere University, Kampala, Uganda

The effect of rainfall on malaria risk may vary across differing transmission and environmental settings and further by the level of intervention deployment. Clarifying this relationship may be informative to malaria control programs. The objective of this study was to determine the effects of change in rainfall on the malaria burden in 2 different endemic settings. The Uganda Malaria Surveillance Project collects daily malaria morbidity data from 6 sentinel health facilities around Uganda. In this ongoing study, we utilized malaria data from the lowest and highest transmission sentinel settings: Kamwezi Health Center in Kabale district (EIR < 1) and Aduku Health Centre, in Apac district (EIR = 1564) respectively. Routinely collected daily rainfall data from Kabale and Apac districts were obtained from Uganda's national meteorological department. We used linear regression models to assess the association between total monthly rainfall and malaria slide positivity rate (SPR) for the next month, adjusting for age and number of malaria laboratory tests done. This preliminary analysis includes data collected over 26 months in Kabale/Kamwezi and 41 months in Apac/Aduku. The median total monthly rainfall in Kabale was 92.7mm (IQR 61-114.6) and 112.3mm (IQR 4.4-202.1) in Aduku. Age-standardized SPRs ranged from 13.4% to 65.6% in Kamwezi (median 29.4%) and from 26.2%% to 57.7% in Aduku (median 46.1%). In Kabale, a 1mm increase in rainfall increased the SPR by 0.002% (p = 0.036). Changes in rainfall were not associated with changes in malaria diagnosed in Apac. These initial findings suggest a modest association between increase in rainfall and subsequent malaria upsurges in areas of low but not high transmission intensity. Data collection on intervention coverage and environmental factors is ongoing.

143

RISK FACTORS FOR ANAEMIA IN CHILDREN WITH *PLASMODIUM FALCIPARUM* MALARIA IN THE MOUNT CAMEROON REGION: ROLES OF NUTRITION, WORMWOOD AND IRON DEFIECIENCY

Irene U. Sumbele

University of Buea, Buea, Cameroon

The lack of up to date epidemiological data on malaria and anaemia in many parts of Cameroon is a serious handicap towards effective control of these conditions. This study had as objectives to identify the risk factors for anaemia and to determine the influence of wormwood, nutritional status and iron deficiency on malaria anaemia. Malariometric and nutritional indices were measured in 817 children between the ages of 6 months to 14 years over a period of 2 years. The prevalence of asexual parasitaemia was 75.0% (613). The overall prevalence of anaemia (Hb < 11g/dl) was 81.3% (664). The prevalence of pyrexia was 24.7% with a significant positive correlation between temperature and malaria parasitaemia density (r = 0.03, P = 0.01). Splenomegaly was significantly prevalent (P < 0.01) in gametocyte positive children (36.2%, 38/105) when compared with gametocyte negative children 19.3%. The prevalence of splenomegaly (23.6%) and gametocytaemia (25.6%) confirms malaria endemicity in this region. The prevalence of gametocytaemia may be due to the observed low parasitaemia and the high prevalence of anaemia. Malnutrition as assessed by a < -2 z-score in any one of the anthropometric indices height-for-age (HA), weight-for-age (WA), weight-for-height (WH), was prevalent in 22.2% of the children. The prevalence of stunting (19.6%) was more common than underweight (7.2%) and wasting (2.2%) which likely reflects the low socio economic status of the inhabitants. Multilinear regression analysis showed the level of education of caregiver (P < 0.05), high WBC count (P < 0.0001), parasitaemia density (P <0.01), length of fever > 2 days (P < 0.01), spleen size (P < 0.05), male sex (P < 0.05), management of onset of malaria by the caregiver (P <0.005), stunting (P < 0.05), ferritin and transferrin (P < 0.001) were the risk factors for anaemia. Iron deficiency had a significant influence on malarial anaemia although a large proportion of anaemia cases could not be explained by iron deficiency indicating that malaria is a significant cause of anaemia in the study population

144

IMPLEMENTING THE FIRST NATIONAL SCHOOL MALARIA SURVEY IN KENYA: PROCESS, MAIN FINDINGS AND IMPLICATIONS FOR SURVEILLANCE AND CONTROL

Caroline W. Gitonga[1], Peris Karanja[1], Mariam Mwanje[2], Jimmy Kihara[3], Akpaka Kalu[4], Elizabeth Juma[5], Abdisalan M. Noor[1], Robert W. Snow[1], Simon Brooker[1]

[1]KEMRI/Wellcome Trust Nairobi, Nairobi, Kenya, [2]Division of Vector Borne and Neglected Diseases, Ministry of Public Health and Sanitation, Nairobi, Kenya, [3]Eastern and Southern Africa Centre of International Parasite Control, KEMRI, Nairobi, Kenya, [4]World Health Organization, Nairobi, Kenya, [5]Division of Malaria Control, Ministry of Public Health and Sanitation, Nairobi, Kenya

National malaria control programmes require up-to-date, sub-national information on malaria transmission in order to guide intervention strategies according to malaria risk. In 2009, the Government of Kenya launched its second National Malaria Strategy for the period 2009-2017 which includes a new Malaria-free Schools Initiative. Here we present results from a national survey of malaria infection and coverage of malaria control interventions among Kenyan 55,737 children in 552 schools. Infection was defined in the field using malaria rapid diagnostic tests (RDT) and haemoglobin assessed using a portable photometer. All RDT-positives and a selection of RDT-negatives were validated through microscopy. A questionnaire was administered to pupils to obtain data on mosquito net ownership and use and when treated, recent travel history, recent history of illness, and socio-economic and household variables. The overall prevalence of *Plasmodium* infection and anaemia was 7.2% and 20.8% respectively. 22.4% of children reported using an insecticide treated net (ITN). Patterns of infection, anaemia and net use varied markedly across the country, with infection prevalence being highest in western Kenya. Malaria risk in the western highlands and along the Kenyan coast was more geographically heterogeneous, whereas there was extremely low malaria risk in central Kenya. Only 1.7% of schools reported ITN use >60%. These data show that there are large areas of Kenya, mainly in Central, Eastern and Rift Valley provinces, that do not merit any direct school-based malaria intervention. School-based interventions, coupled with strengthened community-based strategies, are warranted in western Kenya, whereas a geographic targeting of intervention suites should be considered in the western highlands and along the Kenyan coast. School malaria surveys provide a rapid, cheap and sustainable approach to malaria surveillance and risk mapping and should be seen as an essential component of future monitoring and evaluation strategies in Kenya.

145

MALARIA IN NAIROBI

Sandra A. Mudhune[1], Abdisalan M. Noor[1], Emelda A. Okiro[1], Faith Osier[1], Margaret Mackinnon[1], Pete W. Gething[2], Dejan Zurovac[1], Sophie Githinji[1], Sam A. Ochola[3], Elizabeth Juma[4], Robert Snow[1]

[1]KEMRI-Wellcome Trust Research Programme, Nairobi, Kenya, [2]Department of Zoology, University of Oxford, Oxford, United Kingdom, [3]Ministry of Health, Nairobi, Kenya, [4]Division of Malaria Control, Nairobi, Kenya

Research on the epidemiology of malaria in Africa has traditionally focused on areas of stable, high transmission where infection results in high disease burden. Consequently, little is known about its epidemiology in low transmission settings such as urban areas. Despite documented transmission in the area currently occupied by Nairobi pre-1940 there have only been sporadic studies of malaria risk in recent years. A more detailed examination of the epidemiology of host infection risks is necessary to develop specific treatment and prevention guidelines for Nairobi residents. Four different approaches will be used to address the data deficiencies. First, clinic fever surveys which involve a rapid assessment of the prevalence of malaria infection among patients presenting to 10 clinics with a history of fever. Second, school surveys where children were examined for malaria infection using RDTs. These same 10 schools will be revisited and an additional filter paper blood spot will be collected for PCR and serological analysis. Third, rapid assessment of the quality of care, diagnosis of malaria and case management in health facilities. And finally surveys on EPI attendees where we aim to identify the most plausible estimation of autochthonous transmission by examining the prevalence of infection and history of exposure among resident children. In March 2009 1333 children were examined, 5.5% were identified as having a positive RDT. Among the 74 positive cases 40.5% had travelled outside of Nairobi in the last eight weeks and of these 70% had travelled to an area classified as malaria-risk. However a similar proportion of test negatives had travelled in the last eight weeks 556 (44.5%) and of these 32.6% had travelled to a malaria risk district. In July 926 children were examined. Blood slides were re-examined by expert microscopists for all 17 RDT positives and 10% of RDT negatives. 1.84% of the children had a positive RDT result but this number dropped to 1.08% when the results were confirmed via microscopy and only one child has a history of travel. At this stage the possibility of autochthonous transmission cannot be ruled out and additional results can inform on the true risks.

146

MALARIA HYPO-ENDEMIC FOCI AND EPIDEMIC RISK ON JAVA AND BALI, INDONESIA IN 2009

Iqbal Elyazar[1], David L. Smith[2], Pete Gething[3], Anand Patil[3], Carlos A. Guerra[3], Hanifah Rogayah[4], Desak M. Wismarini[4], Rita Kusriastuti[4], Kevin Baird[1], Simon I. Hay[3]

[1]Eijkman-Oxford Clinical Research Unit, Jakarta, Indonesia, [2]Department of Zoology, University of Florida, Gainesville, FL, United States, [3]Spatial Ecology and Epidemiology Group, Department of Zoology, University of Oxford, Oxford, United Kingdom, [4]National Communicable Disease Center, Indonesia Ministry of Health, Jakarta, Indonesia

Recent calls for strategies aimed at malaria elimination emphasize the importance of prompt identification of persistent or erupting foci of transmission. The challenge is to place limited resources precisely where and when needed. Two essential measures of malaria intensity in elimination stages are the malaria basic reproductive number (Ro) and the reproductive number under some level of control (Rc). Bayesian geostatistical models were used to predict continuous maps of *Plasmodium falciparum* parasite rate (*Pf*PR) from over 200 spatially independent parasite rate estimates from community surveys conducted in Java and Bali in the Indonesian archipelago. Subsequently, stochastics models were employed to define the relationship between *Pf*PR, entomological innoculation rate (EIR) for *P. falciparum* (*Pf*EIR) and the basic reproductive rate for *P. falciparum* (*Pf*Ro). A transmission intensity map for *P. falciparum* (*Pf*Rc) was then generated from the models. The higher spatial resolution *Pf*Rc map is expected to provide a rational basis for malaria elimination planning and setting precise targets of intervention. These results are summarized across Java and Bali and the implications for elimination elaborated.

147

CLINICAL LABORATORY REFERENCE RANGES DERIVED FROM RURAL HEALTHY LOCAL POPULATION OF HEALTH DISTRICT OF SAPONÉ IN BURKINA FASO

Adama Gansane[1], Issa Nebie[1], Amidou Diarra[1], Souleymane Sanon[1], Issiaka Soulama[1], Alphonse Ouedraogo[1], Jean Baptiste Yaro[1], Esperance Ouedraogo[1], Andre Lin Ouedraogo[1], Edith C. Bougouma[1], Amadou T. Konate[1], Alfred B. Tiono[1], Sodiomon B. Sirima[2]

[1]Centre National de Recherche et de Formation sur le Paludisme (CNRFP), Ouagadougou, Burkina Faso, [2]Centre National de Recherche et de Formation sur le Paludisme (CNRFP)/Groupe de Recherche et d'Action en Sante (GRAS), Ouagadougou, Burkina Faso

In Africa, biological parameters that are used in clinical laboratories are from Europe and the United States. The conduct of clinical trials in African sites using references values from others population excludes potential volunteers and makes adverse events assessment challenging as the primary objective of Phase I trials is to demonstrate the safety of the investigational according to CFR and ICH guidelines. For the planning of malaria vaccine trials in Saponé Health District, a malaria endemic area in Burkina Faso, we conducted a study to establish the biological reference ranges for biochemistry, hematology among healthy local population. Two cross sectional surveys conducted respectively during malaria high and low transmission season in healthy adults and children from 14 randomly selected villages out of 89 villages of Sapone Health District and stratified in equal proportions in 7 age-groups and gender for adults: 6 months- 1 year, 1-3 years, 3-6 years, 6-10 years and 10-15 years, 15-45 years male, 15-45 years female. The hematology and chemistry analysis were done with validated analyzer with strong records of internal and external quality controls. Data from the two surveys were pooled and using the methods described in the NCCLS-approved guideline, reference intervals for each measured parameter were calculated non-parametrically by taking the 2.5 and 97.5 percentiles of the observed samples values. From a total of 2520 patients who were screened during both surveys, 2049 were included in the analysis with at least 270 volunteers per age-group and gender for adults. Estimated Hemoglobin and hematocrit references range were lower in our local population than the western ones while alkaline phosphatase, ALT, AST, WBC, lymphocytes were higher in the former. Similar references intervals were found with electrolytes (Na, K, Ca, Cl), creatinin, RBC, total bilirubin, direct bilirubin, albumin and glucose. In conclusion, reference intervals of haematological and biochemical indices based on results from population of developed countries of the same age are different to the estimated values for population of the Health District of Saponé in Burkina Faso. These findings support implementation of malaria vaccines trials in this area using site-specific biological references intervals for enrolment and monitoring of patients.

148

SPONTANEOUS CLEARANCE OF *PLASMODIUM FALCIPARUM* PARASITEMIA WAS MORE COMMON IN UGANDAN CHILDREN WITH SICKLE CELL TRAIT THAN IN THOSE WITH NORMAL HEMOGLOBIN

Lauren S. Gong[1], Catherine Maiteki-Sebuguzi[2], Philip Rosenthal[3], Bryan Greenhouse[3]

[1]University of California Berkeley - University of California San Francisco Joint Medical Program, Berkeley, CA, United States, [2]Makerere University - University of California San Francisco Collaboration, Kampala, Uganda, [3]University of California San Francisco, San Francisco, CA, United States,

The genetic abnormalities glucose-6-phosphate dehydrogenase (G-6-PD) deficiency and sickle cell trait (HbAS) offer protection against *falciparum* malaria. They may protect through improved immune clearance of parasites, but this hypothesis has not been tested clinically. 601 randomly selected children from Kampala, aged 1-10, were followed for a median of 1.4 years. Blood smears were read every 30 days and any time a child presented with fever or history of fever. Children with malaria, defined as asexual parasites on blood smear and fever, were treated after randomization to one of 3 combination therapy regimens. Hemoglobin electrophoresis for HbAS and spectrophotometry to assess G-6-PD activity (deficient: <110 mU/109 erythrocytes) were performed at enrollment. HbSS children were excluded. To follow individual strains, parasitemic samples were genotyped by assessment of polymorphisms in *merozoite surface protein 2* by nested PCR and capillary electrophoresis. Our primary outcome was spontaneous clearance of parasites, a surrogate for effective antimalarial immunity. With HbAS and G-6-PD deficiency as our predictor variables, we used generalized estimating equations to estimate the relative risk of spontaneous clearance of parasites, adjusting for age. Ninety-nine children (16.5%) had HbAS and 62 (10.3%) were G-6-PD deficient. Genotyping revealed 2295 parasite strains in 370 subjects, giving an incidence of parasitemia of 2.8 per person year. Older children were more likely to clear infections once parasitemic (RR = 1.16 / year of age, 95%CI 1.10-1.23, p<0.001). Children with HbAS were significantly more likely to clear infections than those with HbAA (RR = 1.43, 95% CI 1.01-2.01, p=0.04). No association was found between G-6-PD deficiency and rate of clearance of infections. These results support the hypothesis that HbAS is protective against *falciparum* malaria, at least in part, due to increased immune clearance of parasites.

149

SPATIAL ANALYSIS OF PEDIATRIC MALARIA IN WESTERN KENYA

John Waitumbi, **Peter Sifuna**, Carol Tungwony

WRP/KEMRI, Kisumu, Kenya

There is data to suggest that malaria severity is influenced by the force of parasite exposure. The latter is susceptible to a variety of spatial and temporal variables that affect mosquito dynamics. Geographical information systems (GIS) provide an easier way of correlating disease to spatial data. In the present study, spatial information on children presenting at Kisumu District Hospital (KDH), Western Kenya, with either severe or uncomplicated malaria were mapped in relation to rainfall, drainage, altitude and land use. Geographic identifier of patient homes (nearest market center, nearest schools and proximity to known unique features) were collected from 120 subjects attending KDH with either severe malaria (N=60) or mild malaria. Points representing the geographic location of the cases were overlaid onto a map of drainage features (rivers, lake and flood plains), rainfall (800-2000 mM) and topography. Of the spatial features, only rainfall and drainage affected distribution of malaria cases. 93% of patients fell within a distance of 2 km from the rivers basins, lake and flood plains but there was no particular clustering of malaria in relation to disease severity. All the patients fell within the 800-2000 mM annual rainfall belt, with 14% falling in the 800-1200 mM, 55.6% in the 1200-1600 mM and 23.6% within the 1600-2000 mM. In conclusion, drainage and rainfall are the major determinants of exposure to malaria in the holoendemic Lake Victoria basin. Data will be presented to show how GIS can be utilized to describe determinants of malaria exposure and how to achieve targeted interruption of transmission.

150

HUMAN MOVEMENT PATTERNS RELEVANT FOR MALARIA TRANSMISSION IN TANZANIA AND MALI

Deirdre Hollingsworth, Azra Ghani

Imperial College London, London, United Kingdom

Human movement patterns are important drivers of disease transmission, and yet there are few studies which quantify these patterns across large areas, and very few in rural Africa. The demographic and health surveys (DHS) include questions on overnight trips in the previous 12 months, which are asked to adults aged 15-45 of both sexes. These data allow within and between country comparisons of travel patterns relevant for malaria transmission. Here, we compare travel patterns in Tanzania and Mali, as a first direct comparison between east and west Africa. Data on the number of visits in the last 12 months for more than 30,000 adults were analysed using a generalised logit model. Probabilities of making no visits, 1 visit, 2-5 visits and more than 5 visits in the last 12 months were calculated with reference to gender, region of residence, age, occupation and wealth index. In both Tanzania and Mali, all these variables were found to be important predictors of frequency of travel, (p<0.001). Men had higher odds of travelling than women. For example, the odds of travelling more than 5 times (as opposed to 0,1 or 2-5 times) for men are 5.385 (95% CI 4.521-6.415) times those for women in Tanzania (9.917 (95% CI 8.269-11.893) for Mali). Those with a low wealth index were less likely to travel for an overnight visit. For example, those with the lowest income have an odds of 0.487 (95%CI 0.342-0.695) that of the highest wealth class of travelling at least once for Mali when compared with the odds of not travelling. Regions with higher population densities were associated with a lower probability of making no overnight visits. The probability of making at least one visit was significantly different between the two countries, even when accounting for other factors (p<0.0001). Overall 41% of individuals made at least one overnight visit in Tanzania, whereas 32% of those surveyed in Mali made at least one overnight visit. These results show that whilst there are shared determinants of travel patterns, there are important differences in national characteristics. Further study is required to disentangle the reasons for these differences and their consequences for malaria transmission.

151

SITUATION ANALYSIS AND PUBLIC HEALTH INTERVENTIONS TO PREVENT MALARIA IN KENYA

John Mutuku Muli, Peter Kisac, Moses Mbwau, **Vladimir Krcmery**

Mary Immaculate Clinic in Nairobi, Kenya, St. Elizabeth University College of Health and Social Sciences, Bratislava, Slovakia

In Kenya malaria is found in nearly all its provinces except in Nairobi. The leading provinces in malaria endemicity are those near the Indian Ocean and the south and lakes Victoria in western province and Lake Turkana in the north. Nairobi is a focus of our study due to its being the capital but also it is the melting point of the all country, every of Kenya's fourty two communities are found in Nairobi. The site of the study was the Mary Immaculate Clinic in Nairobi. The Clinic provides health care for the urban poor with a population of more than 100,000 inhabitants of Nairobi especially in the informal settlements. We counted the number of positive and negative patients who were tested for malaria since the year 2005 to 2009, a span of five years. The criteria used to according to the clinic protocol for malaria testing is, fever with a high temperature of 38C, joint pains, chills, among others. Testing was done by microscopy. A total of 8157 patients were counted from the laboratory records book. Many

of the patients that were tested have only basic primary education. The malaria species endemic was *Plasmodium falciparum*. In the year 2005 the total number of malaria positive was 17%. A total of 83% patients were negative. The total of positive and negatives were 1860. In the year 2006 the total malaria positives were 242 (16%) out of 1509 (84%), in these two years there was no significant increase in malaria patients with time. In the year 2007 there were 217 (15%) malaria positives and 1488 (85%) malaria negatives. There were 200 (12%) malaria positives in 2008 and 1653 (88%) negative. Despite the increase in the number of patients tested in 2008 there were no significant increase in positivity in those two years. The same can be concluded of the year 2009. In conclusion, the malaria data in the five years confirms that the occurrence of malaria in Nairobi is low. Though only 1218(15%) out of 8157 (85%) had malaria, the positives are clinically significant, but cannot be referred to qualify Nairobi as a malaria zone. It is therefore prudent to for the healthcare system to take more action to educate the public when travelling to use preventive measures and adhere to treatment when sick with malaria.

152

INDIVIDUAL HETEROGENEITY AND THE POTENTIAL REBOUND EFFECT OF MALARIA INTERVENTION STRATEGIES

John M. Williamson[1], Hung-Mo Lin[2], Anna J. Blackstock[3]

[1]Centers for Disease Control and Prevention, Kisumu, Kenya, [2]Mount Sinai School of Medicine, New York City, NY, United States, [3]Atlanta Research and Education Foundatioin, Decatur, GA, United States

Most trials designed to test the effectiveness of a malaria prevention method, such as insecticide-treated nets (ITNs) or insecticide-treated curtains (ITCs), randomize newborns or clusters of newborns to one or more treatment groups. These trials are then analyzed following the intention-to-treat framework. It is hypothesized that infants using malaria prevention methods early in life do not have the opportunity to build adequate immunity to malaria, thus causing higher mortality rates in these infants later in life. This is often referred to as a "rebound" in mortality. We focus on the situation in which the all-cause mortality rates of two groups of randomized infants (e.g., ITNs and no nets) are of interest. We assume that ITNs are given to the group with no nets at the end of year one and that all children, including those initially assigned to the ITN group, are followed for another year. Mortality rates for the two groups are compared for year one and again for year two to assess the treatment effectiveness. If a rebound effect exists, then the children who were initially randomized to the ITN group should have a higher mortality rate in the second year of life than the children initially randomized to the non-ITN group. Biological variation between individuals can account for a large portion of the variability seen in medical and public health studies and can distort observed effects (Aalen, 1998). We demonstrate with randomly generated data that a potential rebound effect can be caused by individual heterogeneity, as treatment groups followed from the end of year one are no longer randomized. We also use data from a randomized, controlled trial conducted in Burkina Faso (Diallo et al., 2004) to illustrate the relationship between individual heterogeneity and the rebound effect. This study found a mortality rate ratio of 1.16 in children aged 24-59 months when comparing original treatment groups after all study participants had been allocated ITCs, and we show that this effect could have arisen from individual heterogeneity alone.

153

SIMULATING MALARIA TRANSMISSION DYNAMICS IN THE PILOT SITES OF THE COLOMBIAN INTEGRATED NATIONAL ADAPTATION PLAN: STEPS FORWARD OF THE INTEGRATED SURVEILLANCE AND CONTROL SYSTEM

Daniel Ruiz[1], Adriana María Molina[1], Viviana Cerón[2], Patricia Gutiérrez[2], Martha Lucía Quiñónes[3], Mónica Marcela Jiménez[4], Madeleine Thomson[5], Stephen Connor[5], Maria Elena Gutiérrez[1], Paula Andrea Zapata[1], Catalina López[1], Remi Cousin[5], Salua Osorio[2], Gilma Mantilla[5]

[1]Grupo de Investigación 'Gestión del Ambiente para el Bienestar Social – GABiS', Escuela de Ingeniería de Antioquia, Envigado, Antioquia, Colombia, [2]Grupo Factores de Riesgo del Ambiente, Subdirección de Vigilancia y Control en Salud Pública, Instituto Nacional de Salud, Bogotá, Colombia, [3]Facultad de Medicina, Universidad Nacional de Colombia Sede Bogotá, Bogotá, Colombia, [4]Instituto Colombiano de Medicina Tropical, Universidad CES, Sabaneta, Antioquia, Colombia, [5]International Research Institute for Climate and Society, Columbia University in the City of New York, New York, NY, United States

Changes in climatic conditions are likely to alter malaria incidence and spatial distribution in Colombia. As part of the Integrated National Adaptation Plan, the Colombian Institute of Health is working on the implementation of a proactive, collaborative, multidisciplinary, integrated surveillance and control system (ISCS). The aim of this initiative is to improve risk assessments of malaria transmission in order to facilitate effective allocation of health resources and more cost-effective preventive responses. One of its key components is an Early Warning System Framework, in which we are proposing several dynamical and statistical models. Dynamical models, in particular, are being used to integrate climatic variables with non-climatic factors in order to simulate malaria transmission dynamics. Twelve process-based models were studied and included in a single multi-model ensemble. Five tools were initially applied in the pilot sites where the ISCS is being implemented. Activities included the characterization of local eco-epidemiological settings and numerical simulations. Characteristics such as general profile (population at risk, natural resources, economic activities), climatic conditions (climatology, long-term trends), entomology (primary and secondary vectors, breeding sites, feeding frequencies, preferences), malaria situation (annual cycles of malaria incidence, stability conditions), and non-climatic factors (including control campaigns) were analyzed to assess local conditions. Simulations included retrospective experiments (base scenarios, changes in initial conditions, local settings, sensitivity analyses, and uncertainties) of at least 8-year simulation periods, as well as short-, medium- and long-term future changing scenarios. Complementary activities included the study of local spatial patterns of vectorial capacity, descriptions of the vulnerability of populations at risk, and a conceptual framework for the analysis of non-climatic drivers. Outreach activities included the design of interactive and online platforms as well as the documentation of our experiences. Dynamical models have improved our understanding of malaria complexity, allowed us to estimate previous malaria outbreaks in the selected pilot sites, and helped us to investigate decision-making processes. All these activities constitute steps forward in the implementation of the Colombian ISCS.

154

MALARIA ASSOCIATED SYMPTOMS IN PREGNANT WOMEN: RESULTS OF A COHORT FOLLOW-UP IN BENIN

Bich-Tram Huynh, Nadine Fievet, Achille Massougbodji, Philippe Deloron, Michel Cot

Research Insitute for Developpement, Paris, France

Little is known on the symptoms of malaria infected pregnant women in stable endemic areas, as it is generally admitted they have acquired an immunity protecting them from acute clinical signs. By following-up

Beninese pregnant women, this study aims to evaluate the clinical burden of malaria in a highly endemic area. An ongoing prospective cohort of 1039 women followed monthly from their first antenatal visit (ANV) until delivery is conducted in three rural dispensaries since August 2008 in Benin. 570 women seen at ANVs, unscheduled visits and at delivery were analysed for the presence of symptoms. We used a multivariate logistic regression to determine the association between symptoms and malaria infection assessed by a positive rapid diagnostic test (RDT). During routine ANVs, headache was the only symptom associated with a higher risk of malaria (aOR=2.6; p<0.001) and was reported by 35% of infected women. On the occasion of unscheduled visits, fever (aOR= 4.1; p<0.001), headache (aOR= 2.1; p=0.01) and shivering (aOR= 3.2; p<0.001) were significantly associated with a malaria infection and 82% of infected women presented at least one of these symptoms. We found an increasing proportion of positive RDTs in late pregnancy more than one month after the last intermittent preventive treatment dose (IPTp); moreover malaria infections during unscheduled visits occurred long after the last IPTp intake. In conclusion, the majority of pregnant women were symptomless during routine visits when infected with malaria in an endemic stable area. Only, during unscheduled visits a significant proportion of infected women were symptomatic. The prevention of malaria in pregnancy could be improved by using systematic RDTs to identify infected women consulting during non routine visits. The design of IPTp could also be optimized by reassessing the number of doses and time of administration of SP.

155

NATIONWIDE PREVALENCE OF MALARIA IN CAMBODIA IN 2007: COMPARISON OF MICROSCOPY AND PCR

Dysoley Lek[1], **William O. Rogers**[2], Frederic Ariey[3], Jane Bruce[4], Sylvia Meek[4], Seshu Babu[1], Socheat Duong[1]

[1]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [2]Naval Medical Research Unit #2, Jakarta, Indonesia, [3]Institut Pasteur du Cambodge, Phnom Penh, Cambodia, [4]Malaria Consortium, London School of Hygiene and Tropical Medicine, London, United Kingdom

In order to assess the current status of malaria in Cambodia and to compare it with the situation found in 2004, a nationwide malaria survey was conducted in November-December 2007, at the end of the rainy season, the time of peak malaria transmission. This was a stratified, multi-stage, cluster sampling survey. The country was divided into three domains based on expected malaria prevalence. The domain that included the provinces immediately around Phnom Penh was not surveyed, due to the very low prevalence found in previous surveys. The remaining provinces were divided into domains 1 and 2. Within each domain 38 clusters (villages) were selected; the clusters were stratified according to risk zones based on the distance from the village to the nearest forest (<250 m, 251-1000 m, 1-2 km, 2-5 km). Within each cluster 40 households were sampled, and from each household, 4 individuals provided malaria smears and filter paper blood spots for PCR-based diagnosis using the mitochondrial cytochrome b gene as a target. Based on microscopy, the overall estimated malaria prevalence and prevalences of *P. falciparum* and *P. vivax* infection in the sampled domains were 2.9% (95% CI, 1.8-4.6%), 1.6% (0.9-2.7%), and 0.9% (0.6-1.6%) respectively. The corresponding prevalences found in 2004 were 4.4% (2.8-6.8%), 2.9% (1.7-5.1%), and 1.3% (0.8-2.1%); this decline in prevalence, while appreciable, was not statistically significant. In order to determine the extent to which microscopy might underestimate the malaria prevalence, we performed PCR on 7707 samples; in these samples the malaria prevalences estimated by microscopy and PCR were 2.8% and 6.9%, respectively; 289 of 7162 microscopy negative samples (4.0%) were positive by PCR. The high prevalence of infection undetected by microscopy suggests that prevalence surveys based only on microscopy may significantly underestimate malaria prevalence. If these sub-microscopic infections contribute to transmission, then mass screening and treatment based on microscopy alone may miss a significant reservoir of infection.

156

SPATIO-TEMPORAL DISTRIBUTION OF MALARIA IN HAINAN PROVINCE, CHINA

Dan Xiao[1], Yong Long[1], Shanqing Wang[2], Guangze Wang[2], Yongping Yan[1]

[1]Department of Epidemiology, Faculty of Preventive Medicine, The Fourth Military Medical University, Xi'an, China, [2]Hainan Center for Disease Control and Prevention, Haikou, China

Hainan Province is one of the regions of the highest malaria incidence in China. Our study analyzed the distribution of malaria and the change of main epidemic areas from 1995 to 2008, to provide basis for the prevention and control of malaria in Hainan Province. The study was based on the data of each county/city between 1995 and 2008. Records of malaria cases were obtained from Hainan Center for Disease Control and Prevention and demographic data from Hainan statistical yearbook. Cluster analysis of time-space scanning was performed with the maximum cluster size of 25% of the population used SatScan 8.0. The temporal cluster analysis of 1995-2008 showed that 2003-2004 was the most likely cluster (*RR*=1.86, *P*=0.001). The space-time cluster analysis of 1995-2008 showed 7 counties/cities (San Ya, Bao Ting, Le Dong, Wu Zhishan, Ling Shui, Bai Sha and Qiong Zhong) in 2003-2004 was the most likely cluster (*Incidence*=2671.0/100,000, *RR*=4.97, *P*=0.001). The space-time cluster analysis of 1995-2002 showed 5 counties/cities (Bao Ting, San Ya, Wu Zhishan, Ling Shui and Qiong Zhong) in 1997-1998 was the most likely cluster (*Incidence*=1852.8/100,000, *RR*=4.49, *P*=0.001) and 3 counties/cities (Chang Jiang, Dong Fang and Bai Sha) in 2001-2002 the secondary one (*Incidence*=1258.6/100,000, *RR*=3.25, *P*=0.001). The space-time cluster analysis of 2005-2008 showed 5 counties/cities (Ling Shui, Bao Ting, Wan Ning, Qiong Zhong and Wu Zhishan) in 2005 was the most likely cluster (*Incidence*=1193.7/100,000, *RR*=4.55, *P*=0.001) and 4 counties/cities (Dong Fang, Chang Jiang Le Dong and Bai Sha) in 2006 the secondary one (*Incidence*=1038.9/100,000, *RR*=3.30, *P*=0.001). In conclusion, during 1995-2008, malaria incidence reached its peak in 2003-2004 and the southern Hainan Province was the main epidemic area. Although the average incidence decreased, the main epidemic area was expanded to the southeastern and southwestern Hainan Province gradually. Hence, future public health planning and resource allocation in Hainan Province should be focused on these areas.

157

INSIGHT INTO ANTIGENIC DIVERSITY OF VAR2CSA-DBL5ε DOMAIN FROM MULTIPLE *PLASMODIUM FALCIPARUM* PLACENTAL ISOLATES

Sédami Gnidehou[1], Leon Jessen[2], Stéphane Gangnard[3], Caroline Ermont[1], Choukri Triqui[1], Mickael Quiviger[1], Juliette Guitard[1], Ole Lund[2], Philippe Deloron[1], Nicaise Tuikue Ndam[1]

[1]Institut de Recherche pour le Développement, Paris, France, [2]Center for Biological Sequence Analysis, Technical University of Denmark, Lyngby, Denmark, [3]Institut Pasteur, Paris, France

High levels of anti-VAR2CSA antibodies levels are associated with protection against pregnancy-associated malaria. VAR2CSA contains molecular signatures associated with parity in one of its domain, and variants preferentially infecting primigravidae are thought to be the most virulent. Therefore it is critical to identify sequence characteristics of this molecule that can interfere with immune response. Highly conserved domains of VAR2CSA such as DBL5ε are likely to contain conserved epitopes, and therefore constitute attractive targets for vaccine development. Sequences of the VAR2CSA-DBL5ε domain obtained from cDNA of 40 placental isolates were analysed by experimental and in silico tools. Competition ELISA assays on two DBL5ε variants, using women plasma samples from two different areas and mice specific antisera, indicated that DBL5ε possess conserved and cross-reactive B cell epitopes. Peptide ELISA identified conserved areas that are recognised by naturally acquired antibodies. Specific antibodies against these peptides

labelled the native proteins on the surface of placental parasites. Despite high sequence homology, both VAR2CSA DBL5ε recombinant proteins displayed different recognition patterns by plasma from malaria-exposed women, and their ability to bind proteoglycans. Sequence analyses showed that, like the previously characterised VAR2CSA DBL3X domain, DBL5ε also contains motifs that discriminate parasites according to donor's parity. In conclusion, this study provides insights into conserved and exposed B cell epitopes in DBL5ε that can act as potential mediator for cross reactivity. The importance of sequence variation in VAR2CSA as a critical challenge for vaccine development is highlighted. As the final conformation of the entire VAR2CSA molecule seems to be essential to its functionality, identification of sequence variation sites in distinct locations within VAR2CSA that affect its antigenic and/or binding properties is of major interest in the effort of developing an efficient VAR2CSA-based vaccine. Motifs associated to parasite segregation according to parity are among these critical issues.

158

SITE CHARACTERIZATION FOR A MALARIA VACCINE TRIAL IN THE SAPONÉ HEALTH DISTRICT IN BURKINA FASO: SEASONAL PREVALENCE OF MAIN PARASITES INFESTATION

Ouedraogo Alphonse[1], Tiono B. Alfred[1], Diarra Amidou[1], Ouedraogo Amathe[1], Ouedraogo Espérence[1], Kabore Youssouf[1], Kargougou W. Desire[1], Kangoye T. David[1], Bougouma C. Edith[1], Soulmana Issiaka[1], Sanon Souleymane[1], Gansane Adama[1], Konate T. Amadou[1], Nebie Issa[1], Sirima B. Sodiomon[2]

[1]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [2]Centre National de Recherche et de Formation sur le Paludisme, Groupe de Recherche Action en Santé, Ouagadougou, Burkina Faso

Populations living in areas where future malaria vaccine trials may be conducted must be characterized not only with respect to the parameters that will be used to establish safety, but also with respect to conditions that may modify the immune response to candidate malaria vaccines. Studies conducted in Africa and Asia indicates that helminths can influence the acquisition of immunity against *Plasmodium* by driving the immune responses towards the production of the non-cytophilic subclasses. The aim of this study was to estimate the prevalence of various parasitic infections according to season in potential participants to malaria candidate vaccine trials in Burkina Faso. We conducted 2 community-based cross sectional surveys in volunteers aged 2 to 45 years in the Sapone health district. Survey 1 was performed during the rainy season, and the second at the dry season. During each survey, clinical examination has been performed and blood samples have been taken for malaria and *Wuchereria bancrofti* diagnosis. Stools and urine were also collected for determination of helminthes and *Schistosoma hematobium*. The diagnosis of intestinal helminthes was done by Kato-Katz thick smear examination technique. The mean age of the volunteers was similar during the 2 surveys (p=0.44). From 1587 stools samples analyzed, 132 (8.3%) had helminth or other intestinal infections. The prevalences were higher at the rainy season as compared to the dry season. The main helminth infections were *Ankylostoma duodenale* (5.9% vs 2.1%; P<0.00), *Ascaris lumbricoides* (1.7% vs 0%; P<0.00), *Trichuris trichiuria* (0.8% vs 0%; P<0.00). Others intestinal parasites were *Hymenolepis nana* and *Taenia* sp. (4.5% vs 2.1%; P<0.00). The seroprevalence of *W. bancrofti* was 11.0% (12.8% vs 9.4%, P=0.03). *S. hematobium* infection was present in 2.3% (1.7% vs 2.9%, P=0.13) of the study population. According to age group the prevalence of malaria infection was higher at the rainy season (< 5years: 68.9%; ≥ 5years: 54.8%) as compared to the dry season (< 5years: 57.8%; ≥ 5years: 46.3%). In conclusion, these data show diversity and intensity of parasitic infections in Saponé health district area according to malaria transmission season. The trends of helminths infections and malaria infection coincide and are both high during the malaria high transmission season. This should be considered when designing future malaria vaccine trial.

159

IMMUNOLOGICAL EFFICACY OF VACCINE-INDUCED ANTIGEN-SPECIFIC CD8+ T CELLS AGAINST *PLASMODIUM YOELII* BLOOD STAGE INFECTION

Takeshi Ono, Yoko Yamaguchi, Jun Yamada, Saori Umemoto, Atsuhiro Kanayama, **Yasushi Miyahira**

National Defense Medical College, Tokorozawa, Saitama, Japan

There is a consensus that CD8+ T cells are critical for conferring hosts' protective immunity against the malarial liver stage; however on the contrary, the lack of MHC molecule on red blood cells has questioned their protective roles against its blood stage infection. This skepticism was supported by an observation that the depletion of CD8+ T cells during the malarial blood stage infection did not affect its natural course and outcome. However, since the CD8+ T cell-mediated vaccine strategy has presented new development in recent years, it is worth elucidating the immunological efficacy of the active induction of antigen-specific CD8+ T cells, particularly the prime-boost vaccination strategy which is the most effective vaccination protocol for the induction of maximal number of antigen-specific CD8+ T cells. To address the question whether the actively induced CD8+ T cells in maximal number are capable for conferring hosts' protective immunity against the malarial blood stage, we have established an experimental system by generating a genetically engineered *Plasmodium yoelii* which expresses a *Trypanosoma cruzi* antigen-derived, H-2Kb-restricted-CD8+ T cell epitope, ANYNFTLV. Expression of the epitope by the transgenic parasites was confirmed by the detection of ANYNFTLV-specific CD8+ T cells in mice, either which were immunized with adjuvant-emulsified parasitized red blood cells or which were cured by the injection of chloroquine after the infection with transgenic parasites. We have then tested the immunological efficacy of the prime-boost recombinant virus vector vaccination, the multiple passive transfers of ANYNFTLV-specific CD8+ T cell line and the combination of both against the challenge infection with the ANYNFTLV-expressing transgenic parasites. The critical roles of CD8+ T cells during the malarial blood stage infection and their background immunological mechanisms will be presented and discussed.

160

HETEROLOGOUS PRIME-BOOST VACCINATION WITH ADCH63 AND MVA EXPRESSING MSP1 CAN INDUCE PROTECTIVE EFFICACY AGAINST SPOROZOITE CHALLENGE IN VOLUNTEERS

Susanne H. Sheehy[1], Christopher J. Duncan[1], Sean Elias[2], Katharine Collins[2], Katie Ewer[2], Nick Edwards[2], Andrew Blagborough[3], Robert Sinden[3], Jitta Murphy[4], Simon Correa[5], Angela Hunt-Cooke[1], Joel Meyer[1], Patrick Lillie[1], Stefano Colloca[6], Riccardo Cortese[6], Alfredo Nicosia[6], Ian Poulton[1], Carole A. Long[7], Sarah C. Gilbert[2], Alison Lawrie[1], Adrian V. Hill[2], Simon J. Draper[2]

[1]Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford, Oxford, United Kingdom, [2]The Jenner Institute, University of Oxford, Oxford, United Kingdom, [3]Imperial College Division of Cell and Molecular Biology, London, United Kingdom, [4]Walter Reed Army Institute of Research, Washington, MD, United States, [5]MRC Laboratories, Banjul, Gambia, [6]Okairos S.R.L, Rome, Italy, [7]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Viral vectored vaccines encoding blood-stage malaria antigens can stimulate potent cellular and humoral immune responses in mice and rhesus macaques and induce protective efficacy in rodent malaria models. We sought to test the safety, immunogenicity and efficacy of this approach in a Phase I/IIa clinical trial using the simian adenovirus 63 (AdCh63) and the poxvirus MVA encoding a novel insert including conserved blocks of sequence and both alleles of the 42kDa C-terminus of the blood-stage malaria antigen MSP1. In a Phase I dose escalation

study in Oxford, UK, 16 healthy malaria naive volunteers were primed with AdCh63 MSP1 and 12 of these volunteers boosted 8 weeks later with MVA MSP1. High level antibody responses were measured against the C-terminal 19kDa region of MSP1 ($MSP1_{19}$) as well as the strongest T cells responses yet reported by subunit vaccination, as measured by ex-vivo IFN-γ ELIspot using peptides spanning the entire vaccine antigen. Given the qualitatively different type of immune responses induced by viral vector vaccines (in comparison to recombinant protein-in-adjuvant vaccines routinely used by other researchers), three vaccinees and six unvaccinated controls underwent sporozoite challenge three weeks after the MVA boost as a Phase IIa safety study. These vaccinees demonstrated a significant delay in time to diagnosis (by positive blood film) compared with the unvaccinated controls (P=0.032). No unexpected adverse events were observed. This is the first demonstration of statistically significant clinical efficacy induced by a vaccine targeting the blood-stage antigen MSP1, and provides the first evidence that vaccines inducing cell mediated responses in conjunction with antibody responses to a blood-stage antigen used alone are safe as well as effective. This AdCh63-MVA viral vectored vaccine regimen also provides a new and safe approach for the development of vaccines for other infectious diseases where it is likely that strong cellular and humoral immunity will be required for protective efficacy in humans.

161

EXTENDED SAFETY, IMMUNOGENICITY AND EFFICACY OF WALTER REED ARMY INSTITUTE OF RESEARCH'S AMA-1 MALARIA VACCINE (FMP2.1) ADJUVANTED IN GSK BIOLOGICALS' AS02A IN 1-6 YEAR OLD CHILDREN IN BANDIAGARA, MALI

Mahamadou A. Thera[1], Drissa Coulibaly[1], Matthew B. Laurens[2], Abdoulaye K. Kone[1], Ando B. Guindo[1], Dapa Diallo[1], Karim Traore[1], Amagana Dolo[1], Youssouf Tolo[1], Mahamadou S. Sissoko[1], Bourema Kouriba[1], Kirsten E. Lyke[3], Shannon Takala-Harrison[3], William C. Blackwelder[3], Johan Vekemans[4], Joe Cohen[4], Tina Dube[5], David E. Lanar[6], Sheetij Dutta[6], Brent House[6], Carter L. Diggs[7], Lorraine Soisson[7], D. Gray Heppner[6], Ogobara K. Doumbo[1], Christopher V. Plowe[2], Idrissa Traore, Issa Diarra, Amadou Niangaly, Modibo Daou, Mady Sissoko, Yukun Wu, Olivier Godeaux, Marie-Claude Dubois, Ripley Ballou, Darby Thompson, Jason W. Bennett

[1]Malaria Research and Training Center, University of Bamako, Bamako, Mali, [2]Howard Hughes Medical Institute/Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, [3]Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, [4]GlaxoSmithKline Biologicals, Rixensart, Belgium, [5]The EMMES Corporation, Rockville, MD, United States, [6]Division of Malaria Vaccine Development, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [7]Malaria Vaccine Development Program, United States Agency for International Development, Washington, DC, United States

The malaria vaccine candidate FMP2.1/AS02A was recently evaluated in Bandiagara, Mali, West Africa. Data collected up to 6 months following the final immunization showed acceptable safety, and high-level antibody responses, but limited efficacy (VE) against first clinical malaria episodes (VE 17.4%, p=0.175) or against any clinical episode (VE 20.0%, p=0.068). Extended safety, immunogenicity and efficacy data were collected for 24 months. Four hundred healthy children aged 1-6 were randomized 1:1 to receive three doses of 50 µg of FMP2.1 in 0.5mL of AS02A or rabies vaccine, 30 days apart. The primary efficacy endpoint is time to first or only clinical malaria episode occurring between randomization and six months after the third immunization. Secondary endpoints include time to first clinical malaria episode and incidence of all clinical episodes (using increasing parasitemia thresholds) occurring during the entire follow-up period. The vaccine showed no safety signal, and was well-tolerated. High-level antibody responses were maintained and boosted during the subsequent malaria season. Extended efficacy of the vaccine against first clinical malaria episodes was 7.6% (p=0.507) and against any clinical episode was 9.9% (p=0.193). In conclusion, the lack of extended efficacy of the vaccine in the second malaria transmission season may be due to waning immunity that is not reflected in anti-AMA1 antibodies as measured by ELISA, or to a shift in AMA1 haplotypes at the site. Studies to determine the precise immune correlates of vaccine-induced immunity and detailed analyses of allele-specific efficacy and vaccine selection may lead to strategies to develop an improved AMA-1 vaccine.

162

CONSTRUCTION AND IMMUNOGENICITY OF A DNA VACCINE PLASMID ENCODING AMA-1 OF THE REEMERGING KOREAN *PLASMODIUM VIVAX*

H.J. Kim[1], B.K. Jung[1], J.J. Lee[1], K.H. Pyo[1], T.Y. Kim[2], B.I. Choi[3], H. Hisaeda[4], K. Himeno[5], E.H. Shin[1], **J.Y. Chai**[1]

[1]Seoul National University College of Medicine, Seoul, Republic of Korea, [2]Yonsei University College of Medicine, Seoul, Republic of Korea, [3]Korea University College of Medicine, Seoul, Republic of Korea, [4]Kyushu University, Fukuoka, Japan, [5]Gunma University Graduate School of Medicine, Maebashi, Japan

A DNA vaccine plasmid encoding *Plasmodium vivax* AMA-1 (PvAMA-1) of the reemerging *P. vivax* in Korea has been constructed and its immunogenicity examined in recipient BALB/c mice. A large AMA-1 protein band of about 56.8 kDa was obtained from COS7 cells transfected with the expression plasmid UBpcAMA-1. In BALB/c mice immunized intramuscularly 4 times with the PvAMA-1 vaccine with or without IL-12, serum IgG titers increased significantly compared to controls. Levels of IL-10, having a T-cell inhibitory function, were significantly depressed in immunized and immunized plus IL-12 treated mice. In contrast, IFN-γ levels showed little changes even in immunized plus IL-12 stimulated mice, and flow cytometry of spleen cells from immunized mice revealed no significant changes in the proportions of CD8+ cells and CD4+ cells. However, when mice were immunized using a gene gun, the proportion of CD8+ cells increased significantly in immunized and immunized plus IL-12 treated mice. The results indicate that the immunogenicity of the PvAMA-1 DNA vaccine was not strong enough when injected intramuscularly but suggest that the immunogenicity could be potentiated using the gene gun injection technique.

163

CHARACTERIZATION OF PFS25-EPA CONJUGATES BY AGAROSE GEL ELECTROPHORESIS AND WESTERN BLOTTING

Daming Zhu, Holly McClellan, Nicholas MacDonald, Patrick Duffy, Yimin Wu

Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States

The Pfs25 protein is a transmission-blocking vaccine candidate against malaria. To enhance the immunogenicity, Pfs25 was conjugated to carrier proteins including a mutant, nontoxic *Pseudomonas aeruginosa* ExoProtein A (EPA) or the outer-membrane protein complex (OMPC) of *Neisseria meningitidis* serogroup B. The Pfs25 conjugates have been demonstrated to be 1000-fold greater in antibody titers when compared to the unconjugated Pfs25 in animal studies. In order to meet the requirements for clinical trials by regulatory agencies, the drug product identity and integrity have to be performed. However, due to the large size of molecular mass of the conjugates (greater than 700 kDa as estimated in this study), the most common characterization methods such as SDS-PAGE (suitable for proteins with molecular masses less than 200 kDa) and SDS-PAGE-based western blots are not suitable for quality control evaluations. The present study utilized the agarose as gel matrix and Pfs25-EPA conjugates as model protein samples for the integrity and identity studies. The Pfs25-EPA conjugates were analyzed by a 5 x 6 cm 2% Seakem ME (Lonza) agarose gel using tris-glycine running buffer conditions and

followed by Coomassie blue staining for visualization. For identity analysis by western blotting, the gels were subsequently transferred to PVDF membranes and probed by mAb 4B7 against Pfs25, mAb against penta-His tag, and polyclonal Ab against exotoxin A. Our results showed that the Pfs25-EPA conjugates had a range of molecular masses, approximately from 500 to 800 KDa as determined by the agarose gel. This molecular mass range was further confirmed by size-exclusion chromatography with multi-angle light scattering (SEC-MALS). Comparable protein conjugate migration patterns were detected by both agarose gel/Coomassie staining and agarose gel/western blotting. The results suggest that epitopes of Pfs25 and EPA remained detectable following the chemical modifications and electrophoresis using agarose gels. Overall, the present studies demonstrate that agarose gel alone or in combination with western blot analysis is a simple, reliable and economic technique to assess the identity and integrity of molecule with large molecular masses and will have a general application for analyzing proteins or their conjugates which are unable to be evaluated using SDS-PAGE.

164

PROTECTION AGAINST MALARIA CHALLENGE BY VACCINATION OF *AOTUS* MONKEYS WITH ADJUVANTED BLOOD STAGE MALARIA VACCINES

Yimin Wu[1], Kelly Rausch[1], Lynn Lambert[1], Sachy-Orr Gonzalez[1], Timothy S. Dutill[2], Christopher B. Fox[2], Kazutoyo Miura[1], Michael Fay[3], David Narum[1], David Jones[1], Robbin Anders[4], Ross Coppel[5], Joan Aebig[1], Ruth Ellis[1], Laura Martin[1], Randall F. Howard[2], Heather Davis[6], Steve Reed[2], Louis Miller[1]

[1]LMIV, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]Infectious Disease Research Institute, Seattle, WA, United States, [3]BRB, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [4]La Trobe University, Melbourne, Australia, [5]Monash University, Melbourne, Australia, [6]Pfizer, Ottawa, ON, Canada

The goal of this study is to evaluate a Blood Stage Antigen Combination (BSAC) containing leading vaccine candidates, AMA1, MSP1, MSP2, MSP3, and MSP4, in an *Aotus* challenge model. Previously we have demonstrated that AMA1 can confer in vivo protection of A. nancymai monkeys against Plasmodium *falciparum* challenge after monkeys received a recombinant AMA1 protein formulated with complete Freund's adjuvant, an adjuvant not suitable for human use. In order to down select a single human-compatible adjuvant system we first conducted an Aotus challenge study using recombinant AMA1 protein. Three groups (N=8) of A. nancymai monkeys received 3 doses of a recombinant AMA1 formulated with a synthetic TLR 4 agonist in an oil-in-water emulsion (EM005), an oil-in-water emulsion alone (EM001), or Alhydrogel+ the TLR9 agonist, CPG 10104. A control group received 3 doses of saline. Three weeks after the third vaccination monkeys were challenged with Aotus red blood cells infected with a homologous P. *falciparum* parasite. Thin blood films from individual monkeys were examined daily for detection of parasites in peripheral blood. Protection was assessed by the monkeys' abilities to control infections. While there were no statistically significant differences between the groups in protection from challenge, it appeared that similar limited levels of protection were observed in monkey groups receiving AMA1/Alhydrogel+CPG 10104 or AMA1/ EM005. In the second challenge study, the BSAC mixture was formulated with EM005. Three groups (N=11) of A. nancymai monkeys received 3 doses of i) an all-5 antigen mix formulated with EM005; ii) a 4-antigen mix (excluding AMA1) formulated with EM005; or iii) saline control. The monkeys were challenged with Aotus red blood cells infected with a homologous parasite, and protection was assessed by monkey's abilities to control infections. Significant protection was observed in both vaccine groups. Details of the study will be presented in the meeting.

165

DEVELOPMENT OF AN AD28-BASED, MULTIPLY-DELETED AND FIBER-MODIFIED, MULTI-ANTIGEN ADENOVIRAL-VECTORED VACCINE FOR MALARIA

Christopher D. Reiter, Svetlana V. Konovalova, Bennett A. Myers, Shaojie Weng, Richard L. Grier, Nathaniel G. Balsley, Joseph Bruder

GenVec Inc., Gaithersburg, MD, United States

Malaria is the most burdensome parasitic disease of man, exacting an estimated toll of 863,000 deaths and 243 million clinical cases per year. Vaccines for malaria are greatly needed, but no technology is yet developed that completely addresses the current need. We are currently developing a capsid-modified, multivalent, adenoviral-vectored vaccine based on the adenoviral serotype 28 against *Plasmodium falciparum* for future human testing. The advantages of using adenoviral-vectored vaccines are several. They generate strong effector and helper T cell responses, strong antibody responses, and produce protective immunity in multiple disease models including against malaria in man. Previously, clinical tests of adenoviral-vectored vaccines for malaria have been based on adenoviral serotype 5. Ad5 has been shown to be safe in extensive clinical testing and produces strong immune responses to the payload antigen; however, the use of Ad5-based vaccines is limited for malaria due to the prevalence of pre-existing neutralizing antibodies to Ad5 in sub-Saharan Africa. To avoid this issue, we are developing our vaccine based on the adenoviral serotype Ad28 as a platform for adenoviral-vectored vaccines. We have found that Ad28 generates stronger immune responses than other alternative serotypes to Ad5, such as Ad35. Recent studies in non-human primates and in humans suggest that a multivalent vaccine may be more effective at protection than vectored, single-antigen vaccines. Along these lines, we have developed a stable, multiply deleted, Ad28 vector that contains multiple *Pf* antigens. We and others have noted that vaccines based on alternative serotypes have generally under-performed Ad5 vectors with regard to induction of antigen-specific immune responses. To address this issue, we are investigating several fiber modifications to the Ad28 fiber that have been shown to increase alternative serotype immunogenicity. In conclusion, our data shows that a multiply deleted Ad28-based vector generates strong immune responses in mice and has potential as a malaria vaccine

166

AN ALL-SYNTHETIC NANOSPHERE VACCINE TARGETING *PLASMODIUM FALCIPARUM* ENOLASE INDUCES POTENT AND LONG LASTING ANTIBODY TITERS IN MICE

Hiroyuki Oku[1], Kazuhiko Yano[1], Megumi Fukumoto[1], Shigeyuki Kano[2]

[1]Gunma University, Gunma, Japan, [2]National Center for Global Health and Medicine, Tokyo, Japan

Enolase catalyzes at the 9th step of the 11 enzymes in the glycolytic pathway. Our field serological studies have suggested that antigens toward *Plasmodium falciparum* enolase were strongly presented by the sera taken from endemic inhabitants who have present and/or recent past infection. To use our findings for vaccine development, we have designed an all-synthetic vaccination material to realize the immunity condition in endemic area, in which residents are sequentially infected and thus sustain immunity against parasite infection. In this presentation, we wish to report (1) nano-encapsulation of a synthetic antigenic peptide based on the enolase, (2) *in vitro* and *in vivo* degradation, and (3) immunological properties of nanospheres. (1) The synthetic antigen consisting of a part of the enolase sequence (22 amino acid residues) was prepared by Fmoc peptide chemistry. The nanospheres were formulated using an oil/water emulsion technique with bioabsorbable synthetic polymers, poly(lactic acid-co-glycolic acid) and poly(vinyl alcohol). The antigen content was adjusted to 4 and 10 μg/mg of the material depending

on the experimental conditions. (2) *In vitro* and *in vivo* degradation of the nanoparticle were observed by monitoring a fluorescence from labeled antigen molecules. In *in vitro*, the antigen was released from the nanospheres slowly and continuously with nearly zero-order kinetics until 40 days. Then, in *in vivo* condition, the antigens were observed even at 28 days after implanting subcutaneously 1 mg of the nanosphere (4 µg antigen) in each nude mouse. (3) Mice were immunized by subcutaneous injection of 5 mg nanoparticle (50 µg antigen). The antibody response of the mice was over 50-fold increase at the 15 weeks if the IgG titer was compared with non-encapsulated control. The titers were increasing through 60 weeks. These results suggest that this synthetic nanoparticle is a promising candidate as a long-lasting antigenic material toward an effective malarial vaccine.

167

INSECTICIDE RESISTANCE IN THE ANTHROPOPHILIC MOSQUITOES *ANOPHELES ARABIENSIS* AND *CULEX QUINQUEFASCIATUS* IN MACHA, ZAMBIA

Laura C. Norris, Douglas E. Norris

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

The mosquito *Anopheles arabiensis* is the major vector of *Plasmodium falciparum* in Macha, Zambia. The arboviral and filarial vector *Culex quinquefasciatus* is also present in high numbers throughout the Macha region. A major portion of Zambia's current malaria control program relies on long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) with insecticides. Insecticide resistance in mosquito populations has the potential to lessen and even eliminate the effectiveness of these control methods. CDC bottle bioassays and LLIN survival assays were used to characterize the *An. arabiensis* colony established at Macha, and this data was used as a baseline against which to compare field mosquitoes. F1 offspring of field-collected adult *An. arabiensis* from and *Cx. quinquefasciatus* from eggs collected from oviposition traps were tested for insecticide resistance. High levels of resistance to DDT, pyrethroids, malathion, and deltamethrin-treated net material were detected in *Cx. quinquefasciatus*, and low levels of resistance to DDT and deltamethrin-treated net material were detected in *An. arabiensis*. Molecular assays revealed that the knock-down resistance (*kdr*) allele was frequent in the *Cx. quinquefasciatus* population, but further investigation is required to determine the level of this mutation in malaria vectors. Continued monitoring and assessment is necessary in these populations in order to determine levels of resistance and appropriately modify vector control operations.

168

SPECIFIC IMMUNO-EPIDEMIOLOGICAL BIOMARKERS OF EXPOSURE TO *AEDES ALBOPICTUS* AND *AE. AEGYPTI* BITES

Souleymane Doucoure[1], François Mouchet[1], Sylvie Cornelie[1], François Favier[2], Phillippe Gasque[3], Jean Sébastien DeHecq[4], Yelin Roca[5], Annie Walter[1], Sirilaksana Patramool[6], Dorothé Misse[6], Jean Pierre Hervé[1], Franck Remoue[1]

[1]Institut de Recherche pour le Développement, UR016, Montpellier, France, [2]Centre d'Investigation Clinique-Epidémiologie Clinique, Saint Pierre La Réunion, Réunion, [3]Université de La Réunion, Saint Denis La Réunion, Réunion, [4]Direction Régionale des Affaires Sanitaires et Sociales, Saint Denis La Réunion, Réunion, [5]Centro Nacional de Enfermedades Tropicales, Santa Cruz, Plurinational State of Bolivia, [6]Institut de Recherche pour le Développement, GEMI, Montpellier, France

Aedes mosquitoes are among the main vectors of mosquito borne diseases. Both *Aedes* mosquitoes and the mosquito borne diseases that they transmit are currently expanding geographically. This situation stresses the need for accurate monitoring of these vectors populations. We aim to develop new methods to evaluate Human/Vector contact by immuno-epidemiological tools complementary to entomological methods. Specifically our aim is to evaluate human IgG responses to *Aedes albopictus* (La Réunion) and *Aedes aegypti* (Bolivia) salivary proteins, to give insights on the population exposed to *Aedes* bites. Our results indicate that assessing human IgG anti *Aedes* whole salivary proteins by ELISA can be used to detect individual exposure to vector bites and can therefore help to evaluate the risk of pathogen transmission. We observe no systematic IgG cross reaction between *Ae. albopictus* and *Ae. aegypti* salivary proteins.

Western blot experiments also reveal different patterns of immunogenic salivary proteins between these two vectors: we find not only common immunogenic salivary proteins to *Aedes* genera, but also specific immunogenic proteins to *Ae. albopictus* and *Ae. aegypti*. In addition, these characteristics may be used to discriminate exposure to *Aedes* vectors and furthermore to develop specific biomarkers of exposure to *Ae. albopictus* and *Ae. aegypti* bites. Characterization of specific immunogenic salivary proteins of *Ae. albopictus* and *Ae. aegypti* is under investigation. Such biomarkers, specific to *Ae. aegypti* and *Ae. albopictus* bites, could be used for monitoring emerging *Aedes* borne diseases and to evaluate efficacy of vector control programs.

169

EVALUATION OF LONG-LASTING BIOLOGICAL LARVICIDE AGAINST *ANOPHELES* MOSQUITOES IN KENYA

Yaw A. Afrane[1], Robert Sjorgren[2], Andrew Githeko[1], Guiyun Yan[3]

[1]Kenya Medical Research Institute, Kisumu, Kenya, [2]FourStar Microbial Products, Sag Habor, New York, NY, United States, [3]University of California, Irvine, CA, United States

Synthetic insecticides are the main chemicals for malaria vector control. Biological insecticides are attractive alternatives for larval mosquito control as they are benign to the environment. However, the currently available bio-larvicide formulations have a short effective duration, and consequently larval control incurs a high operation expense due to requirement for frequent re-treatment of larval habitats. Therefore, formulation of biological larvicides that has long-lasting effects is highly desired. A fourStarTM Single Brood Granules (SBG) of *Bacillus thuringiensis israelenis* (Bti) was evaluated under semi-natural and natural conditions in Kenya. This formulation is designed to be effective against mosquito larvae for up to 6 months. In semi-natural habitats containing soil and rain water, second-instar larvae of *Anopheles gambiae* were introduced, and FourStarTM Bti granules dissolved in rain water with appropriate concentrations were added. The number of pupae produced was recorded daily. We found 100% mortality rate within 48 hrs after fourStarTM Bti was dissolved for two months. The field trial in stable and productive natural habitats is currently ongoing.

170

BIOCHEMICAL MECHANISMS INVOLVED IN DDT AND PYRETHROID RESISTANCE IN TRINIDAD AND TOBAGO STRAINS OF *AEDES AEGYPTI*

Karen A. Polson[1], Samuel C. Rawlins[2], William G. Brogdon[3], Dave D. Chadee[1]

[1]The University of the West Indies, St. Augustine, Trinidad and Tobago, [2]The Caribbean Epidemiology Centre, Port of Spain, Trinidad and Tobago, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

The objectives of this study were to investigate the status of the organochlorine dichlorodiphenyltrichloroethane (DDT) and Pyrethoid (PY) resistance in Trinidad and Tobago strains of *Aedes aegypti* and the underlying biochemical mechanisms. Nine strains of *Ae. aegypti* larvae from Trinidad and Tobago were assayed to DDT and PYs (deltamethrin and permethrin) using the Centers for Disease Control and Prevention (CDC) time-mortality based bioassay method. A diagnostic dosage (DD) was established for each insecticide using the CAREC reference susceptible strain and a Resistance Threshold (RT) - time in which 98-100% mortality was observed in the CAREC strain - was calculated for each insecticide.

Mosquitoes which survived the DD and RT were considered as resistant and the resistance status of each field strain was categorized based on the WHO criteria with mortality <80% indicative of resistance. Biochemical assays were conducted to determine the activities of α and β esterases, mixed function oxidases (MFO) and glutathione-S-transferases (GST) enzymes which are involved in resistance of mosquitoes to DDT and PYs. Enzymatic activity levels in each strain were compared with those obtained for the CAREC susceptible strain and significant differences were determined by Kruskal-Wallis and Tukey's non-parametric tests ($p<0.05$). The established DDs were 1µg/100ml, 20µg/100ml and 100µg/100ml for deltamethrin, permethrin and DDT, respectively; and the RTs for deltamethrin, permethrin and DDT were 30, 75 and 120 mins, respectively. All field strains were resistant to DDT (<80% mortality), two strains were incipiently resistant to deltamethrin and three to permethrin (80-98% mortality). Biochemical assays revealed elevated levels of α-esterase and MFO enzymes in all strains. All, except three strains, showed increased levels of β-esterases and all strains, except Curepe, demonstrated elevated GST levels. Metabolic detoxification of enzymes is correlated with the manifestation of DDT and PY resistance in Trinidad and Tobago strains of *Ae. aegypti*. The presence of this resistance also suggests that knock down (kdr)-type resistance may be involved, hence the need for further investigations. This information can contribute to the development of an insecticide resistance surveillance program and improvement of resistance management strategies in Trinidad and Tobago.

171

COMMUNITY USE OF LONG-LASTING INSECTICIDAL NET IN COMBINATION WITH CARBAMATE TREATED PLASTIC SHEETING FOR INSECTICIDE RESISTANCE MANAGEMENT IN MALARIA VECTORS

Djenontin Armel[1], Chandre Fabrice[2], Osse Razaki[3], Chabi Joseph[1], Akogbeto Martin[3], Corbel Vincent[1]

[1]CREC/IRD, Cotonou, Benin, [2]LIN/IRD, Montpellier, France, [3]CREC, Cotonou, Benin

Due to the spread of insecticide resistance in African malaria vectors, there is an urgent need to develop alternative tools and strategies for the control and management of resistant mosquito populations. In this context, a new Insecticide Resistance Management (IRM) strategy based on the community use of Long Lasting insecticidal Net (LLIN) and Carbamate-Treated Plastic Sheeting (CTPS) was evaluated in southern Benin. A randomized controlled trial (phase III) was carried out in 21 villages in the district of Tori-Bossito. The impact of a full coverage of LLIN, alone or in combination with CTPS, was investigated in terms of malaria transmission and insecticide resistance management in comparison with a control group (i.e. selective coverage of LLIN to children < 5 following the National Malaria Control Program policy). 55,405 mosquitoes of which 1,713 *Anopheles gambiae* and 1,091 *Anopheles* funestus were collected from July 2008 to December 2009. *Anopheles* funestus density was significantly reduced (about 80%) with LLIN and LLIN+CTPS groups compared to the NMCP group ($P<0.001$). No significant reduction of *Anopheles gambiae* density was however observed with a full coverage of LLIN compared to the control ($P=0.061$), whereas combination of LLIN+CTPS significantly reduced the population size of *An. gambiae* (49% reduction, $P<0.001$). The Entomological Inoculation Rate was reduced by 40% ($P=0.010$) and 70% (P0.05). After 18 months intervention, this frequency increased in all treated arms but the frequency evolved faster with a full coverage of LLIN compared to the combination of LLIN+CTPS ($P=0.005$). Regarding carbamate resistance, the frequency of the ace 1R allele was low in the study site (<10%) but did not increase regardless the treatments ($P>0.05$). This study confirmed previous findings in experimental huts showing that a combination of LLIN and CTPS in a same dwelling is promising for the control and management of pyrethroid-resistant malaria vectors in Africa.

172

EFFICACY OF A MOSAIC LONG-LASTING INSECTICIDE NET (PERMANET3.0) AGAINST WILD POPULATIONS OF RESISTANT *CULEX QUINQUEFASCIATUS* IN EXPERIMENTAL HUTS IN TOGO (WEST AFRICA)

Dominic B. Dery

Kintampo Health Research Centre, Kintampo, Ghana

The efficacy of new Long Lasting Insecticide Net; PermaNet3.0, against *Culex quinquefasciatus* was evaluated in six experimental huts (February-March, 2008) in Lomé (Togo). Endpoints of evaluation were deterrence, exophily, blood feeding inhibition and mortality. Also, wash resistance of the net and its efficacy on vectors was compared with commercially marketed PermaNet2.0 net. In parallel, field susceptibility and resistant status of *Cx. quinquefasciatus* and *Anopheles gambiae* local populations were assessed by testing to Permethrin (1%), DDT (4%), Bendiocarb (0.1%), Deltamethrin (0.5%, 0.05%), Carbosulfan (0.4%) and Chlorpyrifos Methyl (0.4%) using WHO test tubes and protocol. Subsequent evaluation of Kdr status was done in *An. gambiae* s.s. 1,223 *Cx. quinquefasciatus* females were collected in six week evaluation period (one Latin square rotation). The unwashed PermaNet3.0 deterred 16.84% of total *Culex* mosquitoes caught. After 20 washes, the net deterred 5.79% mosquitoes compared to 6.84% by unwashed PermaNet2.0 net. Also, the net induced mosquitoes to exit huts by 50.48% and inhibited blood feeding 70.97% in unwashed state. After 20 washes, the net induced 42.91% mosquitoes to exit and inhibited 67.06% of mosquitoes from blood feeding. The new PermaNet3.0 gave 76% personal protection at zero wash and 69% protection after 20 washes. More so, the net retained almost equal its insecticidal effect at zero wash (7.1%) and after 20 washes (6.5%). In susceptibility test, *An. gambiae* populations showed resistance to DDT, Permethrin and Carbosulfan (12%, 61% and 77% respectively) but susceptible to CM (100% mortality) and Deltamethrin (100% mortality). *Culex quinquefasciatus* species however were resistant to all insecticides tested. M molecular form of *An. gambiae* s.s was predominant (97%) with no S form detected. One hybrid form was detected (3%). The kdr resistant genotype frequency F(R) was 0.84 with 70% homozygotes kdrRR. The evaluation depicts the success of vector control innovations using pyrethriods and non-pyrethriods in combination on nets.

173

MULTIPLEX ASSAY DEVELOPMENT FOR SPECIES IDENTIFICATION AND MONITORING OF KNOCK DOWN RESISTANCE IN *ANOPHELES* MOSQUITO VECTOR POPULATIONS OF PAPUA NEW GUINEA

Kogulan Nadesakumaran[1], Cara N. Henry-Halldin[1], John K. Keven[2], Allison Zimmerman[1], Edward Thomsen[1], Lisa Reimer[1], Peter A. Zimmerman[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea

Extensive distribution of indoor residual spraying of insecticides and long lasting insecticide treated bednets for prevention of malaria have created selective pressures resulting in the development of insecticide resistant mosquitoes in malaria-endemic regions of the world. A point mutation in the voltage-gated sodium channel gene (*VGSC), kdr,* is the most common variation associated with resistance to DDT and pyrethroid insecticides used in vector control. In the Papua New Guinean *Anopheles punctulatus* (Ap) species complex (>10 species), species-specific insecticide resistance has not been characterized. As morphological species identification has proved challenging within the Ap complex, we undertook DNA sequence-based strategies to evaluate species-specific differences and *kdr* associated polymorphisms. We observed consistent differentiation among Ap, *A. koliensis*, *A. farauti* 1 &4, revealing species-specific ITS2 and VGSC polymorphisms from DNA sequences of 90 mosquitoes in 7 provinces

of Papua New Guinea. To determine if VGSC sequence polymorphisms distinguish Ap sibling species consistent with ITS2 variation, VGSC and ITS2 sequence specific probes were designed and 237 mosquitoes were evaluated. Results showed that all samples were homozygous wild type at the *kdr* mutation site. Results comparing species-specific polymorphisms were 100% (237/237) concordant between the traditional ITS2 marker and the VGSC sequence variants. Together, VGSC and rDNA molecular methods consistently showed that morphological factors are less reliable in identifying species than DNA based analyses due to the cryptic nature of the *Ap* complex. In addition to monitoring for common insecticide resistant mutations like *kdr*, effective vector control programs must have reliable methods of species identification. Our results suggest that the VGSC gene-based assay allows for the simultaneous evaluation of the *kdr* associated genotype and molecular species identification following a single PCR.

174

SUSCEPTIBILITY STATUS OF *AEDES AEGYPTI* TO INSECTICIDES IN LA GUAJIRA (COLOMBIA)

Ronald Y. Maestre[1], Zulibeth Florez[2], Claudia Cabrera[2], Sergio Goenaga[3], Doris Gómez[1], Claudio Gómez[4]

[1]Universidad de Cartagena, Cartagena, Colombia, [2]Secretaria de Salud de La Guajira, Riohacha, Colombia, [3]Secretaria de Salud del Atlántico, Barranquilla, Colombia, [4]Universidad Nacional, Bogotá, Colombia

Dengue fever keeps an endemic behavior in La Guajira, a location in the northern Caribbean coast of Colombia where insecticides have played an important role in actions towards the control of this disease during the last four decades. However, the real susceptibility of the vector mosquito *Aedes aegypti* to insecticides in this location is still unknown. The aim of this work was to evaluate the susceptibility status to insecticides organophosphorus, organochlorine and pyrethroid of three populations of *A. aegypti* in La Guajira-Colombia during the year of 2009. Biological assays were carried out with adults (F2) and third-instare larva of *A. aegypti* collected in different urban districts of La Guajira (Fonseca, Maicao and Riohacha). For adults, the method of the CDC-Atlanta (Centers for Disease Control and Prevention in Atlanta, GA) was applied using diagnostic doses for malathion (100 µg/ml), fenitrothion (75 µg/ml), DDT (150 µg/ml) and lambdacyhalotrine (6,25 µg/ml). For larva, the World Health Organization method was applied, with a diagnostic dose of Temephos (0,012ppm). Each insecticide was tested three times, with four replicates each time, and a control with no insecticide was also included. 100% susceptibility (100% mortality) was observed with malathion and fenitrothion in the three populations evaluated. Variations in susceptibility/resistance with lambdacyhalotrine (52-100% mortality) and temephos (77-99% mortality) were observed. In contrast, a high resistance was observed to Dichlorodiphenyl-Trichloroethane (DDT) in all populations (2-13% mortality). In conclusion, our results suggest some degree of resistance to insecticides in three populations of *A. aegyptis* in La Guajira-Colombia. This might indicate a growing phenomenon of insecticides resistance in this country area.

175

BASIC EFFICACY OF ORAL INSECTICIDES IN TOXIC SUGAR BAITS TO CONTROL SAND FLIES AND MOSQUITOES

Laor Orshan[1], **Gabriela Zollner**[2]

[1]Laboratory of Entomology, Ministry of Health, Jerusalem, Israel, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States

Mosquitoes (Diptera: Culicidae) and sand flies (Diptera: Psychodidae) are vectors of viruses and protozoan parasites. Finding new ways to prevent exposure of people to these hematophagus biting insects and prevent disease transmission is a continuous challenge. Outdoor control measures are an important component of integrated vector management programs. Targeting adult stages is the only option when breeding places are unknown or inaccessible. Conventional control methods, including the use of residual insecticides and thermal fogging, can cause a wide distribution of harmful insecticides and potential exposure to humans and non-target organisms, in addition to limited efficacy in many areas. The use of oral insecticides added to sugar solution is a new, promising alternative method for outdoor control of adult mosquitoes and sand flies. The choice of insecticides is vital for the successful use of the method in the field. Suitable insecticides should be non-repelling even in high concentrations, have good basic oral efficacy on sand flies and mosquitoes, have low toxicity to mammals and other non target animals and remain potent for a reasonable time in harsh outdoor conditions. The great potential for the widespread use of toxic sugar baits necessitates the alternate use of several suitable insecticides to prevent or significantly slow down the development of insecticide resistance. We designed experiments to test the palatability and basic efficacies of spinosad, thiamethoxam, dinotefuran and boric acid in sugar baits against representative mosquito and sand fly vector species. The feeding rates on a series of toxin dilutions and the resulting mortality rates up to 72h post exposure will be presented for *Culex pipiens, Anophles stephensi* and *Aedes aegypti* mosquitoes and *Phlebotomus papatasi* sand flies. The suitability of these insecticides for use in toxic sugar baits and the framework for further testing their persistence is the field will be discussed.

176

DIFFERENTIAL BEHAVIORAL RESPONSES OBSERVED IN *AEDES AEGYPTI* IN RESPONSE TO REDUCED COVERAGE AND DOSE OF STANDARD VECTOR CONTROL CHEMICALS IN THAILAND

Suppaluck Polsomboon[1], Krajana Tainchum[1], Sungsit Sungvornyothin[2], Wannapa Suwonkerd[3], Nicole L. Achee[4], John P. Grieco[4], Theeraphap Chareonviriyaphap[1]

[1]Kasetsart University, Bangkok, Thailand, [2]Mahidol University, Bangkok, Thailand, [3]Ministry of Public Health, Chiang Mai, Thailand, [4]Uniformed Services University of the Health Sciences, Bethesda, MD, United States

A novel Push-Pull strategy for the reduction of dengue transmission is currently being evaluated in Thailand. One component of this strategy relies on exploiting mosquito behavior in response to vector control chemicals to break human vector contact. In order to achieve the maximum response it is critical to select the appropriate spatial repellent and contact irritant compounds and apply at the correct dose. The current study was aimed at determining the behavioral responses of female *Aedes aegypti* in response to candidate spatial repellent (SR) and contact irritant (CI) compounds. In addition, various treated surface area coverages were evaluated in an effort to reduce indoor mosquito densities while reducing chemical use. Insecticide treated material strips in four different surface area coverage ratios (25, 50, 75 and 100%) were placed on the interior walls of experimental huts in either a vertical or horizontal configuration. The materials used and the configuration of placement were based on data generated from baseline studies conducted on the resting behavior of *Ae. aegypti* in the absence of chemical. Data suggest that there are differential patterns of behavior for *Ae. aegypti* females into (SR) and out of (CI) experimental huts depending upon test chemical, dose applied and treatment coverage. Results from this study will guide the implementation of the Push-Pull control strategy by determining the optimum chemical, dose and coverage to achieve maximum disruption of human vector contact.

177

NEAR-INFRARED SPECTROSCOPY AS A COMPLEMENTARY AGE GRADING AND SPECIES IDENTIFICATION TOOL FOR AFRICAN MALARIA VECTORS

Maggy Sikulu[1], Gerry F. Killeen[2], Leon E. Hugo[3], Peter A. Ryan[3], Kayla M. Dowell[4], Robert A. Wirtz[5], Sarah J. Moore[2], Floyd E. Dowell[4]

[1]Griffith University, Brisbane, Australia, [2]Ifakara Health Institute, Dar es salaam, United Republic of Tanzania, [3]Queensland Institute of Medical Research, Brisbane, Australia, [4]United States Department of Agriculture ARS, Engineering and Wind Erosion Research Unit, Center for Grain and Animal Health Research, Manhattan, KS, United States, [5]Centers for Disease Control and Prevention, Atlanta, GA, United States

Near-infrared spectroscopy (NIRS) was recently applied to age-grade and differentiate laboratory reared *Anopheles gambiae sensu strico* and *Anopheles arabiensis* sibling species of *Anopheles gambiae sensu lato*. In this study, we report further on the accuracy of this tool in simultaneously estimating the age class and differentiating the morphologically indistinguishable *An. gambiae s.s.* and *An. arabiensis* from semi-field releases and wild populations. Nine different ages (1, 3, 5, 7, 9, 11, 12, 14, 16 d) of *An. arabiensis* and eight different ages (1, 3, 5, 7, 9, 10, 11, 12 d) of *An. gambiae s.s.* maintained in 250 x 60 x 40 cm cages within a semi-field large-cage system and 105 female wild *An. gambiae s.l.,* were included in this study. NIR classified female *An. arabiensis* and *An. gambiae s.s.* maintained in semi field cages as < 7 d old or ≥ 7 d old with 89% (n=377) and 78 % (n=327) accuracy, respectively and differentiated them with 89% (n=704) accuracy. Wild caught *An. gambiae s.l.* were identified with 90% accuracy (n=105) whereas their predicted age were consistent with the expected mean chronological ages of the physiological age categories determined by dissections. These findings have importance for monitoring control programmes where reduction in the proportion of older mosquitoes that have the ability to transmit malaria is an important outcome.

178

PREDATION EFFICIENCY OF *ANOPHELES GAMBIAE* LARVAE BY AQUATIC PREDATORS IN WESTERN KENYA HIGHLANDS

Eliningaya J. Kweka[1], Yaw Afrane[1], Guofa Zhou[2], Andrew K. Githeko[1], Guiyun Yan[2]

[1]Kenya Medical Research Institute, Kisumu, Kenya, [2]Program in Public Health, College of Health Sciences, University of California, Irvine, CA, United States

The current status of the insecticides resistance in mosquitoes and effect of insecticides on non-targeted insect' species raised the need of alternative control methods for malaria vectors. Predation has been suggested as one of the important regulation mechanisms for malaria vectors in long-lasting aquatic habitats, but the predation efficiency of the potential predators is unknown. In the current study, we examined predation of backswimmer, tadpoles, Gambusia, Belostoma and dragon nymph on *Anopheles gambiae* larvae in semi-natural habitats. Predators were sampled from the habitats, and starved for 12 hours before experiments. Third instar larvae of *An. gambiae* were introduced into two types of microcosms at various larval densities, and the number of surviving mosquito larvae was monitored after 24 hours. We found that habitat type, larval density and predator species had positive impact on the predation rate of *An. gambiae* larvae. All predators have shown to be actively nocturnal. These results suggest that larval predators play a role in regulating larval population of malaria vectors. We are currently investigating the impact of predators in natural habitats.

179

INSECTICIDE-TREATED BED NET (ITN) OWNERSHIP, USAGE AND MALARIA TRANSMISSION IN THE HIGHLANDS OF WESTERN KENYA

Harrysone E. Atieli[1], Goufa Zhou[2], Yaw Afrane[1], Andrew K. Githeko[1], Guiyun Yan[2]

[1]Climate and Human Health Research Unit, Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya, [2]Program in Public Health, College of Health Sciences, University of California, Irvine, CA, United States

Insecticide-treated bed nets (ITNs) are known to be highly effective in reducing malaria morbidity and mortality. However, compliance of ITN use varies among households and such variations in actual ITN usage may seriously limit the potential impact of the nets and cause spatial heterogeneity on malaria transmission. This study examined the ITN usage and underlying factors for among-household variation in ITN use in two highland sites of western Kenya. Cross-sectional surveys were conducted on ITN ownership (possession) and ITN compliance (actual usage) in occupants of randomly sampled houses in the dry and rainy season of 2009. Despite ITN ownerships reached more than 71%, ITNs compliance was low (<40%). There was also a seasonal variation in ITN compliance rate: compliance rate was significantly higher during rainy than dry season (40% vs. 34%). ITNs were perceived as very important for protection against mosquito bites and malaria by the resident during the rainy season when both malaria prevalence (11.8% vs. 5.1%) and vector densities (1.0 female/house vs 0.4 female/house) were significantly higher than dry season. Other important reasons for higher compliance rate during the wet season include: significantly high numbers of nuisance culicine mosquitoes and low indoor temperatures. Malaria prevalence in rainy season was about 30% lower in ITN users than in non-ITN users, but this was not significantly different during the dry season. In conclusion, in the malaria meso-endemic highland regions of western Kenya, compliance with ITN is relatively higher during rainy season than dry season, but the gap between ITN ownership and usage is high. Reasons for this gap particularly during the rainy season are yet to be known.

180

GENETIC VECTOR CONTROL STRATEGIES TO REDUCE THE BURDEN OF MOSQUITO-BORNE DISEASES

Nina Alphey[1], Luke Alphey[2], Michael B. Bonsall[1]

[1]University of Oxford, Oxford, United Kingdom, [2]Oxitec Ltd. and University of Oxford, Oxford, United Kingdom

Vector-borne diseases impose an enormous health and economic burden around the world and new methods to control vector populations are needed. The Sterile Insect Technique (SIT) is an area-wide method of biological pest control whereby large numbers of a pest insect are bred, sterilized (currently by irradiation) and then released. The sterile insects mate with wild insects, but no viable offspring result from those matings. The SIT has been successful against agricultural pests, but is not in large-scale use for suppressing or eliminating populations of mosquito disease vectors. This is due in part to technical difficulties with the current technology. Genetic RIDL® technology (Release of Insects carrying a Dominant Lethal) is a proposed modification that involves releasing insects that are homozygous for a repressible dominant lethal genetic construct rather than being sterilized by irradiation, and could potentially overcome some of those problems. Using the arbovirus dengue as an example, I combine a vector population dynamics model with an epidemiological model to explore the effect of a programme of RIDL releases on disease transmission, and investigate the potential cost-effectiveness by applying estimates of the costs of RIDL-based SIT. Through mathematical modelling I find that this genetic control strategy could eliminate dengue from a human community in a timescale within one year, and at lower cost than the direct and indirect costs of disease that would be averted by doing so.

181

BLOOD-FEEDING AND IMMUNOGENIC *AEDES AEGYPTI* SALIVA PROTEINS

Pornapat Surasombatpattana[1], Ladawan Wasinpiyamongkol[2], Sirilaksana Patramool[1], Natthanej Luplertlop[2], Souleymane Doucouré[3], François Mouchet[3], Martial Sévéno[4], Franck Remoue[3], Edith Demettre[4], Jean-Paul Brizard[5], Patrick Jouin[4], David G. Biron[6], Frédéric Thomas[1], Dorothée Missé[1]

[1]Laboratoire de Génétique et Evolution des Maladies Infectieuses, UMR 2724 CNRS/IRD, Montpellier, France, [2]Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [3]Institut de Recherche pour le Développement IRD, UR016, Montpellier, France, [4]Plate-forme de Protéomique Fonctionnelle, IFR3, CNRS-UMR 5203, INSERM-U661, UMI-II, Montpellier, France, [5]Génome et Développement des Plantes IRD/CNRS/UP (UMR5096), Montpellier, France, [6]INRA Université Blaise Pascal, Site de Crouelle UMR547 PIAF, Clermont-Ferrand, France

Mosquito-transmitted pathogens pass through the insect's midgut (MG) and salivary gland (SG). What occurs in these organs in response to a blood meal is poorly understood, but identifying the physiological differences between sugar-fed and blood-fed (BF) mosquitoes could shed light on factors important in pathogens transmission. We compared differential protein expression in the MGs and SGs of female *Aedes aegypti* mosquitoes after a sugar- or blood-based diet. No difference was observed in the MG protein expression levels but certain SG proteins were highly expressed only in BF mosquitoes. In sugar-fed mosquitoes, housekeeping proteins were highly expressed (especially those related to energy metabolism) and actin was up-regulated. The immunofluorescence assay shows that there is no disruption of the SG cytoskeletal after the blood meal. We have generated for the first time the 2-DE profiles of immunogenic *Ae. aegypti* SG BF-related proteins. These new data could contribute to the understanding of the physiological processes that appear during the blood meal.

182

THE CONTRIBUTION OF AESTIVATING MOSQUITOES TO THE SUBSEQUENT WET SEASON POPULATIONS IN THE SAHEL

Adamou Abdoulaye[1], Adama Dao[1], Alpha Seydou Yaro[1], Yaya Kassogué[1], Moussa Diallo[1], Sékou Traoré[1], Diana L. Huestis[2], Tovi Lehmann[2]

[1]Malaria Research and Training Center (MRTC)/Faculty of Medicine, Pharmacy and Odonto-Stomatology, Bamako, Mali, [2]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Persistence of African anophelines throughout the long dry season (4-8 months) where no surface waters are available remains one of the last mysteries of medical entomology. Recent studies demonstrate that aestivation (summer diapause) is one mechanism that allows the African malaria mosquito, *Anopheles gambiae*, to persist in the Sahel. However, migration from distant localities - where reproduction continues year-round - might also be involved. To assess the unique contribution of aestivating adults to the build-up of populations in the subsequent wet season, we compared two villages subjected to weekly pyrethrum sprays throughout the dry season with two nearby villages. We predict that in the treated villages, mosquito density during the subsequent wet season would be lower and it would peak later if most aestivating mosquitoes are killed by the insecticide. We selected four small, isolated villages in the Sahel region of Mali located over 10 km away from the nearest permanent larval site. Monitoring started in September 2009 in all villages. It consisted of pyrethrum spray collections conducted once a month in 25 houses selected at random in each village. Insecticide treatment in treated villages started after all larval sites dried up (December). Treatment consisted of four pyrethrum sprayings in all houses every month throughout the dry season, until the first rain. After the first rain, only monitoring was performed every ten days in all four villages. The mosquito density and composition before, during, and after the dry-season treatment was compared in each pair of treated and untreated villages based on their geographical proximity. Currently (March 2010), the dry season treatments are ongoing and house density is 0-0.04/house in all four villages. The complete results will be presented and discussed in respect to the role of aestivation to the persistence of mosquitoes in the Sahel and their implication for malaria control.

183

SIMULATIONS OF MOSQUITO HOST-SEEKING BEHAVIOR

Breschine Cummins, Ricardo Cortez, Justin Walbeck, Ivo Foppa

Tulane University, New Orleans, LA, United States

Models of disease spread commonly make the assumption that susceptible and infected individuals are homogeneously distributed within a population or within subpopulations that are interconnected on a large spatial scale. The effect of small-scale spatial heterogeneity on disease transmission remains a relatively unexplored area, and may be particularly important in diseases where transmission occurs between members of different species. I present a computational model to explore the effect of small-scale spatial heterogeneity on the encounter rate between mosquito vectors and bird hosts in the context of West Nile virus transmission. The model includes behavioral rules for the motion of host-seeking vectors, a spreading odor plume generated by resting hosts, and non-uniform wind conditions. The behavior of the vectors and the spatial arrangement of the resting hosts are varied to measure the number and distribution of mosquito-bird encounters. The results may be used to modify the transmission parameter in models of disease spread, such as SIR and its variants, in order to account for the effects of small-scale spatial heterogeneity in host distribution and differences in mosquito behavior across species.

184

CONTRASTING EXPERIMENTAL HABITAT OPTIMA FOR *ANOPHELES* AND *AEDES*

Clelia Oliva[1], Mark Quentin Benedict[2], Jeremie Gilles[3]

[1]IRD/CRVOI, Saint-Denis, Réunion, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]IAEA - Insect Pest Control Laboratories, Vienna, Austria

The mosquitoes Anopheles arabiensis Patton (Diptera: Culicidae), a vector of malaria, and Aedes albopictus Skuse (Diptera: Culicidae), vector of Chikungunya and Dengue, are targeted for population control programs, such as the Sterile Insect Technique (SIT). These two species coexist in the same areas in Reunion Island but are usually found in different breeding sites. Studies were conducted to assess their optimal food concentration for development and survival in order to optimize mass rearing processes for conventional SIT and to better understand their habitat limitations. For each species, 32 first instar larvae were reared in Petri dishes filled with 32 ml of deionised water, and fed daily with 640 microliters of different concentration (1, 1.5, 2, 4 and 8%) of a diet developed in the IAEA laboratory. Diet concentration tolerance was different for the two species: 2% appeared to be a maximum for An. arabiensis whereas Ae. albopictus survived well until 4% and was still able to develop at 8%. When food concentration increased, the development duration was slightly increased for An. arabiensis but reduced for Ae. albopictus. For both species and sexes, wing length increased with food concentration. Considering all the parameters, the best food concentration was 1% for An. arabiensis and 2% for Ae. albopictus. The sensitivity to the organic content and concentration of the aquatic environment was different between these two species as substantiated by our results. Indeed, An. arabiensis is usually known as a "clean-water" species whereas Ae. albopictus is a "polluted-water" mosquito which can develop well in water with a high organic content.

In order to determine whether inter-specific interactions would expand the optima of the Anopheles, both species were then reared in the same container in a 1:1 ratio and given 1, 2 or 4% of food concentration. Our results suggest that the development of Ae. albopictus larvae in water that would otherwise be too organic-rich for An. arabiensis, would make the environment suitable.These results are discussed in the context of diet concentration for mass-rearing and are linked to specific ecological capacities in the field. The diet provided turned out to be suitable for both species, optimizing all the developmental parameters recorded here, and would be adaptable to any mosquito species rearing.

185

WATER USE PRACTICES LIMIT THE EFFECTIVENESS OF A TEMEPHOS-BASED *AEDES AEGYPTI* LARVAL CONTROL PROGRAM IN NORTHERN ARGENTINA

Fernando Garelli[1], Manuel Espinosa[2], Diego Weinberg[2], Maria Trinelli[3], Ricardo Gürtler[1]

[1]Laboratorio de Eco-Epidemiología, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Fundación Mundo Sano, Buenos Aires, Argentina, [3]Laboratorio de Química del Agua, INQUIMAE, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

A five-year larval control program based on citywide application of temephos every 3 or 4 months significantly reduced *Aedes aegypti* larval indices but failed to maintain them below target levels in Clorinda, northern Argentina. Reduced residuality of temephos has been proposed as a putative factor limiting control in large tanks, the most productive container type in Clorinda. The duration of temephos residual effects in household-owned water-holding tanks was estimated in two longitudinal studies including 18 and 60 tanks followed up during 5 and 14 weeks, respectively. Temephos was applied using spoons or inside small zip-lock bags. Water samples from the study tanks (including positive and negative controls) were collected weekly and subjected to larval mortality bioassays. The trials were concurrent with larval control actions in the entire study neighborhood. Water turnover was estimated quantitatively at the end of the follow-up by adding sodium chloride to a sample of the study tanks and measuring its dilution 48 hs later. Temephos residuality was much shorter than the expected 8-12 weeks and very heterogeneous between tanks. Its mean duration was 5 weeks (range 2-9 weeks) when applied inside small zip-lock bags and 3.5 weeks (range 0.3-10 weeks) when applied using spoons. Water use practices were found associated with loss of residuality via multivariate GEE models. Tanks filled with piped water had high turnover rates and short-lasting temephos effects, whereas tanks filled with rain water showed the opposite pattern. Larval infestation reappeared nine weeks after treatment, five weeks after loss of residuality and most likely originated from newly-laid eggs. High water turnover occurred because the intermittent piped water service forced many householders to refill their tanks almost every night. Limited field residuality of temephos coupled with incomplete coverage of breeding sites explain the inability of the control program to further reduce infestation levels with a 3-4 month treatment cycle period.

186

CHARACTERIZATION OF SLC7-TYPE AMINO ACID TRANSPORTERS IN THE YELLOW FEVER MOSQUITO, *AEDES AEGYPTI*

Lisa L. Drake, Victoria Carpenter, Immo A. Hansen

New Mexico State University, Las Cruces, NM, United States

Mosquitoes are successful as disease vectors because they require vertebrate blood as a nutrient source for egg development. After a blood meal, yolk protein precursor (YPP)-synthesis is up-regulated in the fat body. Amino acid (AA)-transporters, located in the fat body plasma membrane, facilitate blood meal-derived AA import and generate a signal that is transduced to the yolk protein gene via the TOR/S6K signal transduction pathway. YPP gene expression in *Aedes aegypti* is dependent upon the cationic AAs histidine, arginine, and leucine. Arginine is also the precursor to nitric oxide which is an important molecule for the innate immune system of mosquitoes. We identified 68 putative AA transporters in the genome of A. aegypti, eleven members of the subgroup of SLC7-type AA transporters, and five of the subfamily of cationic AA transporters (CATs). We determined fat body expression levels of the eleven SLC7-transporters and found several of them strongly up-regulated after a blood meal. Using RNAi-mediated knockdown and subsequent analyses of reproductive fitness, aging, and immunity we demonstrate the role of SLC7-type AA transporters in adult female *Aedes aegypti*.

187

DISPERSAL OF *CULEX PIPIENS* IN AN URBAN FOCUS OF WEST NILE VIRUS TRANSMISSION: A MARK-CAPTURE STUDY USING STABLE ISOTOPES

Gabriel L. Hamer[1], Tony L. Goldberg[1], Tavis K. Anderson[1], Uriel D. Kitron[2], Luis F. Chaves[2], Jeffrey D. Brawn[3], Marilyn O. Ruiz[3], Bethany Krebs[3], Rebecca Hood-Nowotny[4], Danielle J. Donovan[5], Michael G. Kaufman[5], Edward D. Walker[5]

[1]University of Wisconsin, Madison, WI, United States, [2]Emory, Atlanta, GA, United States, [3]University of Illinois, Champaign-Urbana, IL, United States, [4]International Atomic Energy Agency, Seibersdorf, Austria, [5]Michigan State University, East Lansing, MI, United States

Mosquito movement and dispersal are key determinants of the distribution of arboviruses. Marking and tracking mosquitoes is a long-used strategy to study mosquito dispersal. However, common techniques for marking mosquitoes have significant limitations, reducing their utility for understanding the ecology of certain arbovirus systems, including West Nile virus (WNV). In this study, we deployed isotopic labeling of *Culex* pipiens larvae in aquatic sites and a mark-capture design. We conducted laboratory experiments to identify enrichment levels of 15N-enriched potassium nitrate and 13C-enriched glucose to distinguish marked individuals from natural levels. Mosquitoes reared in enriched environments reached a mean delta 15N of 199.0 and a mean delta 13C of 93.7, compared to natural levels of 4.5 for delta 15N and of -25.5 for delta 13C. The 15N signal maintained its strength up to 25 days post-emergence but the 13C signal declined over the same time period, indicating higher turn-over of carbon than nitrogen during metabolic processes. Stable isotope additions to larval water did not influence mosquito survival or adult body mass. Field deployment of this mosquito mark-capture method in suburban Chicago yielded promising results for measuring arboviral dispersal in an urban landscape. This technique has broad application to the quantification of movement and dispersal of *Culex* pipiens and other disease vectors.

188

PHYLOGENETIC ANALYSIS OF PAPUA NEW GUINEA MALARIA VECTORS (*ANOPHELES PUNCTULATUS* SPECIES COMPLEX) - ASSESSMENT OF BIODIVERSITY AND IMPLICATIONS FOR VECTOR MANAGEMENT

Cara N. Henry-Halldin[1], Kogulan Nadesakumaran[1], Allison Zimmerman[1], John Bosco Keven[2], Edward Thomsen[1], Lisa Reimer[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

Limited studies define relationships among Papua New Guinea (PNG) malaria vector mosquitoes, members of the *Anopheles punctulatus* species complex (*A. punctulatus* [Ap]*, A. koliensis* [Ak]*,* morphologically indistinguishable *A. farauti* [Af] 1-8). Understandably, molecular marker studies are necessary to assess biodiversity of PNG *Anophelines*. To examine species relationships we analyzed the internal transcribed spacer 2 rDNA (ITS2 988bp), cytochrome oxidase I (COI 1277bp) and voltage

gated sodium channel genes (VGSC 1537bp; intronic + coding regions containing mutations associated with knockdown resistance [*kdr*]) of 90 mosquitoes collected from 7 PNG provinces. Phylogenetic analysis was performed using Bayesian and maximum likelihood (ML)-based approaches; including *A. gambiae* (Africa) and *A. longirostris* (PNG) as outgroups. Phylogenetic analysis of single nuclear genes (ITS2 and VGSC) supports the existence of multiple species in the Ap complex, with highly significant internal branch support distinguishing species clusters (ML analysis >92%). Analyses of mitochondrial COI showed different arrangements among species clusters and with significantly lower confidence of species relationships (59-63%). Phylogenetic analysis of the three genes concatenated (3802bp) showed conflicting relationship patterns exist when comparing nuclear and mitochondrial genes. Regardless of analysis technique employed, Ap and Af4 were shown to be most closely and consistently related. Placement of Ak and Af1 varied with gene and analysis method. Some Ap, Af1, and Af4 mosquitoes were shown to stray from their respective clusters, suggesting gene flow between these populations. Ak appeared to be the most conserved cluster, containing only and all Ak samples. Distinct phylogeographic partitioning was observed between Af1 samples from PNG mainland and an island province. Overall, these analyses improve evaluation of species diversity and provide a baseline reference for future comparisons following ongoing distribution of insecticide treated bednets, impacting PNG mosquito populations.

189

CREATION OF A TRANSGENIC MOSQUITO STRAIN FOR USE AS A TOOL IN THE MANIPULATION OF NUTRIENT ACCUMULATION IN THE LARVAL STAGE USING THE HEXAMERIN PROMOTER

Sarah E. Aguirre, Immo A. Hansen

New Mexico State University, Las Cruces, NM, United States

Transgenic mosquitoes have been suggested as an alternative strategy to fight vector-borne diseases like malaria and dengue fever. So far, only two promoters are widely used for transgenic mosquitoes: the vitellogenin promoter (expressed in the adult female fat body after a blood meal) and the early trypsin promoter (found in the adult female gut after a blood meal). In order to characterize a promoter for protein expression in the larval fat body we identified a putative hexamerin promoter. Hexamerins are storage proteins that are highly expressed in the fat body of holometabolous insect larvae. We hypothesize that the hexamerin promoter can be used to express proteins in transgenic mosquitoes in a stage-, tissue-, and sex- specific manner. We identified two hexamerin genes in the published genome sequence of A. aegypti and determined their expression profiles. Next, we identified the transcription start sites via RACE PCR. We performed comparative promoter analysis to identify conserved transcription factor binding sites using the MatInspector® software. A 1.5 kbp DNA fragment containing the putative promoter was cloned in the expression vector pGREEN-Pelican. Next we will test the functionality of the putative promoter by incorporating this construct into the genome of Drosophila and A. aegypti and analysis of EGFP expression during postembryonic development. This transgenic line will be used as a tool for the manipulation of nutrient accumulation during the larval stage in mosquitoes by. Nutrient accumulation is important when considering the development of mosquito control strategies such as sterile insect technique.

190

SEROPREVALENCE OF DENGUE ANTIBODIES AMONG 12 -18 YEAR-OLD STUDENTS IN FOUR SECONDARY SCHOOLS IN TRINIDAD

Karmesh L. Sharma[1], Ron S. Mahabir[2], Dave Chadee[2]

[1]University of the West Indies, Fyzabad, Trinidad and Tobago, [2]University of the West Indies, St. Augustine, Trinidad and Tobago

The aim of this study is to build a dengue seroprevalence data base which can be used to indicate the presence or absence of the circulating infectious agent, among the secondary school population (ages 12-18). This will contribute to evidence-based information to be used in designing a more effective and efficient dengue prevention and control programme. Approximately one hundred and fifty students, between forms 1-6 (ages 12-18), in each of four geographically pre-determined, but randomly selected secondary schools in Trinidad were selected by stratified random sampling. A finger prick was administered by a trained person, subsequent to which a drop of blood (100 microlitres) was placed on a PanBio ICT Rapid Test Card for Dengue. The ICT Cards were then read and the results were recorded. Each ICT card was then re-read by a second person for agreement on the result. Statistical analyses were conducted using the SPSS Software package (version 16.0). Five hundred and ninety eight students between ages 12-18 were selected by stratified random sampling from four geographically predetermined, but randomly selected schools in Trinidad. Of these, two hundred and ninety (48.5%) were positive by PanBio ICT rapid test. While no level of significance was found in the male to female ratio, and by county, a level of significance for positive seroprevalence was found among cases of persons of Indian origin (chi-square value = 0.036; likelihood ratio = 0.031). It was also observed from a crosstabulation between ethic groups and sex, females were more likely to test positive for dengue antigen (chi-square value = 0.028; likelihood value = 0.025). Additionally, seroprevalence rate increased with age (spearman's r value = 0.667; significant 1-tail value = 0.051). In conclusion, a seroprevalence rate of 48.5% is substantial and has critical implications for public health in the light of four circulating dengue viruses in the country. At this level of prevalence, especially among the young age group, one has to be concerned with the high risk of DHF/DSS. Prevention and control efforts should be targeted from a position of integrated management to include re-tooling of current policies, health education, environmental sanitation, source reduction and vector control. Additionally, the concerns of climate change must be well considered in any dengue management strategy. This opens new doors for further investigation and quantification.

191

A SEMI-FIELD, TUNNEL ASSAY FOR THE EVALUATION OF SPATIAL REPELLENTS IN THAILAND

Alongkot Ponlawat, **Prasan Kankaew**, Somporn Chanaimongkol, Brian Evans, Jason Richardson

Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

We describe a semi-field tunnel assay to be used for the evaluation of candidate spatial repellents against mosquitoes. In the absence of a candidate spatial repellent, *Aedes aegypti* released at the midpoint of a 50-m tunnel show relatively equal preference for human "attractants" positioned inside of screened tents at either end of the tunnel. Ongoing studies are determining if equal preference is exhibited by other mosquitoes species. Additionally, the assay is currently being used to evaluate a metofluthrin-based product for its efficacy against *Ae. aegypti.* We propose that this method is an effective, viable alternative to other approaches that are currently employed to evaluate spatial repellents.

192

VECTOR-HOST INTERACTIONS GOVERNING EPIZOOTIOLOGY OF EASTERN EQUINE ENCEPHALITIS VIRUS IN NORTHEASTERN USA

Goudarz Molaei[1], Theodore G. Andreadis[1], Philip M. Armstrong[1], John J. Howard[2], Joanne Oliver[2], Ary Farajollahi[3], Esteban Cuebas-Incle[4], Walter Montgomery[4], Matthew Osborne[5], Sandra Smole[5], Priscilla Matton[6], Wayne Andrews[6], Timothy Deschamps[7], Curtis Best[7], Frank Cornine, III[7], Ellen Bidlack[8], Tony Texeira[8]

[1]The Connecticut Argicultural Experiment Station, New Haven, CT, United States, [2]New York State Department of Health, Syracuse, NY, United States, [3]Rutgers University, New Brunswick, NJ, United States, [4]Northeast Massachusetts Mosquito Control and Wetlands Management District, Newburyport, MA, United States, [5]Massachusetts Department of Public Health, Jamaica Plan, MA, United States, [6]Bristol County Mosquito Control Project, Taunton, MA, United States, [7]Central Massachusetts Mosquito Control Project, Northborough, MA, United States, [8]Plymouth County Mosquito Control Project, Kingston, MA, United States

Recent emergence of eastern equine encephalitis virus (EEEV) in northeastern US, prompted us to renew research on the eco-epizootiology of the virus. Accordingly, we investigated the vector-host interactions and blood feeding behavior of eight mosquito species representing six genera implicated in the transmission of EEEV in New York, Massachusetts, Connecticut, and New Jersey by using a PCR-based assay and sequencing portions of mitochondrial *cytochrome b* gene. Analysis of engorged mosquitoes revealed that *Culiseta melanura* and *Cs. morsitans* acquired blood meals primarily from avian hosts (87-100%). Wood thrushes, American robins, song sparrows, black-capped chickadees, and a few other Passeriformes birds constituted the most common vertebrate hosts suggesting key roles in supporting EEEV transmission. These principally ornithophagic mosquitoes also acquired blood meals from mammals (0.6-4.2%) including humans, and from both birds and mammals (0.3-11.5%) in mixed-blood meals. The frequency of mammalian feedings suggests that *Cs. melanura* and to a lesser extent *Cs. morsitans* may play a role in the transmission of EEEV to equines and humans, in addition to maintaining enzootic transmission among avian hosts. *Anopheles punctipennis*, *An. quadrimaculatus*, *Aedes vexans*, and *Ochlerotatus canadensis* were identified as predominately mammalophagic mosquitoes (92-100%) with no or little inclination for feeding on avian hosts (0-2.5%), or mixed-blood meals (0-6%). *Culex salinarius* and *Coquillettidia perturbans* exhibited a relatively opportunistic blood feeding behavior on avian (36% and 11.8%, respectively), mammalian (53% and 86.7%), and mixed avian-mammalian hosts (11%, and 1.5%). These mammalophagic / opportunistic mosquitoes, may participate as bridge vectors in epidemic / epizootic transmission of EEEV from viremic birds to mammalian hosts. Further details on the resurgence and eco-epizootiology of the EEEV, as well as vectorial capacity of the mosquitoes will be discussed.

193

THE TRANSMISSIVE ROUTE OF *BACILLUS ANTHRACIS* INFECTION

Larissa Lukhnova[1], Alim Aikimbayev[2], Yerlan Pazylov[1], Zaure Zhumadilova[3], Zaurbek Sagiyev[1], Maksim Solomadin[1]

[1]Kazakh Scientific Center for Quarantine and Zoonotic Diseases, Almaty, Kazakhstan, [2]Republican Sanitary – Epidemiological Station, Almaty, Kazakhstan, [3]Committee of Sanitary-Epidemiological Surveillance of HM of Kazakhstan, Astana, Kazakhstan

Many researchers have explained the epizootic spread of anthrax among livestock in pre-vaccinal period by means of activation of infection' carriers, which were considered blood-sucking insects. During a major epizootic of anthrax in Zimbabwe in 1980 amongst wild and domestic animals, accompanied by diseases of over 6000 people, causative vectors of anthrax were flies and gadflies. Epidemiological, epizootological, analytical methods were utilized. Analysis of the spread of anthrax to various natural and geographical areas, epizootic, epidemiological observations suggest that the role of blood-sucking insects in the spread of this disease is possible. Seasonal peak of anthrax animal incidences coincides with the period of maximum activity of arthropods. Anthrax in the past mainly affects horses, cattle, deer, whose predominant the skin carbuncle form. From 1991 to 2009 in various regions of Kazakhstan in 15% cases presumably infection of people with anthrax occurred by the transmissive route. In 1997 in the anthrax foci of Zhambyl region 21 people were infected by anthrax, 107 goals of sheep, 2 horses, 1 pig have fallen. The seven people ill anthrax, were children from 5 to 14 years who had not participating in the slaughter of animals were staying in the anthrax focus. Localization of anthrax carbuncles was not typical for the contact infection route. The carbuncles on the front surface of the tibia, on the flexor surface of the right elbow, on the outer surface of the knee, ankle, on the dorsum of the foot were revealed. In conclusion, the uncharacteristic localization of anthrax carbuncles, presence of the insect bites, and infection with anthrax of children which didn't contact with contaminated objects, but they stayed in the anthrax focus. This is indirect evidence of possible transmission routes of anthrax infection.

194

EFFECTS OF COMBINED SEWER OVERFLOWS ON WATER QUALITY AND *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE) ABUNDANCE IN URBAN ATLANTA

Shirin Jabbarzadeh[1], Gregory M. Decker[1], William Galvin[1], Thomas R. Burkot[2], Rosmarie Kelly[3], Daniel G. Mead[4], Uriel Kitron[1], **Gonzalo M. Vazquez-Prokopec**[1]

[1]Emory University, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Georgia Division of Public Health, Atlanta, GA, United States, [4]University of Georgia, Athens, GA, United States

The factors that favor transmission and amplification of west Nile virus (WNV) infection within urban environments remain poorly understood. We have previously reported that urban streams in the city of Atlanta, GA, receiving sewage pulses from Combined Sewer Overflows (CSOs) are productive habitats for larvae of *Culex quinquefasciatus*, the main vector of WNV in the area. Building upon those findings, we longitudinally investigated the impact of CSOs on water nutrient concentrations and *Cx. quinquefasciatus* productivity and compared these measurements with those taken in a non-CSO affected urban stream. From June to October 2008 we quantified the weekly concentration of ammonia, phosphate, nitrate, dissolved oxygen (DO), PH level and the number of immature *Cx. quinquefasciatus* (sum of I-IV instars larvae and pupae) in a total of 10 pools from two urban streams with similar physical (i.e., water-flow, vegetation cover, waterbed) characteristics. A Prokopack mosquito aspirator was used to quantify the abundance of adult mosquitoes within 5 m of each pool. Two thirds of all identified female mosquitoes at the CSO stream were *Cx. quinquefasciatus*, significantly ($\chi 2=5.425$, P |4.94|, $P<0.05$). In contrast, DO level was significantly higher in the non-CSO stream ($W=3.01$; $P<0.05$). Based on a generalized estimating equation model we determined that larval abundance was positively and significantly associated with ammonia, phosphate, nitrate concentrations and negatively associated with DO concentration. Our study provided further evidence on the factors associated with high Cx *quinquefasciatus* productivity in CSOs. Sanitary sewer management plans in more than 700 US communities relying on CSOs has the potential to reduce the risk of WNV transmission.

195

IMPACT OF VIRUS DOSE, EXTRINSIC INCUBATION TEMPERATURE, AND INCUBATION PERIOD ON VECTOR COMPETENCE OF *CULEX NIGRIPALPUS* (DIPTERA: CULICIDAE) FOR WEST NILE VIRUS AND ST. LOUIS ENCEPHALITIS VIRUS

Stephanie L. Richards, Sheri L. Anderson, Cynthia C. Lord, Walter J. Tabachnick

University of Florida, Vero Beach, FL, United States

Culex nigripalpus, a vector of West Nile and St. Louis encephalitis virus (family *Flaviviridae*, genus *Flavivirus*; WNV, SLEV) in the southeastern US, was characterized for vector competence. For WNV, we assessed the impact of virus dose and incubation period (IP), while for SLEV we assessed the impact of dose and extrinsic incubation temperature (EIT). Vector competence was evaluated with chi-square analyses ($P<0.05$) using rates of infection (% virus-positive bodies out of total tested), dissemination (% virus-positive legs out of those infected), and transmission (% virus-positive saliva out of those infected). Virus titer in bodies, legs, and saliva was also tested. *Culex nigripalpus* were fed blood containing a low dose (LD: 6.3 ± 0.01) or high dose (HD: 7.3 ± 0.1) of WNV (logs plaque-forming units (pfu)/mL ± SE) and held at 28°C for IPs of 6 or 12 d. WNV infection rates were high (100%) and not affected by dose or IP. At 6 d, WNV dissemination rates were highest at the HD, but not different between doses at 12 d. Transmission of WNV was only observed under permissive conditions (HD, 12 d) and was low (11%). *Culex nigripalpus* were fed blood containing a LD (4.0 ± 0.1) or HD (4.6 ± 0.1) of SLEV (logs pfu/mL± SE) and held at 25°C or 28°C for 12 d. SLEV infection rates (≥85%) were not affected by dose or EIT. SLEV dissemination rates were lowest at 25°C for each dose group but rates did not differ between doses, showing a greater impact of EIT than dose. SLEV dissemination rates for the LD (91%) and HD (100%) at 28°C were higher than observed for WNV. Transmission of SLEV occurred at only 28°C. The SLEV doses tested were significantly lower than the WNV doses, yet *Cx. nigripalpus* showed higher SLEV dissemination rates under these conditions. *Culex nigripalpus* vector competence for SLEV and WNV is discussed and compared to previous similar studies of *Cx. pipiens quinquefasciatus* SLEV and WNV vector competence. Studies of environmental and biological factors and their influence on vector competence are essential to understand the role of vectors in virus transmission cycles in nature.

196

HEALTH IMPACT ASSESSMENT (HIA) OF INDIRA SAGAR DAM, MADHYA PRADESH: A CASE STUDY

B.N. Nagpal[1], Aruna Srivastava[1], Neeru Singh[2], S.K. Chand[3], Anushrita Singh[1], Sompal Singh[1], P.W. Kawathekar[4], A.P. Dash[5], V.K. Dua[1]

[1]National Institute of Malaria Research, New Delhi, India, [2]Regional Medical Research Centre for Tribals, Jabalpur, Madhya Pradesh, India, [3]National Institute of Malaria Research, Jabalpur, Madhya Pradesh, India, [4]Narmada Valley Development Authority, Bhopal, Madhya Pradesh, India, [5]World Health Organization, New Delhi, India

Building large dams for the generation of hydropower/ irrigation may transform the flora and fauna of the affected areas and can cause some unforeseen adverse impact on the health of the population. The Health Impact Assessment (HIA) of such projects has been made mandatory by World Health Organization. The study on Health impact assessment of Indira Sagar Dam & Resettlement and Rehabilitation (RR) colonies was initiated in January 2004. The entomological and epidemiological data for all the vector borne diseases i.e. malaria, dengue, JE & Filariasis has been collected in seven districts till March 2010. During these surveys, 32 villages, 18 RR centres, 5 Command area villages and 6 Labour Colonies were surveyed. GIS mapping of all 7 districts was done and is being updated regularly. In October 2008, a special survey focussing on Schistosomiasis was also carried out and snail species found, which has been reported to be specific vectors of cattle. No case for dengue, JE and Filariasis was found in the conducted surveys. A total of 151 samples of drinking water were collected from open wells, tube wells, hand pumps and tanks from all the surveyed areas for detection of coliform and other human pathogenic bacteria using HiWater™ Test Kit (HiMedia). Most of the water samples were positive for harmful bacteria. The information of water testing was promptly given to the concerned PHCs for immediate action. After completing each survey meeting was arranged with Vice-Chairman, Narmada Valley Development Authority (NVDA) and State authorities to intimate the survey highlights and suggest mitigation measures i.e. engineering, epidemiological and entomological to control the vector borne diseases. From October 2005, measures were implemented in the field by state Health Department, National Hydro Development Corporation (NHDC) and NVDA e.g. de-weeding in canals, release of larvivorous fish, source reduction, spray/fogging, use of plastic sheet in the canals, mosquito-proofing of houses. IEC activities and engineering workshop were also carried out. Radical treatment was given to all the Pf cases. Due to implementation of these mitigation measures the density of vectors of malaria, dengue, chikungunya, filariasis and JE has shown a remarkable reduction and also the disease. The project is now extended to cover the entire Narmada basin in Madhya Pradesh.

197

ECO-EPIDEMIOLOGICAL DETERMINANTS ASSOCIATED WITH THE RESURGENCE OF EASTERN EQUINE ENCEPHALITIS VIRUS IN THE NORTHEASTERN UNITED STATES

Theodore G. Andreadis[1], Philip M. Armstrong[1], Goudarz Molaei[1], John J. Howard[2], JoAnne Oliver[2]

[1]Center for Vector Biology and Zoonotic Diseases, The Connecticut Agricultural Experiment Station, New Haven, CT, United States, [2]New York Department of Health, Syracuse, NY, United States

Eastern equine encephalitis virus (EEEV) is a highly pathogenic mosquito-borne virus in North America. The fatality rate in humans approaches 35% to 75%, and most surviving patients experience mild to severe long-term neurologic sequelae. EEEV activity is most common in and around freshwater hardwood swamps in the Atlantic and Gulf Coast states and in the Great Lakes region where there are abundant populations of the primary mosquito vector, *Culiseta melanura*. Outbreaks in temperate regions have been sporadic, both temporally and spatially, highly focal, and largely unpredictable. During the last six years, the northeastern US has experienced a resurgence of EEEV activity throughout the region, including locations where it had not been previously detected, resulting in severe disease in humans (26 cases with 9 fatalities) and domestic animals (126 cases). The underlying causes associated with the introduction, amplification, persistence, and range expansion of EEEV in the region are explored. Factors examined include: 1) Temperature and rainfall: high fall water table and excessive rainfall during the spring and summer are strongly correlated, 2) Vector abundance and distribution: viral amplification appears to be driven by high *Cs. melanura* populations, 3) Species specific avian-mosquito interactions and virus titers in mosquitoes: blood meal analyses suggest that key bird species such as wood thrush and American robin serve as amplification hosts and based on local feeding habits and virus titers in field-collected mosquitoes, *Cs. melanura* likely serves as both the enzootic and epizootic/epidemic vector, 4) Genetic variation of regional EEEV isolates: phylogenetic analyses indicate regional differences in EEEV isolates in the northeastern US and provide evidence for local overwintering, evolution and extinction of EEEV strains, with periodic reintroduction from southern sources.

198

FHV-B2 PROTEIN EXPRESSION IN MIDGUTS OF TRANSGENIC *AEDES AEGYPTI* MOSQUITOES AFFECTS ARBOVIRUS REPLICATION

Cynthia C. Khoo, Joseph Piper, Ken E. Olson, Alexander W. Franz

Colorado State University, Fort Collins, CO, United States

Flock House virus (FHV) is a pathogen of insects. Early in the viral replication cycle, FHV expresses a 12kDa protein named B2. B2 is required by the virus to suppress the RNA interference (RNAi) immune response pathway of infected hosts. Subsequently, B2 has also been shown to suppress RNAi in other animals and plants. B2 specifically binds to long double stranded and short interfering RNA in a sequence independent manner. We hypothesized that B2, expressed in midguts of transgenic *Aedes aegypti* mosquitoes, suppresses the RNAi pathway, therefore lowers the midgut infection and escape barriers for arboviruses. To test the hypothesis, we generated transgenic *A. aegypti* that expresses B2 in the midgut upon ingestion of a bloodmeal. We microinjected a transposable element-based B2 construct into 1820 embryos of the Higgs' White Eye (HWE) strain of *A. aegypti*. Eight transgenic lines were obtained of which, three (B2-133, B2-230, and B2-284) express B2 in the midguts of bloodfed females. When challenged with the recombinant Sindbis virus (SINV) strain TR339, all three lines showed increased midgut and carcass infection rates and higher virus titers compared to HWE, which suggest that expression of B2 in midguts lowers the midgut infection and escape barriers for SINV. Studies to investigate the effects of B2 expression on dengue and chickungunya virus infections in the transgenic mosquito lines are being conducted.

199

NEW LEISHMANIASIS FOCI IN WESTERN GEORGIA

George Babuadze[1], Ketevan Sidamonidze[1], Irine Kalandadze[1], Merab Iosava[1], Ekaterine Giorgobiani[2], Gvantsa Chanturia[1], Nikoloz Tsertsvadze[1], Paata Imnadze[1]

[1]National Center for Disease Control, Tbilisi, Georgia, [2]National Institutes of Health, Washington, DC, United States

As the leishmaniasis is the most serious public health emergences exist in Georgia during the past years it is essential the national authorities to be prepared to react decisively and promptly to control them. According to the 2007-2008 leishmanisis vector surveys in Tbilisi active focuses, number of send flies was decreased. The promoting action for this fact was the enhancement of city cleaning service, initiated by city government. The first news about 4 leishmaniasis cases in Georgia was described in 1913, which presumably was first news about this disease in whole Caucasus region. In 1954, in eastern Georgia were registered 540 cases of visceral leishmaniasis (VL). Most of cases occurred in capital of country -Tbilisi. In 2009, out of 169 registered cases of leishmaiasis in the whole country 89 were located in Tbilisi. In Georgia there are 16 species of leishmaniasis vectors - *Phlebotomine* sand-flies. Due to the significant increase of VL cases in Georgia was initiated a BTEP/ISTC project G 1081 with collaboration of NIH during the 4 years (2005-2009). Mentioned project has been implemented in Tbilisi with the following objectives: Determination of the seroprevalence rate of *Leishmania* infection in humans and dogs (stray and pet); Cultivation, preservation, and identification of isolated parasite strains; Surveillance of Phlebotomine sand fly species within active VL foci, including study of their breeding and feeding behavior and identification of potential or proven vectors. In July 2007 three cases of visceral leishmaniasis were detected in Kutaisi, (all in children) which is the second large city of country by its area and population. Later vector survey was held by NCDC. During this survey several species of *Phlebotomus* genus were collected and identified using the morphology ID keys: *Ph. halepensis, Ph. balcanicus, Ph.sergenti,*. All were adults. Since 1957 none of the leishmaniasis vectors were found in western Georgia. Also no cases of this disease were registered in Kutaisi before and there are no references of existence of its vectors either. In conclusion, in Georgia obviously, climate changes, especially the global warming takes place, which likely can have an influence on various pathogens vectors behaviors, their viability, expansion of the area and prolongation of transmission season.

200

BLOOD-FEEDING PATTERNS IN MOSQUITO COMMUNITIES

Luis F. Chaves[1], Carolyn Keogh[2], Laura C. Harrington[3], Uriel Kitron[1]

[1]Emory University, Atlanta, GA, United States, [2]University of Georgia, Athens, GA, United States, [3]Cornell University, Ithaca, NY, United States

The foraging behavior of blood-sucking arthropods is a key biological event that shapes the transmission cycle of vector-borne parasites. It is also a phenomenon within the realm of community ecology, given that blood-feeding patterns of vectors can occur across a community of vertebrate hosts. Although great advances in knowledge of the genetic basis for blood-feeding choices have been reported for selected vector species, little is known about the role of community composition of vertebrate hosts in determining such patterns. Here, we present an analysis of feeding patterns of vectors across a variety of locations, looking at foraging patterns of communities of mosquitoes, across communities of hosts primarily comprised of mammals and birds. Using null models of species co-occurrence, which do not require ancillary information about host abundance, we found that blood-feeding patterns were aggregated in studies from multiple sites, but random in studies from a single site. This finding can be explained by mosquito species in a community relying primarily on host availability in a given landscape, so that contacts with specific hosts will be influenced more by the presence/absence of hosts than by innate mosquito choices. This host-feeding strategy is a function of blood-feeding plasticity, a key trait of mosquitoes that can explain the emergence of many zoonotic mosquito transmitted diseases. From an epidemiological perspective our findings support the idea that phenomena that enhance synchronization of vectors and hosts can promote the emergence of vector-borne zoonotic diseases, as suggested by observations on the linkages between deforestation and the emergence of several human diseases.

201

MOLECULAR DETECTION AND CHARACTERIZATION OF A *WOLBACHIA* ENDOSYMBIONT IN *AMBLYOMMA AMERICANUM* (IXODIDA: IXODIDAE) IN MARYLAND, USA

Xing Zhang, Jason L. Rasgon, Douglas E. Norris

Johns Hopkins University, Baltimore, MD, United States

Amblyomma americanum, the lone star tick, is very widespread in the United States ranging from Texas to Iowa in the Midwest and east to the Atlantic coast. It is a vector pathogens causing human granulocytic ehrlichiosis, canine and human granulocytic ehrlichiosis, tularemia, and is associated with Southern tick-associated rash illness. *Wolbachia* is a very common endosymbiont of arthropods and filarial nematodes. We identified a *Wolbachia* infection associated with lone star ticks at six locations in Maryland. We screened 92 adults and 10 pools of nymphs (10 nymphs/pool) using multiple primer sets. *Wolbachia* prevalence was low in screened ticks: one adult (1.8%) (n = 92) and two nymphal pools (2 of 10) were infected. The *Wolbachia* strain was characterized using multilocus sequence typing (MLST) with ftsZ, CoxA, GatB, HcpA, and FbpA genes. A phylogenetic tree was constructed using concatenated MLST gene sequences (2019 bp) and indicated that the *Wolbachia* infection of *A. americanum* belonged to supergroup F.

202

DEVELOPMENT OF A MOLECULAR TAXONOMIC KEY FOR THE IDENTIFICATION OF SCRUB TYPHUS VECTORS, MITES WITHIN THE GENUS *LEPTOTROMBIDIUM*

Achareeya Korkusol, Ratree Takhampunya, Taweesak Monkanna, Nittaya Khlaimanee, Brian Evans, Jason Richardson

Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Larval trombiculid mites (chiggers) are important vectors of scrub typhus within Thailand and much of Asia. Identification of mites species is extremely difficult and we have proposed to develop a molecular taxonomic-key for the precise identification of trombiculid mites using the Cytochrome oxidase subunit I (COI) gene of mitochondria. Our aim to develop mtDNA barcoding is to identify the pre-defined species of mites collected from field sites, focusing mostly on the reservoir mites for scrub typhus. Phylogenetic analysis (neighbor-joining algorithms, 1000 bootstrap, Phylip program) of *Leptotrombidium* mites from colonies maintained at AFRIMS was performed using full-length sequence of the mitochondrial COI gene. Five species of *Leptotrombidium* mites, *L. chiangraiensis*, *L. imphalum*, *L. fletcheri, L. deliense, L. scutellare,* are maintained in our lab. Full length sequencing of the COI gene was completed for 66 samples from 5 chigger species and compared to *Leptotrombidium* mite COI gene sequences retrieved from GenBank. Results showed that the deduced amino acid sequence of full-length COI revealed the greatest variation and diversity which can be used to discriminate these five chigger species from each other. Results of the phylogenetic tree and concordance with conventional microscopic species identification were discussed. We are working to develop this tool to allow identification of *Leptotrombidium* species from throughout the region to facilitate improved scrub typhus research in endemic regions.

203

DISTRIBUTION AND IDENTIFICATION OF *ANOPHELES BARBIROSTRIS/CAMPESTRIS* AT SA KAEO PROVINCE IN THAILAND

Prasan Kankaew[1], Somjai Leemingsawat[2], Chamnarn Apiwathnasorn[2], Supatra Thongrungkiat[2], Alongkot Ponlawat[1]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Elucidating vector distribution based on accurate species identification is important for understanding the nature of the species complex and to achieve vector control. The adult morphologies of both *Anopheles campestris* and *An. barbirostris* are difficult to distinguish due to their resemblances. The pupal skins provide better and more reliable stage characteristics for identification of both species. Pupal skin combined with geographical information systems was used to determine the distribution of *An. campestris* and *An. barbirostris*. Breeding habitats were investigated in malarious areas of Sa Kaeo province, Thailand. *Anopheles* larvae were collected from 89 breeding places in five districts (Aranyaprathet. Watthana Nakhon, Khlong Hat, Khok Sung, and Ta Phraya). Seventy six percent of *An. campestris* and *An. barbirostris* collected by larval sampling were correctly identified using pupal skins and 24% misidentified or ambiguously identified based on adult morphology. *An. campestris* larvae collected in a single habitat ranged between 1-15 larvae with a maximum at Nong Yah Plong village, Pa Rai sub-district, Aranyaprethet district. *An. barbirostris* ranged between 1-4 larvae with a maximum at Nong Mak Fai village, Nong Mak Fai sub-district, Watthana Nakhon district. *An. campestris* larvae were found in high numbers in non-drainage habitats, consisting of swamps, flooded areas, and a ground pool. Furthermore, they were found in a pH range of 6.6-7.5, nitrate nitrogen range 5-10 ppm, phosphorus level ≤ 24 ppm, aluminum level ≥ 80 ppm, calcium level ≥ 3,000 ppm, ferric iron level > 25 ppm, humus level < 3 levels, magnesium level 25-79 ppm, manganese ≤ 24 ppm, sulfate < 1,000 ppm, and potassium level 220 ppm. Soil analysis of *An. campestris* breeding habitats, when compared with a previous study at Tak province, showed that a higher proportion was present in non-drainage and semi-drainage habitats, and a pattern of nitrate nitrogen (5-10 ppm), and aluminum (> 80 ppm). A lower proportion was observed in ferric iron < 7.5 ppm. The potassium level was double at Sa Kaeo (225.7 ± 78.2 ppm.) than of Tak province (105.5 ± 66.8 ppm).

204

THE ECOLOGY OF CUTANEOUS LEISHMANIASIS IN ISRAEL: DEMOGRAPHIC AND SPATIAL ASPECTS OF THE VECTOR - RESERVOIR HOST RELATIONSHIP

Gideon Wasserberg

University of North Carolina at Greensboro, Greensboro, NC, United States

Understanding of the ecology of the wildlife reservoir - disease vector interaction is essential for the control of vector-borne zoonotic diseases. In southern Israel cutaneous leishmaniasis is caused by *Leishmania major*, transmitted by *Phlebotomus papatasi*, and maintained by the sand rat *Psammomys obesus*. In this study, I focused on the demographic and spatial aspects of this interaction. With respect to the demographic aspect, using a 1.5 years mark-release-recapture study, I studied the temporal dynamics and distribution of the disease within the host. Most of the transmission occurred following sand fly activity peak of May. Prevalence increases with age but does not differ between sexes. Survival rate is affected by infection and gender: non-infected females have higher survival rate than non-infected males but vice-versa with respect to infected animals. The probability for an individual host to survive long enough to constitute a potential infection source was estimated as 8.2%. With respect to the spatial aspect, I manipulated the degree of burrow isolation by placing artificial burrows at various distances from active host burrows and monitored the rates of their re-colonization by dispersing sand flies. Artificial burrow colonization rates were highest at 0 and 60 meters but even the farthest burrows at 120 and 240 m were frequently colonized. I also conducted a large-scale survey of sand rat burrow distribution after which I trapped and removed sand rats from selected burrows and after three months monitored burrow re-colonization. I used logistic regression to analyze of the relations between the densities of neighboring active host burrow on infection occurrence per host at various spatial scales. Only at the scale of 500-m radius from host burrow, I found significant positive relations indicating that this is a relevant scale for transmission. A risk calibration model, derived from the equation of the logistic model, suggests that even complete host eradication will not nullify transmission risk thus questioning the benefit of local host eradication strategy. The majority of colonizers were juvenile rodents. Burrow re-colonization is dictated by the phenological state of the Chenopodiaceae plants neighboring the burrow. Results indicate that sand flies, more than sand-rats, are responsible for the spatial dynamics of the disease.

205

ECOLOGY AND SPATIAL DISTRIBUTION OF BREEDING SITES OF *ANOPHELES* LARVAE IN LARACHE PROVINCE, MOROCCO

Laboudi Majda[1], Faraj Chafika[2], Sadak Abderrahim[3], Azelmat Mustapha[4], Rhajaoui Mohamed[5], El Aouad Rajae[5]

[1]Faculty of Science, University Mohamed V, Rabat, Morocco, [2]National Institutes of Health, Entomology Laboratory, Rabat, Morocco, [3]Faculty of Science, University Mohamed V, Rabat, Morocco, [4]Direction of Population, Ministry of Health, Rabat, Morocco, [5]National Institutes of Health, Rabat, Morocco

Our study was conducted to characterize larval habitats of Anopheline mosquitoes and to estimate the key ecological factors associated with this mosquito's distribution. The study was carried out during June and July 2009 within 25 localities belonging to 10 sectors of Larache province. The aquatic habitats were sampled by standard dipping techniques. The

habitats were characterised based on depth, pH, temperature, oxygen dissolved, conductivity, salinity, distance to the nearest house, algae and emergent plant (presence or absence), turbidity and habitat type. A total of 54 aquatic habitats consisting of swamps, rivers and rice fields were chosen. Fifty-two percent of all habitats samples were positive for *Anopheles* larvae. From all mosquito larvae gathered, 1145 *Anopheles* larvae were collected, from which 381 (28 %) were early instars and 829 (72%) were late instars. Morphological identification of the III and IV instars larvae revealed that 76 % (n=629) were *An. maculipennis sl* and 24 % (n=200) are *An. cinereus.* The only species belonging to *An. maculipennis* complex was *An. labranchiae*. Statistics analysis showed that the density of *An. labranchiae* was associated with turbidity and depth in aquatic habitats. These findings suggest that the distribution of *An. labranchiae* were driven by different environmental factors. Understanding the relationship between habitats, environmental factors and abundance of *Anopheles* larvae is essential for an efficient application of mosquito control methods.

206

EVALUATION OF A METOFLUTHRIN FAN VAPORIZER DEVICE AGAINST PHLEBOTOMINE SAND FLIES (DIPTERA: PSYCHODIDAE) IN A CUTANEOUS LEISHMANISIS FOCUS IN THE JUDEAN DESERT, ISRAEL

Gabriela Zollner[1], Laor Orshan[2]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Laboratory of Entomology, Ministry of Health, Jerusalem, Israel

Leishmaniasis is a serious public health problem globally and is also a current operational military threat to US military forces deployed in the Middle East and southwestern Asia. *Leishmania* parasites are transmitted by sand flies (Diptera: Psychodidae). In the Judean Desert in Israel, *L. tropica* is vectored by *Phlebotomus sergenti* sand flies that live in rocky hillsides and travel to residential areas at night in search of a blood meal. There is no prophylactic vaccine available for leishmaniasis, so vector control is crucial to prevent parasite transmission. In Israel, sand fly control involves spraying large quantities of residual insecticide on house walls and adjacent surfaces, but insecticidal efficacy is reduced by high UV radiation, temperatures and blowing dust. In many areas, people leave their windows open at night and do not use personal protection (e.g. topical repellents) to protect against bites from sand flies that enter houses. Thus, there is a greater need for passive control measures which effectively repel biting sand flies. Fan vaporizer devices that emanate spatially active pyrethroids are promising tools that might provide long-lasting, passive protection against arthropod disease vectors around the world. The main goal of this study was to evaluate the effectiveness of the OFF! Clip-On device (31.2% w/v metofluthrin a.i.) for repellency against phlebotomine sand flies near a residential area in a cutaneous leishmaniasis as a result of *L. tropica* focus in the Judean Desert, Israel. Sand flies were collected outdoors using modified CDC light traps and sticky traps (unbaited or baited with CO2). The results of this study will be discussed in the context of the effect of metofluthrin and spatial repellents on phlebotomine sand flies.

207

EXPERIMENTAL INFECTION AND TRANSMISSION OF *LEISHMANIA TROPICA* BY LABORATORY-REARED PHLEBOTOMUS DUBOSCQI

Hanafi A. Hanafi, El-Shaimaa M. Nour El-Din, Shabaan S. El-Hossary, Rania M. Kaldas, Jeffrey T. Villinski, Barry D. Furman, Peter J. Obenauer

United States Naval Medical Research Unit Number Three, Cairo, Egypt

This is the first report of laboratory transmission of *Leishmania tropica*, a pathogen causing cutaneous and viscerotropic leishmaniasis, by the sand fly *Phlebotomus duboscqi*. This blood-feeding, anthropophilic, sand fly species is broadly distributed throughout southern Africa, and has been incriminated as a vector of *L. major* in Ethiopia, Kenya, Senegal and Sudan. After colonizing this sand fly species in the lab, we employed the hamster infection model to determine its competence to transmit *L. tropica.* Groups of female sand flies (130-160 flies/group) were fed naturally on infected hamsters, or artificially on blood suspension of infected *L. tropica* tissue amastigotes using a chick-skin membrane apparatus. Samples of blood-fed sand flies from each group were dissected and examined by microscopy at 2, 4, 16, and 20 hrs post-feeding, as well as 1-9 and 11 days post-feeding. Promastigote maturation was observed in 67% (50 of 74) of the artificially infected sand flies, with the promastigotes observed in the thoracic and abdominal midgut at 11 days post-infection. Promastigote infection of the abdominal midgut was observed in 3% (4 of 118) of the naturally infected sand flies. Nine days post-infection, 53% (8 of 15) and 41% (12 of 29) of the remaining blood-suspension infected sand flies were re-fed on 2 uninfected hamsters. Thereafter, we monitored the persistence, dissemination, and visceralization of the parasites in these hamsters. The hamsters were sacrificed at 4 months post-exposure to infected sand flies, and blood, spleen, liver, and bone marrow samples from these hamsters were screened by Polymerase Chain Reaction (PCR). Several of these samples (blood, liver, bone morrow and spleen of one hamster, liver and spleen of the second hamster) were found to be PCR positive for the presence of *L. tropica* DNA. However, no skin lesions developed on these hamsters after being bitten by infected sand flies. Collectively, these preliminary results suggest the potential of *P. duboscqi* to serve as a vector of *L. tropica.*

208

EVALUATION OF *IN VITRO* ANTIMICROBIAL ACTIVITY OF WHOLE BODY EXTRACTS OF *LUCILIA SERICATA* MAGGOTS

Gokhan Dogandemir[1], Ozgur Koru[2], **Orhan Bedir**[1], Abdullah Kilic[1], Remzi Engin Araz[2], Mehmet Tanyuksel[2], Ahmet Celal Basustaoglu[1]

[1]Gulhane Military Medical Academy, Department of Microbiology, Ankara, Turkey, [2]Gulhane Military Medical Academy, Department of Microbiology, Division of Medical Parasitology, Ankara, Turkey

Maggot therapy (MT) has been used for centuries and MT has and is being used extensively in the United Kingdom (UK), Germany, and the United States of America, where sterile maggots are commercially available. Therapeutic maggots used most commonly today are those of the greenbottle fly (*Lucilia sericata*) which only attacks necrotic tissue. The medical literatures have shown that the secretions/extracts of maggots are very effective in the treatment of gram-positive bacterial infections. The aim of this study is to assess the performance of *in vitro* minimal inhibitory concentration from whole body extracts of maggots taken from chronic wounds of treated patients against Gram positive and Gram negative bacteria and also yeast strains. Whole body extracts of maggots harvested by injuring *L. sericata* larvae with a mortar had an inhibitory effect on *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis (mecA)*, *Streptococcus pyogenes*, *Acinetobacter haemolyticus*, moderate effect on methicillin resistant *Staphylococcus aureus* (MRSA), *Enterobacter aerogenes* and no effect on *Enterococcus faecium (VanA)*, *Klebsiella pneumoniae* (ESBL), *Enterococcus faecalis, Pseudomonas aeruginosa,* and *Candida albicans*. Whole body extracts of maggots significantly inhibited the growth of Gram positive bacteria better than Gram negative bacteria and this substance would also be advocated as being useful cost-effective and safe alternative for the management of chronic wound infections because of increasing number and prevalence of antibiotic-resistant microorganisms.

209

CONTINUED INTERRUPTION OF LYMPHATIC FILARIASIS TRANSMISSION ONE YEAR AFTER THE CESSATION OF MDA IN A PREVIOUSLY HIGHLY ENDEMIC AREA OF MALI

Siaka Konate

MRTC/FMPOS, Bamako, Mali

Although cessation of mass drug administration in areas endemic for lymphatic filariasis (LF) is currently recommended when prevalence of microfilaremia reaches < 1% and ICT positivity is <0.1% in children < 5 years of age, the impact of persistent vector infection in this setting is unknown. A pilot study of community-based mass drug administration (MDA) with albendazole and ivermectin was instituted in six *Wuchereria bancrofti (*Wb) endemic villages of the southern part of Mali to provide baseline data and guidance prior to the initiation of the National LF Elimination Program. We have previously reported the results of surveillance performed after 6 rounds of MDA, which indicated persistence of Wb at low levels in the insect vector despite an apparent elimination of infection in human population (0/686 adults positive for microfilaremia and 0/120 children positive by ICT). To assess the durability of transmission interruption in this setting, MDA was stopped in 2 villages (1396 inhabitants) that were free of infected mosquitoes and continued in the remaining 4 villages (3489 inhabitants). Human and vector infection was assessed 12 months after MDA in the 6 villages. Microfilaremia was assessed by finger prick blood collected at night in 800 adults, and circulating antigen (CA) status was determined by ICT in 800 adults and 289 children < 5 years of age, none of the individuals tested was found to be positive for microfilaremia or CA. *Anopheles* vectors were collected monthly by human landing catch from August to December in each village. In all, 1499 mosquitoes were dissected from the untreated villages and 2892 from the treated villages. Two infected vectors were found, one from the untreated villages and one from the treated villages. No infective vectors were detected in any of the villages. These data are consistent with continued interruption of transmission one year after stopping MDA in a previously highly endemic area of Mali with a low level of residual vector infection. A longer follow up period is necessary to confirm the absence of recrudescence.

210

EPITROCHLEAR LYMPHADENITIS INFECTION WITH *DIROFILARIA IMMITIS:* MORPHOHISTOLOGIC FINDINGS

Hernani D. Cualing[1], Ramon L. Sandin[2]

[1]University of South Florida, Tampa, FL, United States, [2]University of South Florida/Moffitt Cancer Center, Tampa, FL, United States

An unusual presentation of a well preserved worm, in a lymph node, from an Indianapolis dog owner, who presented with an enlarged painful epitrochlear lymph node is presented. This node was biopsied and the histological sections of a preserved worm's internal structure is presented and differentiated from other *Dirofilaria spp.* Human infection by filarial worms is unusual in the US. Pulmonary dirofilariasis is the most commonly reported anatomic location in the United States, and is caused by *D. immitis*, also called the dog heartworm. Diagnosis is by histology and serologic diagnosis has not been helpful. Most *D. immitis* agents found in the lungs are necrotic, poorly preserved, and sometimes calcified. (Figure 1a-x) Here we present a histologically preserved *Dirofilaria* in a lymph node. Humans are incidental hosts in the agent's life cycle and terminal hosts. The lymph node shows a necrotizing granuloma with a worm section in the pink necrotic area. A transverse section through a mature adult male shows a pink wall with smooth muscle and regular transverse ridges. Note the even thickness of the cuticles without spikes, the pathognomonic internal cuticular wedged shaped ridges with lateral cords, a muscular layer and reproductive and intestinal tubules. Note the tall coelomyarian muscles and centrally located intestine and reproductive tubes. Worms are 100-350 um in diamater. Projecting into the central worm cavity are internal longitudinal ridges. Unlike *D. immitis,* the cuticle of *D. tenuis* is relatively thick, and shows prominent spike ridges. *D. repens* infect man also and is a more common cause of subcutaneous filariasis, but it has not been reported from the United States. Infection in man presents as lesions of the skin, conjunctivae, arms or legs but rarely in lymph nodes as illustrated here. *D. tenuis* is the species most frequently encountered in the Southeast United States infecting humans and can cause subcutaneous infections. Infections by these filarial worms is uncommon and it is even more uncommon to find their presence in lymph node tissue. A cutaneous mosquito bite adjacent to the lymph node is the purported route of infection rather than hematogenous involvement; this is unlike the more common pulmonary infections where tissue involvement occurs via the blood route.

211

IMPACT OF A COMMUNITY-BASED LYMPHEDEMA MANAGEMENT PROGRAM FOR LYMPHATIC FILARIASIS IN ORISSA STATE, INDIA

Katherine Mues[1], Audrey Flak[1], Aishya Prakash[2], Erin Kennedy[3], Jonathan Rout[2], LeAnne Fox[3]

[1]Emory Rollins School of Public Health, Atlanta, GA, United States, [2]Church's Auxiliary for Social Action, Bhubaneswar, India, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

India has an estimated 7 million people living with lymphedema due to lymphatic filariasis (LF). Lymphedema management has been shown to decrease acute episodes of adenolymphangitis (ADLA) and reduce lymphedema progression, but there are little data on the impact of community-based programs for lymphedema patients. A large community-based lymphedema management program began implementation in Orissa State, India in 2007 through an Indian NGO, the Church's Auxiliary for Social Action (CASA), in consultation with CDC. The program relies on health supervisors and village volunteers to teach lymphedema management techniques to affected patients and has scaled up to enroll over 15,000 patients. A random sample of 376 patients was followed over 6 months to evaluate the clinical benefits of the lymphedema management program. Clinical and questionnaire data were collected at baseline and at 1, 2, 3 and 6 months after the start of the program and were analyzed using longitudinal analysis procedures in SAS 9.2. At baseline, 80 (22.9%) patients reported at least one ADLA episode in the last 30 days compared with 38 (11.7%) at 6 months (P<.0001). Adherence to the program increased over time. At baseline, 185 (55.1%) patients reported washing their legs with soap and water while at 6 months 327 (100%) reported doing so (P<.0001). Sixty six (18%) patients reported treating wounds with antiseptic cream at baseline compared to 188 (57%) at 6 months (P<.0001). A logistic model exploring the effects of program adherence on the odds of at least one ADLA episode in the last 30 days demonstrated an odds ratio for time of 0.86 (95% CI: 0.80, 0.93), indicating that the odds of at least one ADLA episode decreased over time while enrolled in the program. A Poisson model also demonstrated that patients with access to antibiotics (OR=0.64, 95% CI: 0.91, 0.86) had a decreased risk of ADLA episodes. These data highlight the beneficial impact of a community-based lymphedema management program to improve ADLA episodes among lymphedema patients in LF endemic areas.

212

FIRST EVIDENCE OF SPATIAL CLUSTERING OF LYMPHATIC FILARIASIS INFECTION IN AN *AEDES POLYNESIENSIS* ENDEMIC AREA

Hayley Joseph[1], James Maloney[1], Fuatai Maiava[2], Shannon McClintock[3], Patrick Lammie[3], Wayne Melrose[1]

[1]James Cook University, Townsville, Australia, [2]World Health Organization, Apia, Samoa, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

Successful elimination of lymphatic filariasis (LF) requires accurate identification of residual foci of transmission and stringent surveillance

strategies to combat potential resurgence. This is challenging in areas where the day-biting *Aedes polynesiensis* resides, such as Samoa, since in previous studies no geographical clustering of infection has been demonstrated. Another challenge for this low prevalence phase is the choice of diagnostic assay as testing for circulating filarial antigen (CFA) or microfilariae (Mf) alone may not have adequate sensitivity. This could be solved by using the commercially available Filariasis CELISA to measure antibody. In the current study 5 Samoan villages were chosen based on previous epidemiological assessments to represent a range of infection prevalences. CFA, Mf, and antibody levels in children ≤ 10 years of age had been recorded and results linked to household of residence and/or primary school of attendance. To ascertain the location of exposure, two scenarios based on potential foci of transmission around communities and schools were explored. "Community-based" analyses revealed significant spatial clusters of households with infected individuals and a relationship to antibody positive children when they were included in the spatial analysis. Similarly, "school-based" analyses revealed significant clusters of antibody positive children and these were related to CFA positive individuals when they were included in the spatial analysis. These promising findings are the first published evidence of spatial clustering of LF in a day-biting Aedes polynesiensis endemic area. The study provides a key insight into the management of residual foci and potential future surveillance strategies.

213

DECREASED PREVALENCE OF FILARIAL INFECTION AMONG DIABETIC SUBJECTS ASSOCIATED WITH A DIMINISHED PRO-INFLAMMATORY CYTOKINE RESPONSE

V. Aravindhan[1], V. Mohan[1], J. Surender[1], M.M. Rao[1], N.P. Kumar[2], V. Kumaraswami[3], Thomas B. Nutman[4], **Subash Babu**[5]

[1]MDRF, Chennai, India, [2]NIH-TRC-ICER, Chennai, India, [3]Tuberculosis Research Center, Chennai, India, [4]LPD, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [5]NIH-TRC-ICER and SAIC-Frederick Inc., NCI-Frederick, Chennai, India

Epidemiological and animal studies have shown an inverse correlation between the incidence of helminth infections including lymphatic filariasis (LF) and the incidence of atopy and autoimmunity. However, the interrelationship between LF and Type-1 and Type-2 diabetes (T1DM and T2DM, respectively) in humans is not known and hence, two cross sectional studies to assess the baseline prevalence and the correlates of sero-positivity of LF among diabetic subjects were undertaken in Chennai, India. In the first study, there was a significantly lower prevalence (p=0.026) of LF among T1DM subjects (0%; n=200) compared to non-diabetic subjects (2.6%; n=500) providing validation for animal data showing the protective effect of filarial infection on T1DM. More importantly, in the second study, there was a significantly lower prevalence of LF among T2DM subjects (both newly diagnosed [5.7%; n=158] and those under treatment [4.3%; n=161]) compared to pre-diabetic subjects [9.1%; n=154] (p=0.0095) and non-diabetic subjects [10.4%; n=943] (p=0.0463). Among those with filarial infection, there were significantly lower filarial antigen loads among T2DM subjects compared to non-diabetic subjects (Geometric Mean of 354 U/ml in T2DM vs. 1594 U/ml in non-diabetic subjects; p=0.04). Serum levels of the pro-inflammatory cytokines - IL-6 and GM-CSF were significantly lower in T2DM subjects who were LF positive compared to those who were LF negative. There were, however, no significant differences in serum levels of the anti-inflammatory cytokines, IL-10, IL-13 and TGF-beta between the two groups. Thus, there appears to be a striking inverse relationship between the prevalence of LF and diabetes, which is reflected by a diminished serum pro-inflammatory cytokine response in subjects with diabetes and concomitant LF.

214

SEVENTEEN YEARS OF ANNUAL DISTRIBUTION OF IVERMECTIN HAS NOT INTERRUPTED ONCHOCERCIASIS TRANSMISSION IN NORTH REGION, CAMEROON

Moses N. Katabarwa[1], Albert Eyamba[2], Philippe Nwane[2], Peter Enyong[3], Souleymanou Yaya[4], Abdoulaye Yougouda[4], Jean Baldiagai[4], Benjamin Didier Biholong[5], Kambaba Madi[6], Gervais Ondobo Andze[5], Frank O Richards[1]

[1]Emory University/The Carter Center, Atlanta, GA, United States, [2]The Carter Center-Cameroon, Yaounde, Cameroon, [3]Research Foundation for Tropical Diseases and Environment, Buea, Cameroon, [4]Ministry of Health, Garoua, Cameroon, [5]Ministry of Health, Yaounde, Cameroon, [6]Retired (Ministry of Health), Garoua, Cameroon

A contentious issue in the field of onchocerciasis elimination is whether annual mass administration of ivermectin for 15 to 17 years is sufficient to eliminate transmission. We conducted a study to evaluate whether there is ongoing transmission in a hyperendemic focus in North Region of Cameroon, where annual treatment has been administered for 17 years. Surveys were conducted in 12 communities having baseline 1991 microfilaria (mf) prevalence data and nodule data for adults and children. In 2009 we returned to these communities and examined 775 adults for mf, 1015 adults for nodules. The 1991 baseline mf data for 107 children was compared with followed up in 157 children in 2009. 1991baseline data from ocular examinations for onchocerciasis morbidity were also available for 6 communities, and we evaluated 472 persons in 8 communities in the follow up survey. We also conducted entomological studies and determined annual transmission potential, vector infection (all larval stages) and infective rates (L3 only), and annual biting rates. In total, 12,107 flies were examined. Mf prevalence among adults decreased from 70%% to 4.8% (p<0.0001). The mf prevalence in children reduced from 18.7% to 1.3% (p<0.0001). In ocular studies, mf in the anterior chamber dropped from 42.7% to 5.5% mf (p<0.0001), and punctuate keratitis from 33.5% to 3.6% (p<0.0001). *Simulium* vector flies showed 2.1% infection rate, 1.4% infective rate, 39% parous rate, and annual biting rate per person of 24,945. An annual transmission potential (ATP) of 136 in the follow up survey was observed; compared to an OCP standard requiring ATP<100 to avoid new eye disease. This study showed that 17 years was not sufficient to interrupt transmission or stop ocular morbidity from onchocerciasis. Ivermectin treatment should continue in order to avoid the risk of recrudescence.

215

GEOSTATISTICAL MAPPING OF THE PREVALENCE OF INFECTION DURING THE ONCHOCERCIASIS CONTROL PROGRAMME IN WEST AFRICA: IMPLICATIONS FOR ESTIMATING THE GLOBAL BURDEN OF ONCHOCERCAL DISEASE

Simon O'Hanlon[1], Luc Coffeng[2], Hannah Slater[1], Boakye Boatin[3], Sake de Vlas[2], Wilma Stolk[2], Maria-Gloria Basáñez[1]

[1]Imperial College London, London, United Kingdom, [2]Erasmus Medical Centre, Rotterdam, The Netherlands, [3]World Health Organisation, Geneva, Switzerland

Previous Global Burden of Disease (GBD) estimations calculated that 459,000 and 389,000 disability-adjusted life years (DALYs) were lost to onchocerciasis in 2000 and 2004 respectively. These figures were calculated using simplified disease models, which ignored important morbidities caused by onchocercal skin disease and epilepsy, and probably seriously underestimated the true disease burden. Major factors influencing the numbers of DALYs lost to an infectious disease are the adequate delineation of the population at risk and defining the prevalence of infection. The latter can be determined using limited survey data. However because survey data are usually biased towards areas with high disease prevalence, the true population at risk is

likely far greater than the survey data suggest. Estimates of the population at risk in areas under the African Programme for Onchocerciasis Control (APOC) are informed by epidemiological mapping of onchocerciasis, and through census data collected through community-directed treatment with ivermectin (CDTI). However, such detailed estimates are lacking for those countries that were covered by the Onchocerciasis Control Programme in West Africa (OCP). Therefore, we present work to map the prevalence of infection and populations at risk for the time periods 1975 (pre-vector control), and 1990 & 2005 (the two time-points required for the present GBD), in the OCP countries of West Africa based on spatial statistical approaches. We use a generalised linear spatial model, to describe the relationship between several environmental variables (such as altitude, land cover, distance to nearest river) and community-level prevalences of infection (obtained from OCP survey data). We use this model to predict prevalence over the entire geographical area covered by the OCP at different time-points during the control program. These estimates will be helpful in the calculation of the GBD due to the various onchocerciasis sequelae identified in the refined disease model for West Africa.

216

ONCHOCERCIASIS ELIMINATION IN ABU HAMED FOCUS, NORTHERN SUDAN: A 2007 ENTOMOLOGICAL SURVEY

Tarig Higazi[1], Isam Zarroug[2], Hanan Ali[2], Wijdan Abdalla M[2], Tong Schewitaak[2], Nabil Aziz[3], Moses Katabarwa[4], Hassan Hassan[5], Tom Unnasch[5], Charles Mackenzie[6], **Frank Richards**[4]

[1]Ohio University, Zanesville, OH, United States, [2]Ministry of Health, Khartoum, Sudan, [3]The Carter Center, Khartoum, Sudan, [4]The Carter Center, Atlanta, GA, United States, [5]University of South Florida, Tampa, FL, United States, [6]Michigan State University, East Lansing, MI, United States

This paper presents baseline data and assessment study of the first onchocerciasis elimination program in East Africa. The study was carried out in the Abu Hamed focus of onchocerciasis in Sudan, which represents the northernmost focus of the disease in the world. The focus is located in the Sahara desert around the town of Abu Hamed on River Nile and about 700km northwest of the nearest disease focus in the east of the country. The isolated nature of the Abu Hamed focus made it an attractive target for the activities of disease elimination in the new government elimination policy announced in 2006. The strategy involves a switch from annual to semi-annual (six monthly) community-directed treatment with ivermectin (CDTI) and monitoring of *Onchocerca volvulus* indicators of infection and transmission in both human and vector populations. Baseline entomological data were obtained concurrent with switching to twice per year treatment during the 2007-2008 breeding season. Using human landing captures, 29,969 *Simulium damnosum* blackflies were collected from two sentinel villages (Mograt and Nady) within the focus. O-150 repeat PCR screening of these blackflies in 203 pools was conducted in 2009, and analyzed by PoolScreen. 2 positive pools among were found among the 102 pools tested from Mograt and no positive pools were detected in Nady. Overall results showed an infection rate of 0.84 infected flies per 10,000 flies (95% confidence interval of 0.0497 - 1.88 per 10000 flies) for the Abu Hamed focus. This infection rate indicates transmission of the parasite in Abu Hamed may be close to the level below which the parasite population is unsustainable prior to shifting of treatment to twice per year. Control activities in other foci in Sudan resulted in moderate reduction of transmission, as indicated by blackfly vector screening assays. A repeat entomological survey is planned for 2010. Overall assessment of the elimination activities and challenges are discussed.

217

PROTECTION AGAINST ACCELERATED ATHEROSCLEROSIS IN A MOUSE LUPUS MODEL BY ES-62, AN IMMUNOMODULATORY FILARIAL NEMATODE PRODUCT

William Harnett[1], Tamar Aprahamian[2], Margaret M. Harnett[3], Ian R. Rifkin[2]

[1]Strathclyde University, Glasgow, United Kingdom, [2]Boston University School of Medicine, Boston, MA, United States, [3]Glasgow University, Glasgow, United Kingdom

A feature of systemic lupus erythematosus is development of an accelerated atherosclerosis. The gld.apoE-/- mouse represents a good model for this condition, its absence of functional Fas ligand and apolipoprotein E resulting in development of aggravated lupus-like symptoms and accelerated atherosclerosis. Our previous work has shown that the anti-inflammatory statin, simvastatin can reduce onset of both lupus-like symptoms and atherosclerosis in this model animal. We therefore examined the protective effects of another anti-inflammatory molecule, ES-62, a phosphorylcholine (PC)-containing glycoprotein secreted by the filarial nematode Acanthocheilonema viteae. ES-62 was found to have very little effect on lupus-like symptoms as determined by measuring glomerular tuft volume, glomerular cell infiltrates and serum albumin levels but demonstrated a striking 60% reduction in atherosclerotic lesion area. It is known that T15-type antibodies against PC can protect mice from atherosclerosis and hence as ES-62 is a PC-containing molecule, generation of such antibodies was investigated as a mechanism of action for atherosclerosis amelioration. However, ES-62 did not induce T15-type antibodies and thus its protective effects in this model may reflect its known anti-inflammatory properties.

218

IMMUNOGLOBULIN M AND IGG SUBCLASS RESPONSES AGAINST *WOLBACHIA* PEPTIDOGLYCAN-ASSOCIATED LIPOPROTEIN (PAL) IN PATIENTS WITH BANCROFTIAN FILARIASIS

Surang Nuchprayoon, Vivornpun Sanprasert

Chulalongkorn University, Bangkok, Thailand

The presence of large numbers of *Wolbachia* in filarial nematodes raises questions whether *Wolbachia* -derived molecules may contribute to the pathogenesis in lymphatic filariasis. Moreover, the detections of humoral immune responses against the *Wolbachia* antigens may be useful as the immunological marker(s) of morbidity and infection of lymphatic filariasis. The association between anti-peptidoglycan associated lipoprotein (PAL) antibodies and clinical manifestations was studied in 75 individuals from endemic areas (52 individuals with active infection, 4 individuals with clinical manifestations, and 19 individuals were endemic normals). We found that levels of IgG3 antibodies against rPAL were significantly higher in patients with active infection than the endemic normals ($P=0.003$). Furthermore, anti-rPAL IgG3 antibody levels were significantly higher in microfilaremic patients ($P=0.04$). However, the levels of IgM, IgG2 and IgG4 antibodies against rPAL among all groups were not significantly different ($P > 0.05$). The results of this study demonstrate that anti-PAL antibody responses are associated with the presence of filarial infections in humans but not with chronic filarial morbidity. Our results suggest that detections of anti-PAL IgG3 antibodies may be useful for diagnosis of the *W. bancrofti* infection.

219

GENE EXPRESSION IN THE BACTERIAL ENDOSYMBIONT OF THE FILARIAL PARASITE, *BRUGIA MALAYI*

Weam I. Zaky

Smith College, Northampton, MA, United States

The parasitic nematodes *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori* cause a disfiguring disease in humans known as lymphatic filariasis (LF), which affects more than 150 million people worldwide. An intracellular alpha-proteobacterium of the genus *Wolbachia,* which belongs to the family Rickettsiaceae, is found in all life cycle stages of these nematodes. These bacteria are maternally inherited via the egg and are necessary for normal embryogenesis and survival of the parasite. A complete genome DNA sequence for the *Wolbachia* of *B. malayi* was determined in 2005. We used real-time quantitative RT-PCR for comparison of gene expression of *Wolbachia* between two *Brugia malayi* life cycle stages: the L3 vector larval stage and the L4 mammalian larval stage. *Wolbachia* genes significantly upregulated in the L4 stage compared to the L3 stage may be critical for the survival of *B. malayi* in the human host. We tested the number of *Wolbachia* per worm in the L3 and L4 stages by quantitative RT-PCR. We found that there are 100-fold more *Wolbachia* in L4 worms than in L3 worms. Forty-one of the approximately 800 *Wolbachia* genes were selected for study using three biological replicates. Real-time PCR results showed there are expression differences between the biological replicates, but some genes showed consistent differences in expression between L3 and L4 larvae.

220

MOLECULAR CLONING AND CHARACTERIZATION OF A VESICULAR ACETYLCHOLINE TRANSPORTER FROM *ONCHOCERCA VOLVULUS* AND ITS BIOCHEMICAL CHARACTERIZATION IN *HAEMONCHUS CONTORTUS* WORMS

Ann S. Nanteza, George W. Lubega

Makerere University, Kampala, Uganda

Onchocerca volvulus is a human subcutaneous parasitic nematode recognized as the single most common cause of irreversible blindness. No drug currently available is completely safe and effective for mass treatment. The identification of suitable drug targets is essential. Regulated release of acetylcholine for neurotransmission requires the loading of acetylcholine into synaptic vesicles by the vesicular acetylcholine transporter. Vesamicol is a compound that blocks acetylcholine accumulation in cholinergic vesicles by acetylcholine into synaptic vesicles by binding to the VAChT protein. The *O. volvulus* putative vesicular acetylcholine transporter gene was cloned and protein characterized. Total protein from *O. volvulus* and H. contortus worms and rat brain were probed with rat anti-VAChT antibodies from the C-terminus rat VAChT by Western blotting. The protein was characterised biochemically by assessing the effect of vesamicol on motility of H. contortus worms. The predicted partial protein is composed of 404 amino acids and contains 11 conserved transmembrane domains. The cloned cDNA contains the 5' end and forms approximately 75% portion of the gene. The putative protein shows extensive homology to the vesicular acetylcholine transporter, *C. elegans* gene (98%)and closely related to other vesicular acetylcholine transmitter transporters and amine transporters with high conservation within the transmembrane regions with charged amino acids indicating functional significance in substrate transportation. Phylogenetic analysis of VAChTs and MATs clusters the *Onchocerca* protein in the same clade with *C. elegans* showing evolutionary relatedness. The phylogeny revealed that nematodes diverged from the ancestry much earlier in evolution compared to other animals. Since unc 17 mutations protect against organophosphorus toxicity and the Torpedo electric lobe provides extremely dense cholinergic innervation to the electric organ, these relationships support a role of VAChT of *O. volvulus* in neurotransmission. Absence of 70kDa protein band in H. contortus or *O. volvulus* proteins revealed marked variations of the amino acid sequences within at C-terminus. The concentrations of vesamicol and incubation periods caused increased inhibitions of worm motility.

221

SYBR GREEN QPCR ASSAYS FOR DETECTING *WUCHERERIA BANCROFTI* AND *DIROFILARIA IMMITIS* DNA IN BLOOD AND MOSQUITOES

Ramakrishna U. Rao, Yuefang Huang, Gary J. Weil

Washington University School of Medicine, St. Louis, MO, United States

Molecular detection of filarial DNA can be used to map filarial infections and to progress in disease elimination programs. We previously described a highly sensitive real-time PCR method for detecting *Wuchereria bancrofti* (Wb) DNA in blood and mosquitoes. That test employed an expensive TaqMan probe. This study was performed to compare SYBR Green based qPCR assays with TaqMan assays in terms of performance and cost. SYBR Green qPCR assays for Wb and *Dirofilaria immitis* (Di) DNA employed primers specific for LDR (long DNA repeat, 90bp) and MTR (multiple tandem repeat, 84bp; Genbank number M95173) targets, respectively. We compared assays that employed conventional SYBR Green and Power SYBR Green master mixes for detecting filarial DNA isolated from parasites, infected blood, and vectors. The efficiency of qPCR tests with Power SYBR Green to detect both LDR and MTR was close to 100% and better than that observed with conventional SYBR Green qPCR. Ct values with Power SYBR Green assays were 2 cycles lower (4-fold more sensitive) than conventional SYBR Green assays and comparable to TaqMan qPCR assays for these targets. Melting curve analyses showed uniform results for both LDR and MTR with melting temperatures of 73.4C and 72.2C, respectively. Power SYBR Green tests were specific for detecting LDR or MTR with no signals detected with DNA templates from other filarial nematodes, *Plasmodium falciparum*, *Aedes aegypti*, or *Homo sapiens*. Power SYBR Green and TaqMan LDR assay results were identical for 121 pools of gravid mosquitoes collected in endemic areas (40 positive pools) and for 19 blood samples from infected humans (16 positives by qPCR). The two MTR assays produced the same results for *D. immitis* detecting the DNA present in 1/100th of one microfilaria. Both assays also detected *D. immitis* DNA in laboratory infected mosquitoes both immediately after feeding and for 30 days post blood meal. Additional studies are needed to evaluate the sensitivity of the MTR qPCR tests with field collected mosquito and blood samples. The cost of the SYBR Green and TaqMan qPCR assays is approximately the same including the DNA extraction ($3.4/sample). These results suggest that Power SYBR Green qPCR is a promising alternative to probe-based assays for detecting filarial DNA in blood samples and in vectors.

222

GENDER-REGULATED *BRUGIA MALAYI* GENES HAVE *CAENORHABDITIS ELEGANS* HOMOLOGUES WITH GERMLINE (SPERMATOGENESIS AND OOGENENSIS) OR EMBRYOGENESIS-ENRICHED EXPRESSION

Ben-Wen Li, Amy C. Rush, Dao-Jun Jiang, Makedonka Mitreva, Gary J. Weil

Washington Universtiy School of Medicine, St. Louis, MO, United States

Recent advances in sequencing of parasite genomes have outpaced progress in annotation. We previously studied gender-regulated gene expression in *Brugia malayi* adult worms. In this study, gender-regulated *B. malayi* expression profiles were cross-referenced with expression patterns reported for the free-living nematode *Caenorhabditis elegans*. 119 of 605 *C. elegans* homologues of Brugia transcripts that are male-upregulated (20%) and 151 of 850 homologs of female-upregulated transcripts (18%) are annotated as "germline-enriched" in C. elegans hermaphrodites producing both sperm and oocytes. In contrast, only 11%

of total number of Brugia transcripts on the BmV2array has C. elegans homologues classified as germline-enriched. Most C. elegans germline-enriched homologues of Brugia male-upregulated transcripts (73%) are associated with spermatogenesis, while 75% of the germline-enriched homologues of Brugia female-upregulated transcripts are associated with oogenesis. Bioinformatics analysis suggests that many of the proteins encoded by spermatogenesis and oogenesis associated genes have binding and catalytic activities. RNA-binding activity is dominant in the oogenesis gene set, and ATP-binding is associated with spermatogenesis. Ninety-eight Brugia gender-regulated genes have homologues that are required for early embryogenesis in C. elegans, and 80% of these are female-upregulated. Several of the female upregulated genes in this group encode proteins involved in protein synthesis, cell division and regulation of transcription (ribosomal protein large subunit family member rpl-27 and rpl-22, 40S ribosomal protein s27, cell division control protein 2, and histone family member (his-35). Male-upregulated genes include actin, alpha tubulin and beta tubulin. In situ localization results for 5 genes were consistent with the predicted functions of these genes in B. malayi reproduction. This study illustrates the value of comparative genomics for improving annotation of nematode genomes and transcriptomes.

223

MOLECULAR BASIS OF ENDOSYMBIOSIS BETWEEN *WOLBACHIA* ENDOSYMBIONT AND THEIR FILARIAL NEMATODE, A ROLE FOR A FILARIAL PHOSPHATE PERMEASE THAT IS UP-REGULATED IN RESPONSE TO *WOLBACHIA* DEPLETION

Sridhar Arumugam, Kenneth M. Pfarr, Achim Hoerauf

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

Anti-symbiotic approach to control filariasis targeting *Wolbachia* endosymbionts in filarial nematodes has met with promising success, but still the molecular basis governing the endosymbiosis between *Wolbachia* and their filarial host remains unclear. Previously, we found up-regulation of a *Litomosoides sigmodontis* phosphate permease gene (Ls-ppe-1) in response to *Wolbachia* depletion at the m-RNA level and hypothesized that Ls-ppe-1 could have an important role in nucleotide metabolism as depletion of *Wolbachia* induces expression of Ls-ppe-1, perhaps to compensate for lack of nucleotides in the absence of their endobacteria. To test this hypothesis, firstly, the regulation of phosphate permease during *Wolbachia* depletion was studied at the protein level in *L. sigmodontis* and *Onchocerca* volvulus, and secondly, the localization of phosphate permease (PPE) and *Wolbachia* in *L. sigmodontis* and *O. volvulus* were investigated in untreated and antibiotic treated filarial worms. Results show the up-regulation of *L. sigmodontis* phosphate permease (Ls-PPE) both at the m-RNA and protein levels and immunohistology results demonstrate that Ls-PPE is localized to areas of the worms that contain *Wolbachia.* We also found the up-regulation of *O. volvulus* phosphate permease (Ov-PPE) at the protein level during *Wolbachia* depletion by doxycycline treatment of onchocerciasis and Ov-PPE is co-localized to compartments of the worms where *Wolbachia* are in abundance. Up-regulation of PPE in response to *Wolbachia* depletion and co-localization of PPE to *Wolbachia* in filarial worms suggests that PPE could play an important in *Wolbachia* -nematode endosymbiosis and their importance is further indicated in *Caenorhabditis elegans* where knockdown of an orthologous phosphate permease results in embryonic lethality, a phenotype seen when filarial nematodes are depleted of *Wolbachia.* The functions of phosphate permease in the endosymbiosis could involve provision or transportation of phosphate to the *Wolbachia* symbionts, which encode all the genes for the de novo biosynthesis of nucleotides. Further ultrastructural analysis using electron microscopy promises to bring more insight into the molecular interaction between phosphate permease and *Wolbachia* and its role in *Wolbachia* -filarial nematode endosymbiosis.

224

SOCIO-ECONOMIC AND BEHAVIORAL FACTORS THAT INFLUENCE COMPLIANCE WITH MASS TREATMENT IN THE NATIONAL PROGRAMME FOR ELIMINATION OF LYMPHATIC FILARIASIS IN KENYA

Doris W. Njomo[1], Mary A. Nyamongo[2], Sammy M. Njenfa[3], Japheth K. Magambo[1]

[1]Kenya Medical Research Institute, Jomo Kenyatta University of Agriculture and Technology, Institute of Tropical Medicine and Infectious Diseases, Nairobi, Kenya, [2]African Institute for Health and Development, Nairobi, Kenya, [3]Kenya Medical Research Institute, Nairobi, Kenya

In Kenya, mass drug administration (MDA) for Lymphatic Filariasis using was started in 2002. Based on the 2008 MDA, a cross-sectional study to determine factors influencing compliance was conducted and preliminary results of the data which is still being subjected to further analysis are presented in this paper. Two districts were selected for the study and in each district two locations were selected: one with high and the other with low treatment coverage. The study utilized both qualitative (in-depth interviews and focus group discussions) and quantitative tools (interviewer-based questionnaire). For the quantitative data 965 household heads were selected through simple random sampling. The results indicate that some socio-economic factors influenced compliance levels. In areas of low compliance, 30% of the respondents have a main occupation (business, salaried worker and fishing) indicative of higher income level compared to 16% in areas of high compliance. Land ownership, an indicator of high socio-economic status was common in low compared to high compliant areas (95% and 78% respectively). Non- religious believers were more prominent, 62% in low compared to 38% in high compliant areas. In addition, in areas where there was a high perception of risk, there tended to be better compliance although not statistically significant (56% from high and 45% from low). Correct knowledge on cause of LF was more prevalent, 58% in high compared to 42% in low compliant areas. Attitude towards the drug was more positive in high compared to low compliant communities (61.4% and 38%). Problems related to drug's size, number and taste were significantly associated with compliance, more so in low 61.3% compared to 38.7% in high compliant areas. Dislike for the current drug distribution method was significantly associated with compliance; 72% in low and 28% in high compliant areas. Access to information on MDA which seemed to have been better in high compared to low compliant areas (61% and 50% respectively) was another contributing factor.

The study shows that for MDA to be successful, people need adequate information. Information dissemination methods to non-believers should be explored. Alternative methods of drug distribution in higher income areas should be considered. It is important to invest more in sensitization in higher income households to ensure that there is adequate understanding on need for MDA and counter any fears related to drug use.

225

WOLBACHIA-BASED SUPPRESSION OF AN *AEDES POLYNESIENSIS* FIELD POPULATION: A VECTOR CONTROL STRATEGY TO AUGMENT THE LYMPHATIC FILARIASIS ELIMINATION CAMPAIGN

Linda-Lou O'Connor[1], Michel Ayo Cheongsang[2], Herve Bossin[2], Stephen L. Dobson[1]

[1]University of Kentucky, Lexington, KY, United States, [2]Institut Louis Malarde, Papeete, Tahiti, French Polynesia

Lymphatic filariasis (LF), a painful and disfiguring mosquito-borne disease, is the leading cause of disability in the Western Pacific. Mass drug administration (MDA) has effectively reduced LF prevalence. However, historical evidence suggests that the current effort to eliminate LF in the South Pacific is at risk in some regions if it continues to rely solely upon

MDA strategies. This is because the primary vector *Aedes polynesiensis* is a particularly efficient LF vector in areas of low-level microfilaremia. This negative density-dependant transmission complicates the MDA approach. To strengthen the existing MDA program a vector control program is also needed in some regions of the South Pacific. A possible strategy for vector control is the use of Incompatible Insect Technique (IIT) or cytoplasmic incompatibility (CI) which causes embryonic mortality in crosses between individuals of the same species with different *Wolbachia* infection status. Here we will present the results of *Ae. polynesiensis* population monitoring on multiple islands in French Polynesia, including one island that received multiple, inundative releases of cytoplasmically incompatible males. We will provide data relevant to the production, delivery and assessment of released males. To examine for an impact on the targeted population, field collected females were monitored for egg hatch and insemination; broods that did not hatch were interpreted as having mated with an incompatible male. A successful demonstration is that female sterilization through cytoplasmic incompatible male releases will suppress or eliminate the mosquito population.

226

MULTIPLEX PCR-BASED EVALUATION OF *PLASMODIUM* SPP. AND *WUCHERERIA BANCROFTI* INFECTIONS IN PAPUA NEW GUINEA

Zachary A. Kloos[1], Rajeev K. Mehlotra[1], Laurie R. Gray[1], Melinda J. Blood Zikursh[1], Daniel J. Tisch[1], Peter Siba[2], James W. Kazura[1], Peter A. Zimmerman[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea

The parasites that cause malaria and bancroftian filariasis often co-infect humans in Papua New Guinea (PNG). Although programs to eliminate these parasites have met with some success, simultaneous surveillance of infection by these parasites in humans has been limited by lack of efficient diagnostic tools capable of detecting infection with both high specificity and sensitivity. To address this problem, a multiplex, post-PCR ligation detection reaction-fluorescent microsphere assay (LDR-FMA) has been developed to diagnose *Plasmodium* spp. and *Wuchereria bancrofti* (Wb) infections simultaneously in human blood samples. In this assay, multiplex PCR is used to amplify *Plasmodium* spp. small subunit rRNA and Wb long DNA repeat sequences, and the resulting amplicons are used to perform species-specific LDR-FMA. We used this assay to analyze genomic DNA extracted from blood samples (N=2,674) collected in a region of PNG co-endemic for Wb and the four malaria-causing *Plasmodium* spp. Previous calculations of the specificity and sensitivity of Wb detection were 0.94 and 0.86, respectively, while those of *Plasmodium* spp. detection were 0.79 and 0.81, respectively. Analysis of the data generated from application of this assay to PNG samples showed that the prevalence of infection with at least one of the five parasite species was 0.877. More specifically, the prevalence of *Plasmodium* species was 0.861, while that of Wb infection was 0.135. Although the prevalence of mixed-species infection was significantly higher than that of single-species infection (0.531 versus 0.346, $p < 0.001$), the parasite assemblages constituting the former infection type did not, collectively, occur at a frequency significantly different than that expected given the distribution of the parasites in the study population. Revealing marked complexity of infection by *Anopheles*-transmitted parasites in PNG, the assay introduced herein provides an important tool for efficient measurement of the impact of such public health interventions as distribution of insecticide-treated bed nets.

227

MELANIZATION IMMUNE RESPONSES BY *ANOPHELES PUNCTULATUS* INFLUENCE THE TRANSMISSION OF *WUCHERERIA BANCROFTI* IN PAPUA NEW GUINEA

Cheng-Chen Chen[1], Sara M. Erickson[2], **Bruce M. Christensen**[2]

[1]National Yang-Ming University, Taipei, Taiwan, [2]University of Wisconsin-Madison, Madison, WI, United States

Wuchereria bancrofti is vectored primarily by mosquitoes in the *Anopheles punctulatus* complex in Papua New Guinea (PNG). This mosquito complex consists of ~11 cryptic mosquitoes, and also includes the prominent malaria vectors in this area. In PNG, the interaction of *W. bancrofti* with its *Anopheles* vectors has generally been considered one of facilitation, but there is an unexplained reduction in the expected intensity of mosquito infections when mf densities are relatively high. This reduction in parasite intensity has yet to be investigated with vector competence studies. To further evaluate the interaction between *W. bancrofti* and *An. punctulatus*, mosquitoes were collected in the endemic village complex of Drekikire and individually dissected. A total of 418 *An. punctulatus* were captured in the early morning as they rested inside village homes. Seventy-two of the dissected mosquitoes harbored some stage of *W. bancrofti* (17.2% infected) and a total of 242 parasites were recovered (101 mf, 71 L1s, 56 L2s, and 14 L3s). But of these 72 infected mosquitoes, nearly 50% (35/72) elicited a melanization immune response against these parasites. A total of 54 of the parasites were melanized and killed, representing 22% of the entire parasite population recovered. In addition, 14 of the infected mosquitoes killed all of their parasites, giving us an estimate of a refractory rate of 19.4%. This is one of the few instances where melanization has been shown to function as a primary mechanism controlling resistance in a natural vector population. It is possible that the resistance mechanism might be density dependent and even more robust, but controlled laboratory experiments will be required to address these questions. It also is possible that these infection responses by *Anopheles* against filarial worms might influence transmission dynamics for *Plasmodium* in areas where these parasites are co-endemic.

228

IDENTIFICATION OF BENZOXABOROLE PDE4 INHIBITORS AS A THERAPEUTIC AGENTS FOR THE TREATMENT OF LYMPHATIC FILARIASIS

Kurt Jarnagin[1], Judy Sakanari[2], George Lemieux[2], James McKerrow[2], Jacob Plattner[1], Eric Easom[1], Yvonne Freund[1], Fernando Rock[1], Manisha Mohan[1]

[1]Anacor Pharmaceuticals, Palo Alto, CA, United States, [2]University of California San Francisco, Sandler Center, San Francisco, CA, United States

Insect bite transmitted filarial helminthic diseases, such as onchocerciasis and lymphatic filariasis are major health issues in tropical areas. Onchocerciasis is the world's second leading cause of infectious blindness, with a global prevalence of 17.7 million people. Lymphatic filariasis affects ~300 million people worldwide. The current therapy, Ivermectin, kills microfilariae but not adult worms (macrofilariae) Adult worms can live for up to 8-10 years, thus treatment must be given on a regular basis to break the transmission cycle. This highlights the need to identify new macroflariasidal medicines. Anacor has developed a library of about 5,000 boron-based compounds of diverse structures; several of these novel small molecules are under clinical evaluation to treat psoriasis (Phase II, a PDE4 inhibitor), fungi (ready for Phase III), bacteria (Phase I), as well as malaria, Chagas' disease and African trypanosomiasis (Pre-IND). Using a novel whole-organism screening platform, developed at the Sandler Center for the trematode Schistosoma mansoni, a sample collection of benzoxaborole compounds was screened. Among the hit compounds we noted a very strong enrichment for inhibitors of human phosphodiesterase-4 (PDE4), suggesting that Schistosomal PDE4 might be the target. These hits invariably caused parasite hypermotility, 1-2 hours

onset, and morphological derangements that led to parasite death, after 4 days of exposure.. We have taken these observations and extended them by showing that adult nematodes, *Brugia malayi* and *C. elegans*, also show a hypermotile phenotype after treatment, also with a 1-2 hours onset. *B. malayi* was killed after 4 days of exposure. Furthermore, members of the catechol family of human PDE4 inhibitors were not able to cause this phenotypic change, possibly indicating substantial differences between the human enzyme and nematode enzymes. These kinds of difference should allow targeting of therapy to the parasite. In this poster the structure activity relationship between phenotype and PDE4 activity will be presented.

229

MONITORING TRYPANOCIDAL DRUG EFFICACY IN EXPERIMENTAL RODENTS USING THE LOOP MEDIATED ISOTHERMAL DNA AMPLIFICATION (LAMP)

Dianah Katiti, Alex Boobo, Anne J. Kazibwe, Enock Matovu

Makerere University, Kampala, Uganda

Diagnosis of Human African Trypanosomiasis (HAT) relies on conventional parasitological methods to demonstrate trypanosomes in body fluids. These methods are dogged by low sensitivity, thus leave considerable proportions of undetected infections. For the treated patients, in whom relapsing parasitaemia tends to be even lower, they have to be regularly followed up for a period of 18-24 months to confirm cure. The objective of this study was to determine whether the loop-mediated isothermal amplification (LAMP) method can be used as a tool for monitoring treatment success in rodents. Mice were infected with trypanosomes and treated with Melarsoprol or Diminazene Aceturate. Samples were collected from the mice before inoculation, Pre- and post-treatment. Sampling continued at weekly intervals for 4 months; at each occasion microscopy and LAMP were performed to detect circulating trypanosomes or parasite DNA. LAMP remained positive in mice that eventually relapsed after treatment with 14mg/kg Diminazene Aceturate or 10mg/kg Melarsoprol after 4.3±0.5 and 8.2±3.8 weeks respectively. Positive LAMP signals in mice that were successfully treated lasted for a period of 15.2±0.4 weeks post diminazene treatment or 11.4±2.5 weeks for melarsoprol, after which they disappeared. From this study, the LAMP method appears to be a good tool for monitoring trypanocidal efficacy and could confirm cure within 3-4 months. If this can be reproducible in patients, it could cut costs involved in the hitherto lengthy post-treatment follow-up period. Given that LAMP is highly sensitive and has potential to be applicable at treatment centers with basic facilities, it should be further evaluated in treated sleeping sickness patients.

230

TRYPANOSOMA BRUCEI-INDUCED LEUCOCYTE APOPTOSIS AND TRYPANOSUSCEPTIBILITY IN ANIMALS

Anise N. Happi[1], Danny A. Milner[2], Richard E. Antia[1]

[1]University of Ibadan, Ibadan, Nigeria, [2]Harvard Medical School, Boston, MA, United States

The outcome of trypanosomiasis varies with the host, and understanding the mechanism of trypanotolerance is critical to the development of new control strategies. Apoptosis (programmed cell death) has of recent been implicated in the pathogenesis of several infectious diseases. This study was designed to investigate the relationship between *Trypanosoma brucei* - induced apoptosis and trypanosusceptibility. Experiments were conducted in laboratory animals (rats and rabbits) and goats [red Sokoto (RS) and West African dwarf (WAD)] with differing levels of trypanosusceptibility. The effect of diaminazine aceturate (Berenil®) treatment on the level of apoptosis in *T. brucei* infected trypanosusceptible RS goats. Blood and tissues (bone marrow, spleen, thymus, lymph node and liver) were collected for detection and quantitation of apoptosis using, light microscopy (LM), electron microscopy (EM) and DNA gel electrophoresis. Infection was associated with an increase in apoptotic level in all in infected animals. Levels of apoptosis were highest in the spleen. *T. brucei* infected RS and WAD goats showed a significant increase in blood cell apoptosis from 70 days post-infection ($p=0.0092$ and $p=0.0022$ respectively). RS goats showed more severe apoptosis of blood and tissue cells compared to the trypanotolerant WAD. Compared to RS, WAD showed significantly fewer apoptotic cells in the blood ($p=0.0072$). In general, the peak blood cell apoptosis tallied with peak parasitaemia and the lowest leucocyte count. These imply that trypanotolerance is associated with the ability to control apoptotic event during trypanosomiasis. Binucleated lymphocytes were observed in both breeds of goat and correlated with the degree of apoptosis, though the numbers were significantly higher ($p=0.0154$) in RS. Treatment of *T. brucei*-infected RS resulted in a significant decrease ($p<0.05$) in apoptotic cells in blood and tissues. Overall, this study has provided the first evidence of the relationship between blood and tissue leucocyte apoptosis and susceptibility to *T. brucei* infections. Furthermore, this study also provides the first evidence of peripheral blood binucleated lymphocytes during *T. brucei* infections. Future studies have been designed to identify the molecular mechanisms mediating apoptosis of host cells during trypanosomal infections as they may reveal novel therapeutics and vaccine targets for control of the disease.

231

PROPHYLACTIC STEROID THERAPY FOR SAFE AMBULATORY TREATMENT OF SEVERE MUCOSAL LEISHMANIASIS

Braulio M. Valencia, Alejandro Llanos-Cuentas

Institute of Tropical Medicine Alexander von Humboldt, Lima, Peru

Mucosal leishmaniasis (ML) is characterized by scarce parasite load as consequence of a strong TH1 immune response; however, treatment in cases of laryngeal involvement can trigger a local inflammatory response enough severe to cause airway obstruction and even death. For this reason hospitalization could be necessary to minimized risks associated with therapy initiation. Since ML is a neglected disease, patients with mucosal involvement belong to poor, rural and remote areas. Although ambulatory administration of Amphotericin B is available in our center, costs of hospitalization and risk of nosocomial infections are mayor concerns. Patients with confirmed ML (histopathology, culture or PCR) and severe involvement (hoarseness +/- dyspnea + epiglottic and vocal cord infiltration) received 5 days of prednisone 1mg/Kg/d before start treatment with deoxycholate Amphotericin B (AMB). Hydrocortisone 50mg before and after AMB infusion was applied for the first 5 days. Patients were continuously evaluated (vital signs and oxygen saturation every hour) for the first 48 hours of AMB therapy. In absence of complications during this period, treatment continues without closer follow-up. 11 patients were included; all were male and acquired the infection at the central and south jungle of Peru. Mean age was 44 years (SD: 17) and all had previous CL 16 years ago (IQR:10-20). Patients started their symptoms over the nasal area (obstruction + bleeding) 5 years ago (SD: 2,5) and progressively presented hoarseness. 3 received previous treatment with transient improvement. Physical examination revealed extensive compromised included nasal mucosa, palate, uvula and larynx. Even when hoarseness was present in all and dyspnea was present in 2, oxygen saturation was normal in all of them. No changes in vital signs and no signs of respiratory distress were noted during the first 48 hours of treatment. AMB treatment (without steroids) was continued until reach a cumulative dose of 25mg/Kg. In conclusion, individuals with severe ML can be safely treated with AMB as an out-patient if prophylactic steroid therapy is initiated before AMB initiation and if this is continued for at least five days. This regimen can be instituted in endemic regions without "experience and minimal facilities" decreasing costs and probably risks.

232

DRUG DISCOVERY FOR CHAGAS' DISEASE

Patricia S. Doyle, Larissa Podust, Juan C. Engel, James H. McKerrow

University of California, San Francisco, San Francisco, CA, United States

Chagas' disease, a neglected tropical disease prevalent throughout the Americas, is a major cause of cardiomyopathy. Nifurtimox and benznidazole are the only therapies currently available. Both drugs have serious side effects and limited efficacy. The Sandler Center developed the vinyl sulfone cysteine protease inhibitor K777 for the treatment of Chagas' disease. K777 irreversibly inhibits cruzain, a key protease required for viability of the parasite *Trypanosoma cruzi*. The inhibitor prevents cruzain autoprocessing within Golgi cisterns. K777 recently allowed us to elucidate a biological role for cruzain in immune evasion. Cruzain hinders macrophage activation by the proteolytic disruption of an NF-κB P65 mediated signaling pathway allowing *T. cruzi* survival and replication. Therapeutic intervention with the cysteine protease inhibitor prevents normal cruzain expression to the cell membrane of the pathogenic amastigote and leads to host immune cell activation. Studies of K777 have proven that the targeting of cruzain can be done selectively and effectively enough to cure *T. cruzi* infection in acute and chronic models of infection and also ameliorate cardiac damage in dogs. We have documented the efficacy of K777 against various *T. cruzi* strains that represent a spectrum with various tissue tropisms, and even against nifurtimox and benznidazole -resistant parasites. To identify alternative chemotypes with efficacy, we are currently exploring several classes of cruzain inhibitors. A second approach to our drug discovery efforts targets the C14α-demethylase of *T. cruzi*. The lead compound LP10 that disrupts ergosterol biosynthesis showed efficacy in an animal model of disease. By high throughput screening in combination with cell-based assays and animal trials, aided by NMR, crystallography, and molecular modeling, we have selected several top-ranking molecules with high trypanocidal capacity for further development.

233

OPTIMAL DOSING OF MILTEFOSINE IN LEISHMANIASIS PATIENTS

Thomas P. Dorlo[1], Alwin D. Huitema[2], Jos H. Beijnen[2], Peter J. de Vries[1]

[1]Academic Medical Center, Amsterdam, The Netherlands, [2]Slotervaart Hospital, Amsterdam, The Netherlands

Pharmacokinetics and -dynamics (PK/PD) of miltefosine in children with visceral leishmaniasis (VL) remain ill-characterized. In a large phase 4 trial in India, the number of treatment failures was significantly higher in the pediatric population than in adults given a similar dosage of 2.5 mg/kg. Based on this and the previous finding that the mean steady-state concentration in children was almost half of that reached in adults, we hypothesized that the current linear mg/kg dosage is too low for children and that a dose based on allometric scaling might result in a similar exposure to miltefosine between children and adults. A population PK analysis was performed comparing various body size models, based on pooled PK data from three separate studies, including Indian children, Indian adults and European adults. An allometric dosing-formula for miltefosine was proposed. Exposure to miltefosine after the current dose and the proposed new dosing algorithm were compared between adults and children by Monte Carlo-simulations. Modeling and simulations were performed with software packages NONMEM, R and Pirana. The population PK model with allometric power scaling fitted best to the pooled miltefosine data. Moreover, allometric scaling by fat-free mass (FFM) reduced unexplained between-subject variability (BSV): linear scaling by total weight (WT) or FFM, and allometric scaling by WT or FFM resulted, respectively, in a BSV of 50%, 43%, 35% and 32% for CL, and 43%, 37%, 38% and 34% for V. We proposed an allometric miltefosine dose, scaled with a power 0.75 from a standard adult (60 kg) receiving 150 mg (Dose = 150*(Weight/60)**0.75). Simulated exposure to miltefosine was similar between adults receiving 2.5 mg/kg and children receiving the new allometric dose. More importantly, only 74-78% of the children receiving the currently used linear dose of 2.5 mg/kg achieved a similar minimal systemic exposure as 90% of adults receiving 2.5 mg/kg. In conclusion, the currently applied dose of 2.5 mg/kg results in a significantly lower exposure to miltefosine in children than in adults. We recommend the use of an allometric dose formula for miltefosine for leishmaniasis, which results in a similar exposure to miltefosine between adults and children. More data are urgently needed on both PK and PD of miltefosine in VL, certainly in children, to further improve the treatment of this fatal neglected disease.

234

HIT-TO-LEAD AND LEAD OPTIMIZATION OF NOVEL SMALL MOLECULES FOR THE TREATMENT OF HUMAN AFRICAN TRYPANOSOMIASIS

Jennifer C. Freeman[1], Joe B. Perales[1], Cyrus J. Bacchi[2], Beth Beaudet[1], Tana Bowling[1], Eric Gaukel[1], Luke T. Mercer[1], Joseph Moore[1], Bakela Nare[1], Ryan Randolph[1], Cindy E. Rewerts[1], Stephen A. Wring[1], Nigel R. Yarlett[2], Robert T. Jacobs[1], Robert Don[3]

[1]SCYNEXIS, Inc., Research Triangle Park, NC, United States, [2]Pace University, New York, NY, United States, [3]Drugs for Neglected Diseases initiative, Geneva, Switzerland

Human African trypanosomiasis (HAT), or sleeping sickness, is a neglected tropical disease that is transmitted through the bites of tsetse flies infected with the kinetoplastid parasite *Trypanosoma brucei* and is fatal if left untreated. As existing chemotherapies are old, scarce, highly toxic, and encounter parasite resistance, there exists an urgent need for new drugs. As part of our ongoing program to identify new drug candidate chemotypes, we have screened ca. 50,000 novel small molecules in a high throughput *T. brucei* whole cell assay. We report here the identification, hit-to-lead and initial lead optimization efforts opposite one of the chemotypes found by this screening effort. Compounds were first optimized for trypanocidal activity, selectivity vs. mammalian cell toxicity, and *in vitro* ADME properties over the initial screening hits. Several lead compounds were progressed to *in vivo* efficacy and pharmacokinetic assays in rodents.

235

SHORT-COURSE MULTI-DRUG TREATMENT FOR VISCERAL LEISHMANIASIS IN INDIA

Shyam Sundar[1], P.K. Sinha[2], M. Rai[1], D.K. Verma[1], K. Nawin[2], J. Chakravarty[1], S. Alam[1], M. Vaillant[3], N. Verma[2], K. Pandey[2], P. Kumari[1], C.S. Lal[2], R. Arora[1], B. Sharma[4], S. Ellis[4], N. Strub-Wourgaft[4], M. Balasegaram[4], P. Das[2], Farrokh Modabber[4]

[1]Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, [2]Rajendra Memorial Research Institute of Medical Sciences, Indian Council of Medical Research, Patna, India, [3]Centre de Recherche Public – Santé Centre d'etudes en Santé, Luxembourg, Luxembourg, [4]Drugs for Neglected Diseases Initiative, Geneva, Switzerland

Most of the available drugs used as monotherapy for visceral leishmaniasis (VL) are toxic, not well tolerated, require long treatments or are expensive. Better treatment modalities are needed. We conducted a randomized, controlled, non-inferiority trial (Δ= -7% between combinations and standard treatment) in Bihar, India, to compare standard treatment (amphotericin B infusion alternate days for 30 days) with three drug combinations: single injection of 5 mg/kg liposomal amphotericin B (L-AmB) and 7-day miltefosine; L-AmB and 10-day paromomycin; miltefosine and paromomycin for 10 days. Patients were hospitalized for 15 days if on combination therapy or 31 days for standard treatment (end of treatment, EOT). Clinical assessments were performed at EOT, day 45 and 6 months after the start of treatment. Definitive cure was defined as

no sign/symptom of VL and parasitologically cured to the last follow-up. A total of 634 patients were randomly assigned and received amphotericin B (n=157); L-AmB and miltefosine (n=160); L-AmB and paromomycin (n=158); or miltefosine and paromomycin (n=159). 627 patients were included in the per protocol analyses. There were eight relapses, two in each group. The efficacy rates were: amphotericin B 93.0% (93.0% CI 87.50-96.27); L-AmB and miltefosine 97.5% (93.32-99.20); L-AmB and paromomycin 97.5% (93.24-99.19); miltefosine and paromomycin 98.7% (95.06-99.78). Combination therapies were well tolerated and had fewer adverse events. In conclusion, all three combination treatments were highly effective and safe. Due to shorter duration of treatment, combinations can increase compliance as well as reduce emergence of drug resistance.

236

FORMATION OF CYCLODEXTRIN INCLUSION COMPLEXES WITH A BENZIMIDAZOLE DERIVATIVE: THEIR CHARACTERIZATION AND *IN VITRO-IN VIVO* TRYPANOCIDAL ACTIVITY

Yareli Rojas-Aguirre[1], Benjamin Nogueda-Torres[2], Adrián Márquez-Navarro[2], Alicia Hernández-Campos[1], Rafael Castillo[1], Francisco Hernández-Luis[1]

[1]Universidad Nacional Autónoma de México, México, Mexico, [2]Escuela Nacional de Ciencias Biológicas, México, Mexico

Trypanosoma cruzi is the etiological agent of Chagas' disease, a chronic illness affecting many people principally in Central and South American countries. According to the World Health Organization, an estimated 20 million people are infected with this parasite and about 25% population of Latin America is at risk of being infected. Treatment of Chagas' disease is still unsatisfactory. Nifurtimox and benznidazole have been widely used as trypanocidal agents, however, both have significant activity only in the acute phase of the disease and, when associated with long term treatments, give rise to severe side effects. Additionally, *T. cruzi* resistance to these nitroderivatives constitutes an important factor in the low rate of cure in treated patients; therefore, there is an urgent need to develop new antiparasitic leads with improved pharmacological and pharmacokinetic characteristics. In this sense our research group found a benzimidazole derivative (G2) in preliminary *in vitro* trypanocidal screening. However, in these studies G2 showed poor aqueous solubility, which hampered the subsequent *in vivo* experimental trials. Furthermore, the lack of water solubility reduces flexibility for drug administration. To overcome these drawbacks, increasing the aqueous solubility of G2 was performed through the formation of inclusion complexes with cyclodextrins (CD). In this work, we report the change of the solubility profile of G2 when is complexed with three different CD, the physicochemical characterization of the complexes and their *in vitro-in vivo* activity against *T. cruzi*. Additionally, we show toxicity results in human lymphocytes and erythrocytes.

237

IN VITRO EVALUATION OF SOME NOVEL IMIDO-SUBSTITUTED 1,4-NAPHTHOQUINONE DERIVATIVES AS ANTITRYPANOSOMAL AGENTS

Mozna H. Khraiwesh[1], Clarence M. Lee[1], Yakini Brandy[2], Emmanuel S. Akinboye[2], Solomon Berhe[2], Genelle Gittens[2], Oladapo Bakare[2]

[1]Department of Biology, Howard University, Washington, DC, United States, [2]Department of Chemistry, Howard University, Washington, DC, United States

Chagas disease is a tropical disease, caused by the protozoan *Trypanosoma cruzi* and transmitted by triatomine bugs. It commonly occurs in poor and rural areas of Central and South America. Chagas disease is expanding beyond its endemic area as a result of migration from and to the endemic countries. Currently used drugs have been reported to have undesirable side effects including gastrointestinal, neurological and mutagenic effects. In addition, problems such as bone marrow depletion, skin rashes, weight loss and dizziness have been reported for these drugs. Consequently, there is a search for safer drugs with more selective mode of action. Several classes of drug-like molecules have been studied for their antitrypanosomal activity. One of the most interesting ones is the quinone family of compounds. This class of compounds incorporates several diverse structural types including the naphthoquinones, which are known to possess a number of useful biological activities. We have developed some imido-substituted 1,4-naphthoquinones as a unique class of compounds with antitrypanosomal activities. Cytotoxic activities on Balb/C 3T3 mouse fibroblasts cell lines revealed excellent selectivity index for four of these compounds. Initial attempt to understand the mechanism(s) of action of these compounds appears to point to possible modulation of tubulin polymerization.

238

ANTILEISHMANIAL ACTIVITY OF NOVEL ARYLIMIDAMIDES

Xiaohua Zhu[1], Moloy Banerjee[2], Abdelbasset A. Farhat[2], Carolyn Reid[1], Trupti Pandharkar[1], Donald A. Patrick[3], Richard R. Tidwell[3], Michael Wang[3], Dennis Kyle[4], David Boykin[2], Karl Werbovetz[1]

[1]The Ohio State University, Columbus, OH, United States, [2]Georgia State University, Atlanta, GA, United States, [3]University of North Carolina, Chapel Hill, NC, United States, [4]University of South Florida, Tampa, FL, United States

We recently showed that the arylimidamide DB766 (2,5-bis[2-(2-propoxy)-4-(2-pyridylimino)aminophenyl]furan) possessed excellent activity against several *Leishmania* species in intracellular assays and displayed good efficacy in murine and hamster models of visceral leishmaniasis when given orally, as reported previously. A series of DB766 analogs have since been prepared and tested for their effect on intracellular *Leishmania in vitro*. Several classes of these analogs exhibited potency similar to that of DB766. Compounds possessing isosteric substitution of fluorine for hydrogen in the alkoxy linker moiety were active, as exemplified by DB1961 (2,5-bis[2-(1,3-difluoroproan-2-yloxy)-4-(2-pyridylimino)aminophenyl]furan, IC50 = 98 ± 33 nM). Molecules bearing an unsymmetrical linker showed potent activity, illustrated by DB1967 (2-[2-(2-propoxy)-4-(2-pyridylimino)aminophenyl]-5-[4-(2-pyridylimino) aminophenyl]furan, IC50 = 93 ± 28 nM). 35DAP081, an arylimidamide compound possessing a terphenyl linker, also displayed sub-micromolar potency (3,4"-bis-[N-(2-pyridylimidoyl)]amino-*m*-terphenyl, IC50 = 260 ± 130 nM). A new class of arylimidamides also showed submicromolar *in vitro* antileishmanial activity. Furthermore, compounds in this class do not possess overt toxicity to mice when administered at a dose of 30 mg/kg/day × 5 by the intraperitoneal route. Members of this new class of arylimidamides are being tested in our murine model of visceral leishmaniasis in comparison to the reference arylimidamide DB766 and the oral antileishmanial drug miltefosine.

239

STUDY OF THE POTENTIAL RESISTANCE OF *LEISHMANIA AMAZONENSIS* TO A THIOSEMICARBAZONE DERIVATIVE

Renata O. Soares[1], Aurea Echevarria[2], Vanessa Picolo[1], Denise B. Castro-Pinto[1], **Leonor Laura P. Leon**[1]

[1]Oswaldo Cruz Institute, Rio de Janeiro, Brazil, [2]Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, Brazil

The chemotherapy of leishmaniasis is based until now in drugs which are not totally efficient and present severe side effects and in some case are able to induce resistance to treatment. This resistance could be related to the volume of the drug (dose and frequency) and the time of administration, among other factors. The mechanism of resistance have been associated to the increased expression of a transmembrane protein (Pgp), that act as a efflux pump for a wide spectrum of drugs and

depends on energy (from ATP) and must be phosphorylated to be active. As part of our research program on chemotherapy against diseases caused by trypanosomatids we have been studied several thiosemicarbazones and semicarbazones derivatives, which have a medical interest because of their capacity of inhibit the growth of several pathogens. Studies concerning its biological activity show that these compounds are active against trypanosomatids, such as *T. cruzi, T. brucei* and *Leishmania* sp. In the present work, it was used a thiosemicarbazone [(3-methoxy-4-hyidroxy-estiryl)-thiosemicarbazone], that showed to be very active against *L. amazonensis* promastigotes and Pentamidine as a reference drug. Parasites were grown in Schneider´s medium, pH7.2, temperature of 26ºC and resistance was induced by the compounds in the presence of the compounds for several passages in culture. During this process, it was evaluated the potential acquired resistance by new screenings in each passage (new LDs50), besides the assay of infectivity of the parasites through complement lysis test (to detected metacyclic forms) and *in vitro* infection. The results showed that a significant increase in the LD50 was observed at passage number 10 and the parasites were able to maintain its infectivity, even after several passages in culture.

240

COMPARISON OF REAL-TIME PCR ASSAYS FOR DETECTING *TRYPANOSOMA CRUZI* DNA IN CLINICAL SAMPLES

Yvonne Qvarnstrom[1], Alejandro Schijman[2], Vincent Veron[3], Christine Aznar[3], Alexandre J. da Silva[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]INGEBI-CONICET, Buenos Aires, Argentina, [3]Université des Antilles et de la Guyane, Cayenne, French Guiana

Chagas disease is caused by the parasite *Trypanosoma cruzi* and is characterized by chronic infection of the heart. Vectorial transmission is the main route of infection in endemic areas. Other routes of infection include congenital transmission, blood transfusion, organ transplantation and oral ingestion of foods contaminated with the parasite. Diagnosing Chagas disease is challenging, in part because of the varying clinical manifestations during different phases of the disease. Serology is the preferred method for patients in the chronic phase, whereas PCR can be successful in acute and congenital cases. Quantitative PCR can be especially powerful to monitor changes in parasite blood levels during re-activation or drug treatment. Here we present data comparing three TaqMan PCR assays (one previously published and two new methods) and a well-established conventional PCR targeting the kinetoplast minicircle. Included in the analysis were DNA extracted from 191 EDTA blood samples, 12 heart biopsies, 9 umbilical cord blood samples, 1 skin tissue sample and 2 CSF samples. The sources of the samples were patients with suspected exposure to *T. cruzi* through organ transplantation, bug contact or laboratory accidents, and from immunosupressed patients with suspected re-activation. The assays had differing sensitivities (ranging from 63% to 97%) and specificities (96% to 100%). The most sensitive assay was a TaqMan PCR targeting the kinetoplast minicircle; however, that assay also had the highest number of false positives. The published TaqMan assay, targeting the TCZ microsatellite region, was 84% sensitive and 98% specific in this evaluation. The third TaqMan assay was designed to be very specific but unfortunately had the lowest sensitivity. These data strongly suggest at least two PCR assays with different performances should be used to obtain accurate results and are consistent with the conclusions from the recent multicenter study by the Consortium for standardization and validation of clinical use of PCR for *T. cruzi* DNA detection in Chagas disease.

241

ADMINISTRATION OF TOMATO EXPRESSING INTERLUKIN 12 CONTROLS *TRYPANOSOMA CRUZI* INFECTION IN ORALLY TREATED MICE

Julio V. Cruz[1], Manuel A. Sansores[2], Maria J. Ramirez[1], Miguel G. Gomez[3], Eric O. Dumonteil[1]

[1]Universidad Autonoma de Yucatan, Merida, Mexico, [2]Instituto Tecnológico de Mérida, Merida, Mexico, [3]Cinvestav, Irapuato, Mexico

Chagas Disease (American Tripanosomiasis) is an anthropozoonotic illness by the *Trypanosoma cruzi* parasite. The disease is endemic in the Americas and there are 10 -15 million existing cases. A third of infected people develop cardiomyopathy or mega-digestive syndrome. Interleukin IL-12 is a cytokine that is induced early when a host is challenged by a pathogen. Additionally, IL-12 is required to elicit a Th1 immune response, which is responsible for eliminating *T. cruzi* parasite through the activation of T lymphocyte. In previous studies, oral administration of tomatoes expressing murine IL-12 allowed the control of a *Mycobacterium tuberculosis* infection. Here we tested if this treatment could control an infection with *T. cruzi* in mice. Balb/c mice were infected with 500 *T. cruzi* parasites via intraperitoneal and they were treated during 30 days with daily doses tomatoes pure expressing IL-12 (240 ng IL-12) via oral. The control group received tomatoes without cytokine. Every three days, parasitemia was recorded in both groups. The density of inflammatory cells in cardiac tissue was measured in tissue section stained with hematoxilin using Multispec software 3.0. Amastigote nests were also counted from cardiac tissue. Administration of tomatoes expressing IL-12 significantly decreased the parasitemia in treated mice. These also presented significantly less cardiac inflammation than the control group and had fewer amastigote nests. Results show that oral administration of tomatoes expressing IL-12 at least partially control *T. cruzi* infection in mice, which opens new opportunities for the control of Chagas Disease.

242

EVALUATING THE IMPACT OF AN INTERVENTION ON PEDIATRIC MALARIA CASE-MANAGEMENT PRACTICES IN PUBLIC HEALTH FACILITIES IN KENYA

Beatice Wasunna

KEMRI-Wellcome Trust Research Programme, Nairobi, Kenya

An intervention to improve effective case management of febrile children under 5 years was implemented in public health facilities in Nyanza Province, Kenya between 2008 and 2009. An evaluation of the intervention using pre & post intervention cross sectional health facility surveys was conducted in one district of the Province The surveys included: audit of the health facility; health worker structured interviews; exit interviews with caretakers of sick children under 5 years of age. The primary outcome indicator was the proportion of febrile children managed in accordance with national recommended treatment guidelines. At baseline 33 government health facilities, 48 health workers and 560 febrile children consultations were evaluated. At follow-up the same health facilities were surveyed and 36 health workers and 423 febrile children consultations evaluated. Our findings show : 1) the proportion of health workers who had received any malaria case-management training increased from 46% to75% (P=0.01); 2) the proportion of health workers who received the intervention specific training was 61% 3) the proportion of febrile children with uncomplicated malaria treated with the first-line antimalarial drug, artesunate-lumefantrine (AL), at health facilities where AL was in stock increased from 74% to 84.6% (P=0.007) 4) The proportion of caregivers who knew the correct AL duration increased from 67% to 80% (P=0.009). However, when the analyses were restricted to health workers who received the intervention training versus those not trained, there were no significant differences. In conclusion, although there were significant improvements in case management, these could not be attributed to the intervention.

243

ANTIPLASMODIA ACTIVITIES OF METHANOLIC EXRACT OF *ANOGEISSUS LEIOCARPUS* AND ITS PATHOLOGICAL EFFECT ON MALARIA PARASITE INFECTED MICE

Olusegun Akanbi, Akhere Omonkhua, Mojisola Cyril-Olutayo, Rotimi Fasimoye

Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria

Methanolic extract from *Anogeissus leiocarpus* has been considered locally to have the same antimalarial activities as artemisinin combination theraphy newly introduced by WHO. Therefore this work studies the *in vivo* antiplasmodia activities of extract of *A. leiocarpus* and its pathological effect on some ectopic organ of malaria parasite infected mice. Mice used for this study were infected with *Plasmodium berghei* and divided into 5 groups. The first group was not infected with parasite. The second group was infected with parasite and was not treated with antimalarial drugs. The third group was infected and treated with artesunate at 5mg/kg body weight. The fourth and fifth groups were infected and treated with 100 and 200mg/kg body weight of extract of *A. leiocarpus* respectively. Thick and thin films were prepared and used for malaria parasite counts. High density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride levels were determined from plasma. MDA and Catalase levels were also determined from the plasma and homogenates from kidney, liver and heart. The full white blood counts were also determined. The parasite density was significantly higher ($P < 0.05$) in group infected with the malaria parasite but without treatment than other infected groups which were treated. The rate of parasite clearance was higher in the group treated with artesunate than the groups treated with *A. leiocarpus*. MDA level was significantly higher in serum, liver and heart of mice infected with artesunat than mice in other groups. This could be as a result of increase in lymphocyte, neutrophils, eosinophils and basophils levels in group treated with artesunat. Catalase level was significantly higher in the homogenate from liver and heart of the mice treated with 200 mg/kg body weight of *A. leiocarpus* than other groups. LDL and total triglyceride were significantly higher in group treated with artesunat than other groups, while HDL was significantly higher in the two groups treated with *A. leiocarpus* as compared with the group treated with artesunate. This study shows that extract of *A. leiocarpus* has antimalarial activities with minimal adverse effect as compared with artesunate.

244

EFFECTIVENESS AND TREATMENT ADHERENCE TO ARTEMETHER/LUMEFANTRINE UNIT DOSE AGE SPECIFIC PRE-PACKS VERSUS BLISTER PACKS IN THE TREATMENT OF UNCOMPLICATED MALARIA IN UGANDA

Joaniter I. Nankabirwa[1], Samuel Gonahasa[2], Grace Nakanwagi[2], Daniel Kyabayinza[2], James Tibenderana[2]

[1]Makarere University Kampala, Kampala, Uganda, [2]Malaria Consortium, Uganda, Kampala, Uganda

Prompt and adequate treatment of clinical malaria episodes remains one of the key elements of malaria control and this partly depends on effectiveness of the drugs and patients' compliance to treatment. Uganda adopted Artemether/Lumefantrine (AL) 6 dose unit dose age specific pre-packs as first line treatment for uncomplicated malaria, however, concerns about the costs and stock-outs of these packages have been raised. This has led to a need for equally efficacious alternatives drugs in order to reduce these problems. We are currently conducting a randomized, open label trial to compare the effectiveness and treatment adherence to AL unit dose age specific pre-packs to AL blister packs plus instruction leaflets for the treatment of uncomplicated malaria. An interim analysis including 100 participants (target sample size = 702) is presented here. Children aged 4 months to 5 years with history of fever/axillary temperature > 37.5 and a positive malaria blood smear were randomized to receive one of the study regimens and were followed for 28 days. Participants were assessed for treatment outcomes over 28 days according to modified World Health Organization criteria. Of 100 participants enrolled in the study, 94% completed follow-up and were assigned a treatment outcome; 6 participants were withdrawn or lost to follow-up. By day 28, clinical failure (CF) occurred in 21% of the children and parasitological failures (PF) occurred in 42% of the participants. All treatment failures occurred between days 14 and 28. At least one adverse event was reported in 53% of the participants, but no serious adverse events occurred. Treatment failure rate (CF + PF) unadjusted by genotyping was 65% for the unit dose age specific pre-pack group compared with 55% for the blister packs plus instruction leaflet group. The adherence to unit dose age specific pre-packs was 92% compared with 96% to the AL blister packs plus instruction leaflets. Complete results, including assessment of parasite isolates by genotyping, and full results of safety will be presented.

245

IMPROVING RELATIVE BIOAVAILABILITY AND PROPHYLACTIC EFFICACY OF ORAL WR299958 BY REDUCING PARTICLE SIZE USING AN ULTRA-SONICATOR

Sean Reyes, Jing Zhang, Qiang Zeng, Erin Harris, Ping Zhang, Lisa Xie, Jason Sousa, William McCalmont, Michael Kozar, Qigui Li

Walter Reed Army Institute of Research, Silver Spring, MD, United States

Particle size is an important determinant of gastrointestinal absorption in human and animal species by oral administration. Although the use of particle size reduction to increase bioavailability of compounds has been reported in the literature, the effect of a reduction in particle size on the bioavailability of WR299958, a new antimalarial compound, is unclear. Suspension and emulsion formulations of WR299958 were made using a homogenizer and an ultra-sonicator, respectively, and the particle sizes of each formulation were measured by a LA-950 laser particle size analyzer. The mean particle size of the suspension and emulsion formulations were measured and showed the particle size of 102.2 and 0.085 µm, respectively. The two new suspension and emulsion formulations of WR299958 at various doses were administrated intragastrically to infected- or uninfected-mice for efficacy and pharmacokinetic (PK) evaluations. For the PK assessment, the plasma and liver samples were collected and drug concentrations were analyzed by LC-MS/MS. For the efficacy test, blood was taken from the mice through tail nicks and the parasitemia was determined by flow-cytometry. The results indicated that the particle size reduction resulted in significant differences in PK and efficacy evaluations. If bioavailability of 100% was set for the emulsion formulation, the relative bioavailability of WR299958 for the suspension formulation was only 30.8% *in vivo*. With the same oral dosage, the peak concentration of the emulsion formulation (Cmax) was 14.75 ng/ml in mice which was 2.32 times higher than the peak concentration of the suspension group at 6.20 ng/ml. Similarly, the area under the curve (AUC) of 60.33 ng·h/ml after administration of the emulsion was 3.27 fold higher than in animals treated with the drug in suspension with 18.60 ng·h/ml. The initial efficacy of these formulations was also tested and full causal prophylaxis in mice treated with emulsion WR299958 was two-fold stronger than that of the suspension in *Plasmodium berghei* sporozoite-infected mice. Although the bioavailability of WR299958 was significantly increased by using the emulsion formulation with a nanoparticle size, the drug bioavailability remains very poor. Therefore, further improvement in the oral bioavailability of WR299958 will require additional work.

246

A NEW METHOD SUITABLE FOR HIGH THROUGHPUT SCREENING OF *PLASMODIUM FALCIPARUM*

Cristina de Cózar, Laura Sanz, Francisco-Javier Gamo

Diseases of the Developing World (DDW), Medicines Development Campus, GlaxoSmithKline, Tres Cantos (Madrid), Spain

The standard *in vitro* protocol currently used for assessing susceptibility of drugs against *Plasmodium falciparum* is based on the incorporation

of radioactive 3H-hypoxanthine. This methodology relies on the use of 96-well plates and together with the inherent problems of the use of radiolabelled material, makes this assay unsuitable for use in a *Plasmodium* high-throughput whole cell screening. Alternative methodologies, amenable for use in a high-density format (384-well) and preferably non-radioactive, are required to tackle a whole cell screening of *P. falciparum* in a high scale. We have implemented conditions for growing *P. falciparum* cultures in a 384-well format. Using optical microscopy, we have demonstrated that growth rates observed in these conditions are equivalent to the ones occurring in a 96-well format. Parasite lactate dehydrogenase activity (PfLDH) is a good surrogate of *P. falciparum* growth and can be used to determine susceptibility to antimalarial drugs. APAD+ (acetyl pyrimidine adenine nucleotide) is an analogue of NAD+ cofactor. During enzymatic L-lactate oxidation, APAD+ is used 300 times more efficiently by PfLDH than by its human counterpart. To determine parasite growth in a semiautomatic way, we have adapted a colorimetric method that takes advantage of the described structural differences of *P. falciparum* and human lactate dehydrogenase enzymes. This assay is non-radioactive and suitable for use in a high-density format without the need for filtration or centrifugation steps, making it useful for low technology settings. The new method has been validated using known antimalarial compounds. Drug sensitivity results (IC50) obtained with this protocol compared well to that of the traditional isotopic method.

247

EFFECT OF ARTESUNATE ON DISPOSITION OF ORALLY ADMINISTERED AMODIAQUINE IN PATIENTS WITH UNCOMPLICATED MALARIA

George O. Ademowo[1], Olumuyiwa N. Adedeji[1], Catherine O. Falade[1], Oluseye O. Bolaji[2]

[1]Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria, [2]Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Ile Ife, Nigeria

The emergence of drug resistance in *Plasmodium falciparum* has necessitated that *falciparum* malaria be treated with Artemisinin-based Combination Therapy (ACT). Amodiaquine (AQ) is one of the drugs used in combination with artesunate for malaria treatment. We assessed the pharmacokinetics of AQ and the effect of artesunate on its disposition in patients with malaria. A liquid chromatographic method was developed for analysis of AQ and its metabolite, desethylamodiaquine (AQm). Twelve patients positive for malaria parasite were randomized to receive either AQ or AQ plus artesunate (AS). The doses were AQ 600mg once daily and fixed-dose AQ/AS daily for 3 days. Blood samples were collected before and at 0.5, 1, 2, 4, 6, 12, 24, 48, 72, 144 and 336 hr after drug intake. Plasma was separated and used to assay for AQ. The analytical method was highly sensitive and specific. Calibration curves were linear (r2 > 0.99) in the range of 100 - 1000 ng/ml for AQ and AQm. The intra-assay coefficients of variation were 1.87-4.94% for AQ and 0.49-5.34% for AQm. While inter-assay coefficients of variation was 1.67-6.37% for AQ and 2.49-6.89% for AQm. The mean values of Peak Plasma Concentration, Cmax (22.7±0.01 vs 20.43±0.12 ng/ml) and Area Under the Plasma Concentration-time Curve, AUC (59.63±0.05 vs 57.52±0.24 ngh/ml) of AQ were significantly (P<0.05) higher in the AQ alone compared with AQ/AS group. Terminal half life, t1/2 was longer (2.83±0.02 vs 2.69±0.01 hr) and oral clearance, Cl/F (9729.54±7.61 vs 9857.24±42.5 ml/h) was significantly lower in AQ/AS when compared to AQ group (P<0.05). The mean values of peak time of plasma concentration (Tmax) of AQ in the two groups were the same at 2hr. There were however no statistically significant differences in the values of Tmax, Cmax, t1/2, Cl/F and AUC of AQm in both treatment groups (P > 0.05). Artesunate significantly affected the disposition of the parent drug, amodiaquine but not the metabolite, desethylamodiaquine when orally administered in combination in patients with malaria.

248

THE ANTIMALARIAL EFFICACY OF PRIMAQUINE: THE ROLE OF CYTOCHROME P450-MEDIATED METABOLITES

Charlotte A. Lanteri[1], Colin Ohrt[1], Montip Gettayacamin[2], Victor Melendez[1], Brandon Pybus[1], Jason Sousa[1], Anchalee Tungtaeng[2], Karen White[3], Larry Walker[4], Susan A. Charman[3]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]Monash Institute of Pharmaceutical Sciences, Parkville, Victoria, Australia, [4]University of Mississippi, University, MS, United States

Primaquine (PQ) is currently the only FDA-approved drug to treat relapsing malaria, but PQ causes hemolytic toxicity in glucose-6-phosphate dehydrogenase deficient individuals. Metabolic activation of PQ appears necessary for antimalarial efficacy and hemotoxicity. The link between cytochrome P450 (CYP450)-mediated metabolism of PQ and causal prophylactic efficacy was investigated in a murine (ICR stain) *Plasmodium berghei* (ANKA strain) sporozoite challenge model. 1-Aminobenzotriazole (ABT) was used to irreversibly inhibit multiple CYP450 isoforms to determine the effect of inhibition on antimalarial efficacy. Mice were treated with ABT two hours prior to oral dosing with a single (37.5 mg/kg) or 3 day (25 mg/kg and 40 mg/kg x 3 days) curative dose of PQ. Comparator groups were treated with the same curative doses of PQ without ABT. Parasitemia was monitored over 31 days as an indicator of protective action or failure in this lethal model. ABT blocked the prophylactic activity of PQ, suggesting that CYP450 metabolites of PQ contribute significantly to exo-erythrocytic efficacy. To compare plasma exposures of PQ and metabolites ± ABT, terminal bleeds were conducted on the day that mice succumbed to infection in the ABT treatment groups. A pharmacokinetic and metabolism profile of PQ ± ABT in non-infected mice was constructed to interpret results attained in the murine malaria model. Taken together, these results have prompted further exploration of the CYP450 pathway as being critical to PQ's efficacy. Experiments are underway to investigate the effect of ABT on radical cure (i.e. anti-hypnozoite activity) of PQ in a Rhesus monkey model of relapsing malaria.

249

NOVEL BORON-CONTAINING SMALL MOLECULES DEMONSTRATE POTENTIAL FOR MALARIA THERAPY: EXCELLENT *IN VIVO* EFFICACY IN MURINE *PLASMODIUM BERGHI* MODELS AND FAVORABLE PHARMACOKINETICS

Yvonne R. Freund[1], Liang Liu[1], Y. K. Zhang[1], Wei Bu[1], Anne Wu[1], Eric Easom[1], Charles Ding[1], Jake Plattner[1], Jiri Gut[2], Philip J. Rosenthal[2], Belen Jimenz-Diaz[3], Maria Santos[3]

[1]Anacor Pharmaceuticals, Inc., Palo Alto, CA, United States, [2]Department of Medicine, Division of Infectious Diseases, University of California, San Francisco, CA, United States, [3]Diseases of the Developing World (DDW), Medicines Development Campus, GlaxoSmithKline, Tres Cantos, Spain

Recent suggestions of resistance to artemisinin-based combination therapies in Southeast Asia underscore the ongoing need for discovery of new chemical entities for treatment of *falciparum* malaria. New therapeutics need to be orally active, effective in short-course therapy, inexpensive to produce, and safe for use in developing world populations. We have discovered a series of novel boron-containing small molecules with excellent *in vitro* potency against *Plasmodium falciparum* and have evaluated pharmacokinetic properties and *in vivo* efficacy of several potent scaffolds. Greater than a thousand members of the Anacor compound library were screened *in vitro* against cultured W2-strain *P. falciparum* at a single concentration of 10 µM. Activities of hit compounds were then titrated and numerous compounds with IC50 values <500 nM were observed. The most potent compound, AN3661 (IC50 = 26 nM against W2-strain *P. falciparum*) showed high plasma clearance (4513 mL/h/kg) with reasonable oral bioavailability when dosed to mice at 30 mg/kg IV and PO. AN3661 demonstrated *in vivo* efficacy after oral treatment

in a 4-day murine model of *P. berghi* infection, where parasitemia was detected by flow cytometry on Day 4 (ED90< 3 mg/kg). In addition, AN3661 showed 100% cure when dosed twice-daily for 4 days at 100 mg/kg, in a 42-day model, with no parasitemia detected after 42 days. Lower doses of AN3661 significantly extended mouse survival, although didn't cure. The high required dose for cure may be attributed to high clearance in mice. Analogues of AN3661 are being synthesized to optimize pharmacokinetic properties. Initial PK analysis of the new designs revealed significant reduction of plasma clearance in mice. In summary, novel boron-containing small molecules offer promising potential as new orally-active antimalarials.

250

POPULATION SCREENING FOR GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCIES IN ISABEL PROVINCE, SOLOMON ISLANDS, USING A MODIFIED ENZYME ASSAY ON FILTER PAPER DRIED BLOODSPOTS

Melissa Kuwahata[1], Rushika Wijesinghe[2], Mei-Fong Ho[1], Anita Pelecanos[1], Albino Bobogare[3], Losi Landry[3], Hugo Bugora[3], Andrew Vallely[2], **James S. McCarthy**[1]

[1]Queensland Institute for Medical Research, Herston, Australia, [2]University of Queensland, Herston, Australia, [3]National Vector Borne Disease Control Program, Ministry of Health, Honiara, Solomon Islands

Glucose-6-phosphate dehydrogenase deficiency poses a significant impediment to primaquine use for the elimination of liver stage infection with *Plasmodium vivax* and gametocyte clearance, because of the risk of life-threatening haemolytic anaemia that can occur in G6PD-deficient patients. Although a range of methods for screening G6PD deficiency have been described, almost all require skilled personnel, expensive laboratory equipment, freshly collected blood, and are time consuming; factors that render them unsuitable for mass-screening purposes. We have adapted a published WST8/1-methoxy PMS method to assay G6PD in a 96-well format using dried blood spots, and used it to undertake population screening alongside a malaria survey undertaken in Isabel Province, Solomon Islands. The assay was validated by comparing it to biochemical screens and a recently marketed rapid diagnostic test. The overall prevalence of G6PD deficiency was determined to be 20.3% by mass-screening approximately 8541 people from 41 villages in Isabel Province, Solomon Islands. Comparative testing with biochemical and rapid diagnostic test indicated that results obtained by filter paper assay were accurate. The assay enabled simple and quick semi-quantitative population screening in a malaria-endemic region. The study indicated a high prevalence of G6PD deficiency in Isabel Province and highlights the critical need to consider G6PD deficiency in the context of *P. vivax* malaria elimination strategies in Solomon Islands, particularly the potential role of primaquine mass drug administration.

251

AN LC-MS BASED METHOD FOR THE MICRO-SAMPLING AND MEASUREMENT OF COMMON ANTIMALARIAL DRUGS *IN VIVO*

Brandon S. Pybus, **Duke D. Poore**, Jason Sousa, Dustin Carroll, Michael P. Kozar, Victor Melendez

Walter Reed Army Institute of Research, Silver Spring, MD, United States

Rodent PK models often require population sampling due to volume constraints. Furthermore, efficacy models can be confounded by excessive blood drawing in unhealthy animals, depending on the experimental end-point. To circumvent these issues, and facilitate measurement of drug levels in efficacy models on single living animals, an LC-MS based method for the micro sampling (15µl) and extraction of PK samples collected *in vivo* was developed and the limits of detection and quantification for a number of common antimalarials, including drugs from the 8 and 4-aminoquinoline classes, were compared. In this method, a droplet of blood is dried on paper (GE Healthcare FTA DMPK-B) at the time of collection and extracted into acetonitrile. Multiple combinations of solvent and paper type were tested, and the combination with maximum signal for extracted drug was chosen. Liquid liquid extraction from whole blood methods were also compared to the solid liquid extraction used in the microsampling technique to assess extraction efficiency. This procedure can be used to increase sampling rates in population PK models, collect single animal PK data, or to correlate drug levels to endpoints in efficacy models with little or no perturbation on the animal model itself. Although drug levels (Mefloquine) as low as 30-50 ng/ml can be measured reproducibly in this method, compared to single digit ng/ml values in currently used liquid liquid extraction protocols, the method is being refined to try to enhance sensitivity as the volume required for reproducible sampling in many animal models is a critical and often limiting factor.

252

THE USE OF A PRODRUG APPROACH TO MINIMIZE POTENTIAL CNS EXPOSURE OF NEXT GENERATION QUINOLINE METHANOLS WHILE MAINTAINING EFFICACY IN *IN VIVO* ANIMAL MODELS

Jason Sousa, Dustin Carroll, Erin Milner, Diana Caridha, Sean Gardner, Charlotte Lanteri, Quiqui Li, William McCalmont, Jay Moon, Nicanor Obaldi, Anchalee Tangteung, Norma Roncal, Bryan Smith, Qiang Zeng, Michael P. Kozar, Geoffrey Dow

Walter Reed Army Institute of Research, Silver Spring, MD, United States

Among the drug development programs currently under way at the Walter Reed Army Institute of Research is an effort to produce an analog of mefloquine (MQ) that is less susceptible to penetration of the blood-brain barrier (BBB) while maintaining levels of efficacy that are equal to or greater than MQ. To that end, a library of MQ analogs was synthesized to explore the relationship between a range of physiochemical properties and efficacy/BBB permeability (1,2). One of the compounds from this library, WR308245, initially generated interest due to its promising *in vitro* efficacy and toxicity values. However, its MDCK-MDR1 permeability values suggested high BBB penetration. *In vivo* mouse PK confirmed this finding with drug levels in the brain approximately six times that of MQ at the Cmax after IV dosing. Another drug in this class of analogs, WR319670, was found to have *in vivo* activity against *Plasmodium berghei* in a mouse model of anti-malarial efficacy while exhibiting poor activity levels in hypoxanthine and SYBR Green *in vitro* efficacy screens. These data suggested that the activity of WR319670 in the *in vivo* model was due to drug metabolism, i.e., that WR319670 behaved as a prodrug. Based on the structure of WR319670 and common Phase I biotransformations, it was postulated that one of the metabolites would be WR308245. *In vivo* mouse PK confirmed the formation of WR308245 upon IV administration of WR319670. In addition, drug brain levels for both WR319670 and WR308245 achieved a Cmax of approximately 1/3 that of MQ. In order to support the assertion that the *P. berghei* efficacy of WR319670 would translate to human malaria parasites, the drug's efficacy was tested in an Aotus monkey *P. falciparum* model. The *Aotus* model demonstrated sufficient efficacy to confirm the correlation between the *P. berghei* and *P. falciparum* models and, consequently, the use of a prodrug approach to minimize drug brain levels while achieving antimalarial efficacy.

253

IN VITRO AND *IN VIVO* METABOLIC AND PHARMACOKINETIC PROFILES OF WR283194, A NOVEL ANTIMALARIAL DRUG CANDIDATE

Dustin Carroll, **Brandon S. Pybus**, Jason Sousa, Raul Olmeda, Quiqui Li, Ai J. Lin, Michael P. Kozar, Victor Melendez

Walter Reed Army Institute of Research, Silver Spring, MD, United States

The imidazolidinedione (IZ) class of compounds has a long history with many pharmaceutical and industrial uses including herbicides, polymers, antibacterials, and anticonvulsants. Several compounds of the class have also demonstrated causal activity in *in vivo* malaria models, and the IZ compounds have been reported to be the first class of compounds demonstrated to possess activity exclusively against liver stage malaria (Guan et. al, 2002). The novel antimalarial drug candidate WR283194, of the imidazolidinedione class, was evaluated for its *in vitro* metabolic characteristics and *in vivo* pharmacokinetic (PK) properties in Rhesus monkeys. *In vitro* metabolic stability assays predict stability >60 min in both human and mouse liver microsomes, and drug-drug interaction screening against a panel of CYP isoenzymes and known subtstrates showed no significant interaction with each of the five primary abundance CYPs (3A4, 1A2, 2C9, 2C19, and 2D6). In a three day dosing model (PO 2.5 mg/kg), the average Cmax was determined to be 6546 ng/ml with a Tmax of 8 hrs in plasma and an average VD of 2225 ml/kg. The compound cleared relatively rapidly, with an average half life of 4.6 hrs and an observed average AUCinf of 156,411 hr*ng/ml. Preliminary data identified WR283246 as a major metabolite in Rhesus plasma and red blood cells. Metabolite ID studies in progress will help understand the compound's metabolic profile. These studies help define the pharmacokinetic and metabolic characteristics of compounds in the IZ class to guide future antimalarial drug efforts.

254

DHA INHIBITS HUMAN ERYTHROID CELL DIFFERENTIATION BY ALTERING THE GATA SWITCH

Sara Finaurini[1], Luisa Ronzoni[2], Alessandra Colancecco[2], Sarah D'Alessandro[1], Yolanda Corbett[1], Maria Domenica Cappellini[2], **Donatella Taramelli**[1]

[1]Università di Milano, Milan, Italy, [2]Università di Milano,Fondazione Policlinico Mangiagalli, Regina Elena, IRCCS, Milan, Italy

WHO recommends to avoid artemisinin treatment during the first trimester of pregnancy, because animal models showed a significant depletion of embryonic red cells, which occurs only during a specific days of gestation. We recently demonstrated for the first time that DHA, which is the *in vivo* metabolite of many artemisinin derivatives, inhibits human erythroid cell differentiation, as well. We showed that DHA specifically targets the pro- and basophilic erythroblasts during *in vitro* erythroid cell differentiation of CD34+ stem cells. By using K562 cells differentiated toward the erythroid lineages by chemical inducers and by comparing the effects of several artemisinins, we confirmed that DHA is the most toxic compound of this drug family. Significant reduction by DHA was observed not only of cell growth, but also of erythroid cell maturation, as shown by the changes in cell viability, cell cycle progression, GpA expression, inhibition of γ-globin gene and GATA-1 mRNAs. In addition, we observed that the toxicity is related to pathways which regulate the haemoglobin synthesis. In fact, DHA rapidly induces the release of Cytochrome C from the mitochondria, which in turn, activates Caspase-3 and, together with the HSP70 down regulation, induces the GATA-1 cleavage and the up-regulation of GATA-2. In conclusion, altering the GATA switch, DHA modifies the fate of the erythroid cell: it prevents the erythroid cell differentiation and simultaneously causes the arrest of cell growth and, eventually, the cell death through apoptosis. This dual effect is clearly dose-dependent. In conclusion, our results support WHO recommendations and the urgent need to better define the risk-benefit of the use of artemisinins treatment for malaria during the first trimester of human pregnancy.

255

PERMEABILITY OF ANTIMALARIAL DRUG CANDIDATES USING CACO-2 AND MDCK CELL LINES

Xiannu Jin, **Vanessa Collazo**, ThuLan Luong, NeCole Reese, Brandon S. Pybus, Jason Sousa, Dustin Carroll, Constance Asher, Raul Olmeda, Michael P. Kozar, Victor Melendez

Walter Reed Army Institute of Research, Silver Spring, MD, United States

Malaria figures amongst the major health and developmental challenges in the world. There is a need for orally active, liver stage antimalarials without CNS adverse effects. The use of cell lines such as CACO-2 and MDCK in permeability assays serve as surrogate indicators of absorption and transport; with the two approaches often used interchangeably. The growth period for the CACO-2 cells is at least 2 weeks before their trans-epithelial electrical resistance (TEER) reaches its optimal level of 300-500 ohms. On the other hand, MDCK cells reach their optimal TEER (>800 ohms) in 4 days; making MDCK more desirable in terms of time needed to cultivate and maintain them. We sought to characterize both approaches in support of our antimalarial drug development paradigm. Accordingly, the bi-directional transport using both CACO-2 and MDCK cells was evaluated for over 20 candidate antimalarial compounds and the permeability coefficient (Papp) values were calculated based on liquid chromatography/tandem mass spectrometry (LC-MS/MS) analyses. The result showed that the Papp results were similar in CACO-2 and MDCK permeability assay with low and medium permeable antimalarial compounds. However, there were variations of Papp result and efflux ratio between the CACO-2 and MDCK approaches. This could be due in part to P-gp mediated efflux in apical-basolateral or basolateral-to-apical transport, as well as, the tight junctions found in the MDCK permeability model. While the use of MDCK cells may be a "fast-growing" alternative to CACO-2 cells for measuring compound CNS transport, the later assay may still desirable permeability model for measuring intestinal transport of antimalarial candidates.

256

IN VITRO AND *IN VIVO* METABOLIC PROFILE OF TWO DEOXO-IMIDAZOLIDINEDIONE ANALOGS WITH ANTIMALARIAL PROPERTIES

ThuLan Luong, Ai J. Lin, Jason Sousa, Xianjun Liu, Qiqui Li, Raul Olmeda, Lisa Xie, Jing Zhang, Michael P. Kozar, Victor Melendez

Walter Reed Army Institute of Research, Silver Spring, MD, United States

A series of newly synthesized Deoxo-Imidazolidenedione (IZ) analogs are being evaluated by the U.S. Army for development as candidate antimalarials. This study provides *in vitro* and *in vivo* metabolic profile based on mass spectrometry analyses for two IZ analogs: WR308449 and WR308597. For the *in vitro efforts, compounds were* incubated for up to two hours in the presence of pooled microsomes originated from human, monkey or rat livers. The samples were extracted by protein precipitation and analyzed by LC-Trap/MS. The *in vivo* metabolic profile work analyzed samples from a mouse pharmacokinetic study following a single 50 mg/kg oral dose. Plasma and liver tissues were collected for up to 48 hours. Plasmas were extracted and analyzed as described above, while the liver tissues were homogenized prior to extraction. *In vitro* microsomal incubations of WR308449 yielded four putative metabolites while WR308597 yielded two putative metabolites. For WR308449, hydroxylation (+16) appears to be the major metabolite compared to bis-oxidataion (+32), methylation (+14), and metabolite 388 (-40). For WR308597, hydroxylation (+16) appears to be the major metabolite followed by bis-oxidation (+32), regardless of the specie evaluated. *In vivo,* WR308449 and WR308597 yielded more metabolites than the *in vitro* microsomal assays. WR308499 had 10 metabolites in plasma and 11 metabolites in liver, while WR308597 had 5 metabolites in plasma and 10 metabolites in liver. The *in vivo* metabolites of WR308499 and WR308597 included masses consisting with glucuronidation (+176), product of phase II metabolism. Based on the *in vitro* and *in vivo* results obtained, metabolic

profiles were postulated for WR308499 and WR308597. The relative contribution of the putative metabolites to efficacy and/or toxicity is yet to be characterized. The findings contribute to the development of new IZ analogs with desired attributes and facilitate characterization of their metabolic pathways.

257

ANTIMALARIAL ACTIVITY OF INDIVIDUAL ENANTIOMERS OF 8-AMINOQUINOLINES

N.P. Nanayakkara, H.M. Herath, Babu L. Tekwani, Larry Walker

University of Mississippi, University, MS, United States

8-Aminoquinolines are a group of important antiparasitic agents with broad activity and excellent efficacy against malaria, leishmania and *Pneumocystis jirovecii* pneumonia. However, a serious limitation to widespread use of this class of compounds is that they produce reversible methemoglobinamia and hemolysis in individuals who suffer from glucose-6-phosphate dehydrogenose deficiency. Primaquine (1), the only drug currently approved in this class, is utilized as the racemate. Previous studies using animal models have shown that an enantiomer of primaquine or NPC1161C (WR233078) (2), another drug candidate of this class, has a better therapeutic index than the racemate. In order to further study these observations, we resolved primaquine and two 8-aminoquinolines (3 (WR225448) and 4 (WR247705)) with potent antimalarial activity, and evaluated them for antimalarial activity using a mouse model infected with *Plasmodium berghei.* Comparison of these results with those data we previously reported for 2 indicated that the (-)-*(R)*-enantiomer had better activity than the (+)-(*S*)-enantiomer or the racemate, except for primaquine in which the converse was true. The racemate, (-)-(*R*)- and (+)-(*S*)-enantiomer of primaquine, 2 and 3 were not toxic at the highest dose (16 mg/kg/day) tested. However, the racemate and both enantiomers of 4 were toxic at this dose and the (-)-(*R*)-enantiomer was more toxic than the (+)-(*S*)-enantiomer.

258

A MATHEMATICAL MODEL TO DESCRIBE THE ARGININE CATABOLISM IN MALARIA

Indri R. Supriadi[1], Tsin Wen Yeo[2], Nicholas M. Anstey[2], Stephen B. Duffull[3]

[1]National Institute of Health Research and Development, Jakarta, Indonesia, [2]Menzies School of Health Research, Charles Darwin University, Darwin, Australia, [3]School of Pharmacy, Otago University, Dunedin, New Zealand

Decreased nitric oxide (NO) is associated with severe disease in *falciparum* malaria. Possible etiologies for low NO in malaria are decreased plasma L-arginine concentrations, the substrate for NO production, and NO quenching by cell-free hemoglobin (Hb) released during hemolysis. L-arginine infusion has been shown to increase vascular NO in moderately severe malaria but the optimal dosing regimen remains unclear. A mathematical model was developed, building on the work of others to describe L-arginine catabolism in endothelial cells in malaria. The model included the time course of hemolysis and subsequent release of arginase and cell free Hb. This model was used to investigate the optimal dosing schedule of arginine infusion in malaria to achieve maximal production of NO. Additional simulations were conducted in order to predict the extra- and intracellular concentrations of arginine, ornithine and citrulline as well as the cumulative NO molecules reach vascular muscle cells in both healthy volunteers (HV) and those with moderate severe malaria (MSM). The model described adequately the data collected from our previous study in Timika, Indonesia. The cumulative NO molecules produced by endothelial cells was significantly increased with supplementation of extracellular arginine. The choice of dose (3, 6 or 12 g) was less important than the duration of the infusion over which the dose was administered. Additionally, an increase in cell free Hb decreases cumulative NO molecules reaching vascular smooth muscle cells in an approximately inverse exponential manner. In conclusion, the model provided an adequate description of the time course of arginine catabolism in HV and MSM and results were in agreement with the current *in vivo* and *in vitro* data. The administration of arginine is schedule-dependent, i.e. how the arginine is administered is at least as important as how much arginine is administered, and this should be taken into account in the design of future clinical trials.

259

COMMUNITY BASED PHARMACOVIGILANCE, A WAY FORWARD FOR STRENGTHENING THE PHARMACOVIGILANCE SYSTEM IN AFRICAN UNDERSERVED AREAS: EXPERIENCE IN SARAYA HEALTH DISTRICT IN RURAL SENEGAL

Youssoupha Ndiaye

Ministry of Health and Prevention, Dakar, Senegal

Following the Abuja Conference, many strategies have been put in place for malaria control in Senegal. ACTs were implemented for the treatment of uncomplicated malaria and Intermittent Preventive Treatment for Pregnant women (IPTp) with Sulfadoxin Pyrimethamin (SP) was also adopted. In addition, from November 2006, Intermittent Preventive Treatment for infants (IPTi) has started in an operational research held in Saraya and two other districts. The objective of the study was to assess the monitoring of pharmacovigilance at the community level. Saraya district is located in southeasterner Senegal, a rural area where patients' ability to have access to health facilities is extremely limited. Health staff (14) and community health workers (30) have been trained in passive and active pharmacovigilance. Trainings were reinforced by supervision; follow up meetings, social mobilization and information through community radio broadcasts from July 2007 to December 2009. In a 38000 estimated population, 7067 ACT treatments were administered and 53 notifications of adverse events completed; 21/53 notifications that were completed by health staff were related to ACT administration; among them 7/21 were put under observation. The other 32 notifications were brought to the attention of the health staff by the community (Community Health Workers, matrons, volunteers, leaders); they concerned SP in IPTi (12/53), Ivermectin (14/53), Cotrimoxazole (3/53), Mebendazole (1/53), Anti inflammatory (1/53), unknown drug (1/53). In conclusion, to successfully implement a pharmacovigilance program, it is fundamental not only to reinforce health staff training but to involve communities by engaging leaders, families, schools, and traditional healers. It is also urgent to ensure the validity of information related to Pharmacovigilance.

260

A STRUCTURE BASED DRUG DESIGN APPROACH TO REPURPOSE DRUGS AGAINST *PLASMODIUM FALCIPARUM* HSP90 (PFHSP90)

Dea Shahinas[1], Tony Taldone[2], Gabriella Chiosis[2], Dylan R. Pillai[3]

[1]University of Toronto, Toronto, ON, Canada, [2]Memorial Sloan-Kettering Cancer Centre, New York, NY, United States, [3]Ontario Agency for Health Protection and Promotion, Etobicoke, ON, Canada

Plasmodium falciparum heat shock protein 90 (PfHsp90 or PfHsp86 PF07_0029) is essential for the development of the parasite during the intra-erythrocytic cycle and has the potential to serve as a drug target and circumvent antimalarial resistance when targeted in combination. In fact studies in Candida albicans have shown that Hsp90 inhibitors are able to reverse resistance to common antifungal agents such as cyclosporine A and echinocandins, as reported previously. Based on the conservation of the Hsp90 binding pocket and its central role in folding resistance associated proteins, our central hypothesis is that PfHsp90 inhibitors can reverse antimalarial resistance. Furthermore, evidence from the literature suggests that ATP mimetic inhibitors of the Hsp90 ATP-binding pocket target the phosphorylated "active form" of the protein in abnormal cancer cells. Regulation of PfHsp90 by phosphorylation by casein kinase II has

been previously reported suggesting that such a mechanism of selectivity of Hsp90 inhibitors for infected cells may be in place and may account for specificity of these inhibitors. Based on this hypothesis, we used the anticancer inhibitor of Hsp90 PU H71 for activity against malarial Hsp90. PU H71 inhibits ATP binding on the PfHsp90 GHKL domain and provides inhibition of parasite growth in cell culture in the nanomolar range. In addition, PU H71 exhibits synergistic activity with mefloquine and is able to reverse chloroquine resistance in the chloroquine resistance parasite line W2. Crystallization of PfHsp90 with PU H71 was achieved in order to understand the interactions of PU H71 with the PfHsp90 binding pocket and to further optimize this inhibitor for malaria. In conclusion, we are presenting a synergistic inhibitor of malaria hsp90 that can reverse resistance. Crystal data are being used to further optimize this inhibitor for malaria. Doses at which synergy is obtained are likely not toxic and will prevent emergence of resistance.

261

IN VIVO ANTIMALARIAL, SERUM LIPID PROFILE AND HEMATOLOGICAL EVALUATIONS OF *ANOGEISSUS LEIOCARPUS* (DC.) GUILL. AND PERR. IN *PLASMODIUM BERGHEI* INFECTED *MICE*

Mojisola C. Cyril-Olutayo, Olusegun M. Akanbi, A. A. Omonkhua

Adekunle Ajasin University, Akungba- Akoko, Ondo State, Nigeria

Malaria is a public health problem most especially in the tropical countries where majority bear the burden of the disease. It is one of the six killer diseases in the world to-day and it has been estimated that 40% of the world's population is at risk and 500 million people suffer from the disease annually. Symptoms of malaria include fever, shivering, vomiting, anemia (caused by hemolysis), hemoglobinuria, retinal damage and convulsions. *Anogeissus leiocarpus* is an evergreen tree native to the savannas of tropical Africa. The inner bark is used as a chewing stick in Nigeria and extracts of the bark is used locally to treat malaria and show antibacterial properties. *Plasmodium berghei strain NK 65* was used to infect mice grouped into five and left to establish for 7 days. On the seventh day, groups A, B and C were treated with Artesunate 5mg/kg, *A. leiocarpus* 100mg/kg and A. leiocarpus 200mg/kg respectively while groups D and E were negative control and uninfected mice respectively. The parasite density was monitored daily for five days and on the 5th day, haematological and serum lipid profile parameters were assessed. Treatment of malaria infected mice with artesunate 5mg/kg and *A. leiocarpus* reduced the parasite density compared with the negative control. Also treatment with artesunate and *A. leiocarpus* extracts increased the packed cell volume and red blood cell count which decreased in the negative control mice. Neutrophil and lymphocyte counts of the treated infected mice were brought to the levels of the uninfected mice. SOD level was significantly higher in the homogenate from liver and heart of the mice treated with 200mg/kg of *A. leiocarpus* than other groups. LDL, Total cholesterol, triglyceride were significantly higher in mice treated with artesunate than other groups, while HDL was higher in the group treated with 200mg/kg *A. leiocarpus.* The study justifies the traditional use of the extract of *A. leiocarpus* as an active antimalarial and has antianaemic as well as an antioxidant properties.

262

EVALUATION OF THE ANTI-MALARIA, HAEMATOLOGICAL AND ANTI-OXIDANT PROPERTIES OF METHANOLIC EXTRACTS OF *TERMINALIA AVICENNIOIDES* IN *PLASMODIUM BERGHEI BERGHEI* INFECTED MICE

Akhere A. Omonkhua, Olusegum Mathew Akanbi, Mojisola C. Olutayo

Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria

Various extracts of *Terminalia avicennioides* (Combretaceae) are used in Nigeria to treat ailments such as rheumatic pain, helminthiasis, gastric and peptic ulcers. *T. avicennioides* has also been shown to have significant activities against *Salminella typhi*, *S. paratyphi* and *Vibrio cholera*. This study investigated the *in vivo* anti-malarial effects of different methanolic crude extracts of *T. avicennioides* in *Plasmodium berghei berghei* infected mice. The haematological and oxidative statuses of the mice were also evaluated. 25 mice in 5 different groups were used for this study. The parasites density of *P. berghei* infected mice was monitored daily for five days (by thin and thick blood films stained with Leishman's stain) upon treatment with artesunate (5mg/kg body weight) and *T. avicennioides* (100 and 200mg/kg body weight). Parameters to assess haematological and oxidative status were measured after five days. The parasite density of artesunate and *T. avicennioides* treated malaria parasite positive (MP+) mice decreased compared to untreated MP+ mice. The decreases seen in the haemoglobin (Hb) and red blood cell (RBC) count, as well as the increased neutrophil and decreased lymphocyte counts, of the untreated MP+ mice was restored to normal control levels in the artesunate and medicinal plant treated MP+ mice. Serum MDA levels of the treated MP+ mice were significantly ($p<0.05$) lower than untreated MP+ mice, while increases were observed in serum and liver superoxide dismutase (SOD) activities of the treated MP+ mice. In conclusion, oxidative stress during acute malaria infection, including depletion of antioxidants and increased plasma lipid peroxidation, has been documented. Oxidized molecule thus produced may play a role in the pathogenesis of malaria. The restoration of oxidative status, as well as, haematological parameters to normal values may reduce the severity of malaria infection.

264

RELATIONSHIP BETWEEN AGE AND BODY WEIGHT GROUPS IN CHILDREN WITH *FALCIPARUM* MALARIA RECEIVING ARTEMETHER-LUMEFANTRINE (AL) AND ANALYSIS OF AL EFFICACY AND SAFETY ACCORDING TO BODY WEIGHT GROUPS

Quique Bassat[1], Clara Menéndez[1], Sonia Machevo[2], Alain Nahum[3], David Ubben[4], Verena Walter[5], Obiyo Nwaiwu[6], Chemtai Kipkeu[7], Bernhards Ogutu[8]

[1]University of Barcelona, Barcelona, Spain, [2]Manhiça Health Research Center, Maputo, Mozambique, [3]Centre de Recherche Entomologique de Cotonou, Cotonou, Benin, [4]Medicines for Malaria Venture, Geneva, Switzerland, [5]Novartis Pharma AG, Basel, Switzerland, [6]Novartis Pharma AG, Lagos, Nigeria, [7]Novartis Pharma Services AG, Hospital Road, Kenya, [8]Walter Reed Project, Kenya Medical Research Institute, Kisumu, Kenya

Artemether-lumefantrine (AL) is the current standard of care for uncomplicated *Plasmodium falciparum* malaria. Dosing by body weight is recommended but can not be used if weighing scales are unavailable e.g home-based management. Therefore age by body weight (BW) data can provide some guidance for optimal dosing when information on weight is not available. Data from a randomized, multicenter, investigator-blinded study in 899 infants and children (≥5kg and <35kg) with uncomplicated *falciparum* malaria in five African countries (Benin, Kenya, Mali, Mozambique, and Tanzania) was analyzed. AL was dosed according to body weight groups (BWG): BWG1, 5kg to <15kg (1 tablet/dose); BWG2, 15kg to <25kg (2 tablets/dose); or BWG3, 25kg to <35kg (3 tablets/dose). The primary analysis population included 477, 277, and 58 patients in the

BWG1: BWG2: and BWG3 respectively. PCR-corrected cure rate at day 28, the primary end point, was similar across all BWGs: BWG1 98.3%, BWG2 97.8% and BWG3 98.3%. Median times to parasite clearance and fever clearance were comparable between all BWG groups. There were no unexpected differences in safety or tolerability between the three BWG groups. Age by BW data showed that 80% (10-90th percentile) of the patients in the BWG1, BWG2 or BWG3 were aged 10-50 months (median 27, range 0-70; mean 29.3±15.4), 46-100 months (median 71 months, range 31-148, mean 71.7±22.4) and 90-147 months (median 122 months, range 78-152, mean,119.3±20.5) respectively. In conclusion, the efficacy of AL in treating uncomplicated *falciparum* malaria is similar across body weight dosing groups in infants and children, with no clinically relevant differences in safety or tolerability. Analysis of age by BW group in this population of children from sub-Saharan African countries showed there to be only a minor overlap of age ranges between BWGs based on the 10-90th percentiles.

265

ANTI-MALARIAL ACTIVITY OF CEM-101, A FLUOROKETOLIDE ANTIMICROBIAL, IN BOTH BLOOD STAGE AND PRESUMPTIVE CAUSAL PROPHYLACTIC MOUSE MODELS

Susan Fracisco[1], Montip Gettayacamin[2], Anchalee Tungtaeng[2], Michael Kozar[1], Michael O'Neil[1], Carl Craft[3], Prabha Fernandes[3]

[1]Walter Reed Army Institute of Research Experimental Therapeutics, Silver Spring, MD, United States, [2]Armed Forces Research Institute of the Medical Sciences, Bangkok, Thailand, [3]Cempra Pharmaceuticals, Inc, Chapel Hill, NC, United States

CEM-101 is a new broad spectrum macrolide that has completed Phase 1 trials that acts to inhibits protein synthesis through binding to bacterial ribosomal RNA. A comparator drug, azithromycin, causes a delayed death effect *in vitro Plasmodium falciparum* blood stage assays and demonstrates antimalarial activity against liver stage parasites. CEM-101 was recently shown to be active *in vitro* against *P. falciparum* in extended incubation assays which measure the potency of inhibitors that demonstrate delayed death effects. CEM-101 is also active against blood stages in *P. berghei*-infected mice. Dose-response for CEM-101 was characterized in both blood stage treatment and causal prophylactic *P. berghei*-infected mice models using 3 day PO or SC dosing. Efficacy was measured by number of mice with delayed parasitemia and mice that were malaria free at day 31. Antimalarial liver stage activity was assessed in mice infected with luciferase expressing *P. berghei* parasites using an *in vivo* imaging system. For blood stage infections, the minimum curative SC dose was 40 mg/kg/d X 3 days, while 80 mg/kg/d X 3 was the minimum active dose for PO route. In the *P. berghei* causal mouse model, CEM-101 was curative at 40 mg/kg/d X 3 days with SC or PO dosing. No systemic toxicity was observed with SC or PO dosing as high as 160 mg/kg/d X 3 days. No demonstrable antimalarial activity against liver stage parasites was observed by *in vivo* imaging analysis of luciferase-expressing *P. berghei* with PO dosing at 40 mg/kg/d X 3 days. While drug activity against liver stage parasites could not be measured by *in vivo* imaging, no blood stage infection was detected in mice dosed as low as 40 mg/kg/d X 3 days and the minimum active dose was 20 mg/kg/d X 3 days. In conclusion, CEM-101 shows 100% prophylactic activity in causal mouse malaria models with PO dosing at 40 mg/kg/d X 3 days and 3/5 mice remain parasite free at 20 mg/kg/d X 3 days. The *in vivo* imagining analysis of liver stage parasites suggests that CEM-101 does not affect parasite growth at 40 mg/kg/d X 3 days. Based on *in vitro* blood stage drug assays and the mechanism of inhibition of this class of compounds, the lack of demonstrable liver stage activity was probably due to dosage. These results suggest that CEM-101, like azithromycin, demonstrates a delayed death effect; that is, developing liver stage merozoites are effectively non-viable blood stage parasites.

266

ANIMAL MODELS FOR SCREENING OF EXOERYTHROCYTIC ANTIMALARIAL DRUGS

Montip Gettayacamin[1], Anchalee Tungtaeng[1], Rawiwan Imerbsin[1], Jetsumon Sattabongkot[2], Pranee Hansukjariya[1], Srawuth Komcharoen[1], Laksanee Inamnuay[1], Sarah B. Hinds[1], Joseph J. Novak[1], Susan D. Fracisco[3], Geoffrey S. Dow[3], Ai J. Lin[3], Charlotte A. Lanteri[3], Sandi K. Parriott[3], Colin K. Ohrt[3], Michael P. Kozar[3], Michael T. O'Neil[3], Alan J. Magill[3]

[1]Department of Veterinary Medicine, United States Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Department of Entomology, United States Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]Division of Experimental and Therapeutics, Walter Reed Army Institute of Research - ET, Silver Spring, MD, United States

AFRIMS has used both mouse and monkey models for screening new antimalarial compounds from Walter Reed Army Institute of Research. Currently, novel antimalarials are screened first in the *Plasmodium berghei* (ANKA strain)-ICR mouse malaria model at AFRIMS and/or the new *in vivo* imaging system (IVIS) at Walter Reed Army Institute of Research. The active compounds are selected for testing in the *P. cynomolgi*-Rhesus monkey (*Macaca mulatta*) relapsing malaria model. The infection has been achieved through standard intravenous inoculation doses of 1 x 105 and 1 x 106 sporozoites from *Anopheles dirus* mosquitoes for the mouse and monkey models respectively. In 2008-2010, >200 new compounds and human approved drugs were screened in >80 mice experiments, and >20 compounds were tested in the monkey model in 5 experiments. More than 25 compounds showed causal prophylaxis activity in the mouse model and these included WR283205, WR296580, WR301855, WR299548, Clindamycin, Mirincamycin-*Trans*, Mirincamycin-*Cis*, Mirincamycin-racemic mixture, WR308499, WR308597, WR308271, WR308272, WR308145, WR308482, WR308658, Primaquine (+), Primaquine (-), WR279825, WR299958, WR319568, Tafenoquine (TQ), WR319590, CEM101, WR319673, WR319718 and WR282315. In addition, the mouse model was further validated with known antimalarials, Atovaquone (AT), Azithromycin (AZ), TQ, and Primaquine (PQ) to determine standard minimum effective oral (PO) doses. The minimum effective doses (MEDs) of AT, AZ, PQ and TQ for the oral three-day regimen (Days -1, 0 and 1), two-day (0, 1) and one-day (Day 0) were 0.5, 0.5 and 2.5 mg/kg; 120, 160 and 240 mg/kg; 25, 30 and 35 mg/kg; and 3.0, 2.5, and 7.5 mg/kg, respectively. In the monkey model, active tissue schizontocidal compounds in combination with chloroquine treatment were Tinidazole 300 mg/kg/PQ 0.3 mg/kg, PO for 7 days and twice weekly for 4 weeks; WR299548 (Decoquinate) 15 mg/kg, IM for 7 days; WR308499 at 30 mg/kg, IM for 3 days; PQ (-) at 0.6 mg/kg, PO for 7 days; and PQ (+) at 1.3 mg/kg, PO for 7 days. There are several compounds in the process for screening in both models and updated results will be discussed.

267

NOVEL INHIBITORS OF *PLASMODIUM FALCIPARUM* DIHYDROOROTATE DEHYDROGENASE EXHIBIT ANTI-MALARIAL ACTIVITY IN MURINE MODELS

Michael L. Booker[1], Cecilia M. Bastos[1], Martin Kramer[1], Robert H. Barker Jr[1], Joseph Cortese[2], Cassandra Celatka[1], Benito Munoz[2], Jose E. Guerrero Bravo[3], Keila N. Krespo Llado[3], Adelfa E. Serrano[3], Renato Skerlj[1], Iñigo Angulo-Barturen[4], María Belén Jiménez-Díaz[4], Sara Viera[4], Helen Garuti[4], Sergio Wittlin[5], Petros Papastogiannidis[5], Ian Bathurst[6], David Floyd[6], Dyann F. Wirth[7], Roger Wiegand[2], Jeffrey D. Klinger[1], Edmund Sybertz[1]

[1]Genzyme Corporation, Waltham, MA, United States, [2]Broad Institute of Harvard and MIT, Cambridge, MA, United States, [3]University of Puerto Rico School of Medicine, San Juan, PR, United States, [4]GlaxoSmithKline, Tres Cantos, Spain, [5]Swiss Tropical Institute, Basel, Switzerland, [6]Medicines for Malaria Venture, Geneva, Switzerland, [7]Harvard School of Public Health, Boston, MA, United States

Dihydroorotate dehydrogenase (DHODH) catalyzes the rate-limiting step in the *de novo* pyrimidine biosynthetic pathway, in which dihydroorotate is formed through a coupled redox reaction utilizing a mitochondrial respiratory chain ubiquinone. *Plasmodium falciparum* is unable to salvage pyrimidines and must rely on *de novo* biosynthesis for survival. DHODH represents the ultimate target of atovaquone via that agent's disruption of the electron transport chain, and it offers a viable target for additional chemotherapeutics. A high-throughput screen and subsequent medicinal chemistry program identified two promising series of compounds: 5-benzimidazolyl-N-alkylthiophene-2-carboxamides and 5-(4-phenyl)-imidazolyl-N-alkylthiophene-2-carboxamides. Compounds from each of these series demonstrated double-digit nanomolar *in vitro* potency against DHODH from *P. falciparum, P. vivax,* and *P. berghei*, with selectivity for the parasite enzymes over human DHODH. The activity against the *P. falciparum* enzyme was well correlated with *in vitro* potency against the *P. falciparum* 3D7 and Dd2 parasites. Several of the most potent compounds demonstrated good tolerability and oral exposure in the mouse, as well as ED50 values in the 4-day murine *P. berghei* (N strain) model of 10-15 mg/kg/day with oral b.i.d. dosing. Furthermore, oral b.i.d. dosing of the benzimidazole compound Genz-667348 at 100 mg/kg/day in the *P. berghei* (ANKA strain) model resulted in sterile cure, as defined by absence of recrudescence during a 30-day period following the cessation of dosing. This compound exhibited comparable activity in the *P. falciparum* humanized NOD-*scid* mouse model. An iterative lead optimization process is continuing, and closely related analogs with good potency and improved ADME properties are currently under investigation.

268

THAI MULTIDRUG-RESISTANT (MDR) C2A STRAIN OF *PLASMODIUM FALCIPARUM* ADAPTED FOR USE IN THE *AOTUS LEMURINUS IN VIVO* MODEL

Bryan L. Smith[1], Nicanor Obaldia, III[2], Lucia Gerena[1], Geoffrey S. Dow[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Gorgas Memorial Institute of Health Studies (ICGES), Panama City, Panama

Aotus monkeys infected with adapted human *Plasmodium falciparum* strains have been used for more than 40 years to study the pathogenesis, efficacy and pharmacokinetics of antimalarial compounds. The emergence of resistant malaria strains makes adaptation of recent strains from geographically relevant areas especially important. A recent preliminary report indicates that a multidrug-resistant (MDR) C2A strain originally obtained in Thailand may have been successfully adapted. Six splenectomized animals were inoculated with 5,000,000 parasites IV from a donor animal previously inoculated with a preserved aliquot of the 2008 Level VIII C2A. Daily parasite densities were obtained until > 100,000 parasites/µL. Animals randomized to each of the three treatment arms [mefloquine (MQ) 40 mg/kg orally X 1, artesunate (AS) 33 mg/kg orally daily X 3, or MQ 40 mg/kg orally X 1 plus AS 33 mg/kg orally daily X 3] were then started on their respective treatments. Parasitological and clinical responses were followed for 100 days. Animals in which primary treatments failed were administered the rescue regimen of AS+MQ. All 6 animals showed parasite patency at Day 1- 2 and reached peak parasite levels of > 100,000 parasites/µL by Day 9-11. One animal given AS+MQ was cured. The regimens administered to four of five of the remaining animals failed to adequately clear their parasitemia (late treatment failures) and required treatment the rescue treatment. All had final clearance by Day 23-28. The remaining MQ treated animal failed to clear by Day 9 and required rescue treatment (late parasitological failure). The rescue treatment was curative. *In vitro* IC50 and IC90 values obtained via a labeled hypoxanthine assay showed preserved to increased values of 29.6 ± 1.3 and 138.8 ± 8.7 ng/ml respectively when compared to the standard lab strain C2A values of 20.9 ± 1.0 and 93.9 ± 4.7 ng/ml. In conclusion, the current strains of the Thai MDR C2A strain has been successfully adapted to growth within splenectomized *Aotus* monkeys.

269

CEM-101, A NEW FLUOROKETOLIDE WITH ANTIMALARIAL ACTIVITY

J.C. Craft[1], Sergio Wittlin[2], Julie Lotharius[3], Ian C. Bathurst[3], Prahavathi Fernandes[4]

[1]Cempra Pharmaceuticals, Libertyville, IL, United States, [2]Swiss Tropical and Public Health Institute, Basel, Switzerland, [3]Medicines for Malaria Venture, Geneva, Switzerland, [4]Cempra Pharmaceuticals, Chapel Hill, NC, United States

CEM-101(CEM) is a new fluoroketolide antibiotic under clinical development for the treatment of community-acquired bacterial respiratory tract infections. In this study, we evaluated its *in vitro* and *in vivo* activity against different species of *Plasmodium* parasites to see if there is a potential for use in the treatment of malaria. CEM was tested by the semi automated microdilution assay against intra-erythrocytic forms of *P. falciparum* derived from asynchronous cultures of the strain NF54, essentially as described previously. Parasite growth over 120h was measured by the incorporation of radiolabelled [3H]hypoxanthine (in hypoxanthine-free culture medium) added after 96h of drug incubation and 24h prior to the termination of the test. Because of its slow mode of action, CEM was followed for 120 hrs vs the usual 72h assay. *In vitro P. falciparum* data for CEM in the 120h assay (96h + 24h). Cem showed an IC50 against NF54 of 2.4ng/ml compared to artesunate with an IC50 of 3.3ng/ml, clindamycin 5.3ng/ml and chloroquine 4.7ng/ml. Based on its promising *in vitro* activity, CEM was tested in the murine *P. berghei* model as described previously. CEM was first studied after a single dose of 100 mg/kg either in DMSO or HPMV. In the second experiment CEM was given at a dose of 100 mg/kg daily for 4 days in both vehicles. Following the single dose, CEM showed antiparasitic activity of 80.05% and 81.45% and mouse survival in days was 15.2 and 12.7 days, respectively. Following daily doses of 100 mg for 4 days CEM showed antiparasitic activity of 99.79% in both vehicles. The mice survived for 30 days in both experiments and thus CEM is considered to be fully curative in this model. CEM has been shown to have excellent *in vitro* and *in vivo* activity against *Plasmodium* species. This data would support future studies to determine CEM potential for the treatment of blood stage malaria in combination with a fast-acting antimalarial. It may also have additional benefits because of its activity as an antibiotic.

270

PRIMAQUINE AND TAFENOQUINE IN THE *PLASMODIUM CYNOMOLGI* CAUSAL PROPHYLACTIC MALARIA MODEL

Charles A. DiTusa[1], Montip Gettayacamin[2], Michael P. Kozar[1], Ai J. Lin[1], Susan D. Fracisco[1], Colin Ohrt[1], Alan Magill[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

The relapsing malaria model consisting of *Plasmodium cynomolgi bastianellii* (B strain) in the rhesus is a valuable tool for identifying causal prophylactic drug candidates against *P. vivax* in humans. Historically, the 8-aminoquinolines (8-AQs) primaquine and tafenoquine have been protective at oral doses administered on days -1, 0 and 1 against sporozoites inoculated on day 0, presumably due to drug action against pre-erythrocytic stages. However, recent data suggest that the historically effective dosing regimens are not protective in the modern model. At historically effective doses of the two 8-AQs on days -1, 0, and 1, development of parasitemia was delayed slightly when compared with the untreated animals, but was not prevented. Delay in parasitemia averaged 5 days for the primaquine group (1.78 mg/kg/day) and 3 days for the tafenoquine group (0.316 mg/kg/day) when compared to the controls (vehicle only). Increasing the dose of tafenoquine to 6.0 mg/kg/day has provided complete protection to date, study day 53. While increasing the length of primaquine dosing from 3 to 10 days also provided protection to one monkey in the group, the other developed parasitemia on day 49. In this model, the primary attack is observed in untreated monkeys between days 8-10, the monkeys are treated with chloroquine for 7-10 days, and relapse occurs approximately 10 days after the last chloroquine dose. In the case of drugs with long elimination half-lives, such as tafenoquine, a lengthy delay in development of parasitemia may be attributed to drug still in the system. For primaquine, with a 2 hr elimination half-life in monkeys, other possibilities must be considered; the most likely being hypnozoite latency period. Comparison of 8-AQs to atovaquone-proguanil, an antimalarial with no antihypnozoite activity, will be presented, as well details of the analysis of plasma drug concentration-time data to determine dosing-exposure profiles and plasma drug levels associated with protection.

271

SPATIAL AND TEMPORAL PATTERN OF ANTI-MALARIA ANTIBODY RESPONSES AS EVALUATION OF HUMAN EXPOSURE IN THE WESTERN KENYAN HIGHLANDS

Kingsley Badu[1], Yaw Afrane[1], John Larbi[2], Ann Stewart[3], John Waitumbi[3], Goufa Zhou[4], Andrew Githeko[1], Guiyun Yan[4]

[1]Kenya Medical Research Institute, Kisumu, Kenya, [2]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [3]Walter Reed Project, United States Army Medical Research Unit, Kisumu, Kenya, [4]Program in Public Health, College of Health Sciences, University of California at Irvine, Irvine, CA, United States

Assessment of exposure to malaria at different altitudes and transmission intensities will inform the implementation and evaluation of malaria control programs. Recently anti malaria antibodies to merozoite surface protein 1 (MSP-1) have been described as the best immunological marker for estimating malaria exposure as a proxy for transmission intensity across various altitudes. The purpose of this study was to determine if the spatial and temporal patterns of antibody (Ab) responses are consistent with varying transmission intensities in the highlands of western Kenya. We measured total IgG levels to *Plasmodium falciparum* MSP-119 in an age stratified cohort (1= ≤1, 2=2-3, 3= 4-14, 4=15-45) of 900 participants from uphill and valley bottom residents at highland site during a low transmission and high malaria transmission season. Total IgG levels to salivary gland peptide gSG6 -P1 were also measured to determine whether micro-heterogeneity exposure to *Anopheles* bites correlates with MSP-1 IgG titers. Significantly higher proportions of sero-positives and total IgG titers were observed in valley bottom residents and in high transmission season. Age stratified cohort revealed intriguing differences; higher titers in 1 yr olds, a decrease in 2-3yr olds with a non significant increase in 4-14 yr old before rising to significantly higher levels in the 15-45yr olds. No significant differences between age groups 1, 2, and 3 across all parameters compared except for seasonal variation, however significant differences were observed between each younger age group and group4. In conclusion, this data confirms a highly heterogeneous malaria exposure at this highland site possibly due to clustered vector densities around major breeding sites near valley bottoms. Whether the high level of Ab in infants is as a result of exposure or exclusively due to maternal antibodies is yet to be elucidated.

272

INFLUENCE OF EXPOSURE TO *ANOPHELES* BITES ON THE DEVELOPMENT OF ACQUIRED ANTIBODY RESPONSE TO *PLASMODIUM FALCIPARUM* IN CHILDREN

J.B. Sarr[1], B. Samb[2], OI. Dia[3], S. Senghor[4], C. Thiam[4], A.M. Schatch[4], S. Guindo[4], F. Simondon[1], L. Konaté[2], G. Riveau[4], F. Remoue[1]

[1]UR016" Institut de Recherche pour le Développement", Montpellier, France, [2]Université Cheikh Anta Diop, Dakar, Senegal, [3]Institut Pasteur, Dakar, Senegal, [4]ONG EPLS "Espoir Pour La Santé", Saint-Louis, Senegal

Numerous ecological and epidemiological factors could modulate the anti-malaria immunity. Among these factors, the exposure to *Anopheles* bites, especially by active components of *Anopheles* saliva, could play a key role on the development of human immune response to *Plasmodium falciparum*. We investigated here the influence of exposure to *Anopheles* bites on the acquired antibody (Ab) response specific to *P. falciparum* whole schizont extract (WSE) and to CSP vaccine candidate, in children (1-9 years) living in malaria area. A multi-disciplinary and longitudinal study was conducted in two Senegalese villages where intensity of exposure to *Anopheles* bites was clearly different: Mboula, presenting low exposure (BHN =3) versus Gankette, with high exposure (BHN = 120). IgG, IgG1, IgG3 response directed to WSE and CSP antigen were determined before (June), at the peak (September) and after (December) the period of malaria exposure. In Mboula, the peak of exposure was followed by increase of anti-WSE IgG levels whereas low and constant specific IgG response was observed in Gankette. Interestingly, anti-WSE and anti-CSP IgG1 levels were higher in Mboula, whereas specific IgG3 response predominated in Gankette. Specific IgG1 response appeared therefore observed mainly in area presenting low exposure to *Anopheles* bites, whereas IgG3 isotype predominate in high exposure area. In addition, Ab response to WSE and CSP antigens decreased progressively with the season of exposure to *Anopheles* bites and this decrease appeared dependent to IgG1/IgG3 balance and to the level of exposure. Altogether, these results show that the development of anti-malaria Ab response was profoundly different according to areas where the level of *Anopheles* bites exposure was dissimilar. This influence of exposure to bites appeared to differently regulate the balance between specific IgG1 and IgG3 isotype levels, known to be associated with anti-malaria protective immune response. One hypothesis is that the influence of *Anopheles* saliva could be involved in the observed anti-malaria immune regulation.

273

STUDIES ON ABO BLOOD GROUPS, HAEMOGLOBINOPATHIES AND G6PD GENOTYPES, AND *PLASMODIUM FALCIPARUM* INFECTION IN KPONE-ON-SEA, GHANA

Samuel Armoo[1], Michael Wilson[2], Daniel Boakye[2], Isabella Quakyi[3]

[1]Council for Scientific and Industrial Research, Accra, Ghana, [2]Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Accra, Ghana, [3]School of Public Health, College of Health Sciences, University of Ghana, Legon, Ghana, Accra, Ghana

Erythrocyte variants such as the ABO blood groups, haemoglobinopathies and G6PD genotype are known to be associated with naturally acquired immunity against malaria. Despite some evidence of their protection, other epidemiological studies have provided evidence to the contrary, therefore their associations with malaria at Kpone-On-Sea, a coastal fishing village with high malaria incidence, was investigated. The design was cross-sectional, 592 individuals were randomly selected from whom 0.5ml of blood was collected and human DNA extracted using DNeasy Kit (Qiagen, USA). Blood groups and haemoglobinopathies were determined by standard agglutination method and cellulose acetate haemoglobin electrophoresis respectively. G6PD genotypes were determined by a PCR-based method using primers 5'-CCTGTTCCCTCTGCCACA-3' and 5'-GGGGGTCTCAAGAAGTAC-3', followed by restriction of the amplified product with Hsp 92II enzyme. Parasitaemia was determined using microscopy. Among the study participants, 60.5% were females and 39.5% males. The distribution of the blood groups O, A, B and AB were 44.76%, 20.61%, 31.25% and 3.38% respectively. The prevalence of HbAA, HbAC, HbAF, HbAS, HbSC and HbSS were 71.28%, 8.11%, 1.18%, 16.89%, 1.35% and 1.01% respectively. Of all study participants, 50.68%, 35.81%, 8.11%, 1.69% and 3.72% were G6PD homozygous normal, hemizygous normal, heterozygous deficient, homozygous deficient and hemizygous deficient respectively. Only 72 individuals among the total study participants were parasitaemic. The geometric mean parasite density was 829.7 parasites/µl of blood (95%CI, 574.0-1199.40). Blood group O was not associated with reduced parasitaemia (t = -0.546, P = 0.587). HbAS was not associated with reduced parasitaemia (t = -1.262, P = 0.212). HbAC was not associated with reduced parasitaemia (t = 0.189, P = 0.851). The heterozygous G6PD deficiency was also not associated with reduced parasitaemia (t = 0.437, P = 0.664). Sample collection occurred in a period following a long dry season, resulting in low parasite prevalence rates being recorded, therefore the need for more studies to further explore the associations of these RBC variants and parasitaemia in the area. A more sensitive diagnostic technique such as PCR should be used in future studies to determine parasitaemia. There may be a clinal trend in the distribution of HbS and HbC in the country so the need for nationwide screening.

274

ANTIBODIES THAT INHIBIT BINDING OF *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTES TO CSA ARE ACQUIRED DURING PREGNANCY AND CORRELATE WITH ANTI-VSA AND ANTI-VAR2CSA

Justin Doritchamou[1], Firmine Viwami[1], Lise Deneoud[2], Sédami Gnidehou[3], Azizath Moussiliou[1], Achille Massougbodji[1], Ali Salanti[4], Philippe Deloron[3], Nicaise Tuikue Ndam[2]

[1]University of Abomey Calavi, Cotonou, Benin, [2]Institut de Recherche pour le Développement, Cotonou, Benin, [3]Institut de Recherche pour le Développement, Paris, France, [4]Kobenhavns Universitet, Kobenhavns, Denmark

Pregnant women are infected by *Plasmodium falciparum* presenting with unique adhesion properties that allow them to specifically bind Chondroitin sulphate A proteoglycan in the placenta. Acquisition of protective immunity over successive pregnancies is attributed to antibodies that block the adhesion of infected erythrocytes to CSA. In this study we analysed plasma samples of women of various parity enrolled from their first trimester of pregnancy till delivery in the ongoing STOPPAM project in Benin. The plasma level of anti-VSA antibodies and adhesion inhibitory activity were measured on two parasite lines selected for CSA binding on Bewo cells (FCR3 and HB3). Specific antibodies to var2csa were measured on recombinant proteins of the DBL5 domain and the full-length extracellular part of the VAR2CSA. The majority of primigravidae had low levels or no anti-adhesion antibodies at enrollment compared to multigravidae. Women who experienced a detected parasitemia during the follow up significantly increased their levels of anti-VSA, anti-var2csa as well as their plasma inhibitory activity. However a difference in the kinetics of antibody production was observed between primigravidae and multigravidae following an infection. Women infected with HIV displayed an antibody acquisition pattern similar to that of HIV negative primigravidae. Overall, a significant correlation was found between the plasma level of anti-VSA and anti-var2csa IgG and the plasma anti-adhesion activity. The results from this study suggest that the anti-adhesion antibodies play a significant role in the protective immunity acquired against pregnancy malaria and should be considered a priority in strategies aiming at developing a vaccine against this pathology.

275

DEVELOPMENT OF ANTIBODY RESPONSES AND RESTRICTED GLOBAL DIVERSITY OF *PLASMODIUM FALCIPARUM* ERYTHROCYTE MEMBRANE PROTEIN-1 IN MALARIA ENDEMIC REGIONS

Kiprotich Chelimo

Kenya Medical Research Institute, Kisumu, Kenya

Clinical cases due to *Plasmodium falciparum* malaria in areas of high stable transmission reduce with age partially due to acquired humoral immunity to parasite proteins exposed on the surface of infected erythrocytes. To better understand the development of immunity to *P. falciparum*, we measured antibodies among a cohort of Kenyan children and adults to surface antigens expressed by the trophozoite stages of *P. falciparum* using five *P. falciparum* parasite isolates from different geographic origins. The isolates were selected for adhesion to ICAM-1, thought to be an important receptor for endothelial adhesion. Furthermore, we quantified the importance of *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) as a target of acquired antibodies by using transgenic parasites with altered expression of PfEMP1. IgG was measured by flow cytometry. Most adults had IgG antibodies that reacted with the surface of infected erythrocytes, and all isolates were well recognized by serum antibodies. In contrast there was very low to no antibody reactivity in children aged below three years. PfEMP1 appeared to be the dominant target of antibodies among adults and children. Results suggest that there is restricted global diversity or common antigenic determinants in PfEMP-1 antigens and antibody reactivity increases with age and/or exposure. Further studies on PfEMP-1 are required to define the common epitopes for development as correlates of immunity and potential blood stage vaccines.

276

PHENOTYPE AND ACTIVATION LEVELS OF DENDRITIC CELLS (DC) AND MONOCYTES IN PREGNANCY-ASSOCIATED MALARIA DURING A FOLLOW-UP IN BENIN

Samad Ibitokou[1], Nadine Fievet[2], Bertin Vianou[1], Carine Agbowai[1], Sem Ezinmegnon[1], Mayke Oesterholt[3], Achille Massougbodji[1], Thor Theander[4], Stefania Varani[5], Marita Troye-Blomberg[6], Adrian J.F. Luty[3], Philippe Deloron[7]

[1]University of Abomey Calavi, Faculté des Sciences de la Santé, Cotonou, Benin, [2]IRD UMR216, Mother and Child Health in the Tropics, Cotonou, Benin, [3]Department of Medical Microbiology, Radboud University, Nijmegen Medical Centre, Nijmegen, The Netherlands, [4]Department of International Health, Institute of International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark, [5]Department of Laboratory Medicine, Division of Clinical Microbiology, Karolinska University Hospital, Huddinge, Sweden, [6]Department of Immunology, Wenner-Gren Institute, Stockholm University, Stockholm, Sweden, [7]Université René Descartes, Faculté de Pharmacie, Paris, France

Dendritic cells (DC) are important both in amplifying the innate immune response, and in initiating adaptive immunity and shaping the type of T helper (Th) response. Although the role of DC in immune responses to many intracellular pathogens has been delineated and research is underway to identify the mechanisms involved, relatively little is known concerning the role of DC in immunity to malaria. We evaluated the immunophenotype of antigen presenting cells (APC) in peripheral blood of pregnant Beninois women from the area of Come, southwestern Benin, where we are conducting a longitudinal prospective study of 1000 mothers. Pregnant women are enrolled ≤ 24 weeks of pregnancy and followed at each ante-natal visit until delivery. Cellular immunological assessments have been performed with samples from a subgroup of 149 women at enrolment and 106 at delivery, with or without active *Plasmodium falciparum* infection detected by a rapid diagnostic test. Immunophenotyping of APC and their level of activation (HLA-DR, CD86 expression) are being evaluated using flow cytometry. *P. falciparum* infection was associated with DC altered maturation in pregnant women, as reflected by lower frequencies of MDC and PDC and their downregulated expression of HLA-DR but not CD86, whether in early pregnancy or at delivery. DC of pregnant women with anaemia were present at low frequency during pregnancy. In conclusion, HLA class II expression on DC is fundamental for presenting antigens to T cells and inducing their activation. Therefore, impaired DC activation upon malaria infection in pregnant women may result in a deficient and delayed adaptive immune response to the parasite and/or to other pathogens. Therefore, through an inhibitory effect on DC, *P. falciparum* may impair cell mediated immunity in pregnant women leading to a reduce response against the parasite itself and possibly rendering pregnant women more susceptible to other infections.

277

INHIBITORY HUMORAL RESPONSES TO THE *PLASMODIUM FALCIPARUM* VACCINE CANDIDATE EBA-175 ARE LINKED TO ERYTHROCYTE RECEPTOR USAGE

Aida S. Badiane[1], Amy Bei[2], Ambroise Dioum Ahouidi[3], Nichole Salinas[4], Daouda Ndiaye[1], Ousmane Sarr[1], Omar Ndir[1], Niraj Tolia[4], Souleymane Mboup[1], Manoj Duraisingh[5]

[1]Cheikh Anta Diop University and Le Dantec Hospital, Dakar, Senegal, [2]Harvard School of Public Healt, Boston, MA, United States, [3]Cheikh Anta Diop University, Dakar, Senegal, [4]Washington University School of Medicine, St. Louis, MO, United States, [5]Harvard School of Public Health, Boston, MA, United States

Plasmodium falciparum utilizes multiple ligand-receptor interactions for invasion. The invasion ligand EBA-175 is being developed as a major blood-stage vaccine candidate. It is located in the apical micronemes of merozoites and mediates parasite invasion of host erythrocytes in a sialic acid dependent manner. In this study, we seek to address the ability of naturally acquired antibodies raised against the EBA-175 RII erythrocyte binding domain to inhibit parasite invasion, in relationship to its sialic acid dependence. To address this hypothesis, we have taken two primary approaches. We have determined the presence of antibodies to the PfEBA-175 RII domain by ELISA in individuals from malaria endemic areas of Senegal with high or low transmission. We have tested the plasma of those individuals for their specific EBA-175 inhibitory potential by performing invasion assays using *P. falciparum* EBA-175 KO transgenic parasites. We have also affinity purified antibodies to the EBA-175 RII domain from pooled patient serum for the invasion inhibition of uncultured Senegalese parasite isolates in *ex vivo* assays. Our results suggest that naturally acquired anti-EBA-175 RII antibodies significantly inhibit invasion of Senegalese parasites and this inhibition is dependent on the sialic acid dependence of the parasite strain. This work has implications for vaccine design based on EBA-175 in the context of alternative invasion pathways.

278

IMPACT OF MATERNAL CYTOKINE GENE POLYMORPHISMS ON MOTHER AND FETUS BIOLOGICAL AND IMMUNOLOGICAL PARAMETERS IN THE CONTEXT OF PLACENTAL MALARIA

Adjimon Gatien Lokossou[1], Célia Dechavanne[1], Aziz Bouraïma[2], David Courtin[2], Agnès Le Port[1], Rodolphe Ladékpo[2], Julien Noukpo[2], Désiré Bonou[2], Claude Ahouangninou[2], Franck Loumedjinon[2], Messanh Daoudou[2], André Garcia[1], Florence Migot-Nabias[1]

[1]Research Institute for Development, Paris, France, [2]Research Institute for Development, Cotonou, Benin

Some single mutations in cytokines genes are related to modifications of the protein production. For cytokines involved in the regulation of the antibody production, particular gene polymorphisms influence the antibody levels. We investigated the consequences of some cytokine gene polymorphisms on the antibody levels of mothers at delivery, and on fetal immunity, in the context of *P. falciparum* placental malaria infection. We hypothesized that if some maternal cytokine gene polymorphisms lead to an increased production of maternal specific antibodies, they could help to lower the *in utero* sensitization of the fetus to plasmodial antigens, and contribute to delay the occurrence of the first malaria attack in early life. Six-hundred pairs of mothers and children were recruited in south-west Benin, where malaria is endemic. At delivery, peripheral blood was drawn from mothers, as well as corresponding cord blood. Eleven percent of mothers had a placenta infected with *P. falciparum*. From the maternal genomic DNA, 5 mutations occurring in genes coding for IL-4, IL-10 and IL-13 were genotyped by quantitative PCR. High frequencies were observed for genotypes IL-4-590 TT (61.8%), IL-4 +33 CT (50.5%), IL-10-1082 AA (52.5%), IL-10-592 AC (51.3%) and IL-13-1055 CT (51.0%). We evaluated the influence of these mutations on the ability of maternal mononuclear cells to produce the cytokines of interest, following stimulation by mitogens. Finally, we determined maternal and fetal plasmatic levels of IgM, IgG and cytophilic isotypes IgG1 and IgG3 directed against recombinant proteins from the MSP1, MSP2, MSP3, AMA1 and / or GLURP antigens, which are candidates for inclusion into a multivalent vaccine against malaria. The analysis of the relationships between i) maternal cytokine gene polymorphisms, ii) maternal cytokine and related antibody production, and iii) fetal specific antibody production, may help to understand the strength of the mother and child immunological interactions during pregnancy, depending on the presence or not of a plasmodial placental infection.

279

INFLUENCE OF IPT ON THE ACQUISITION OF ANTI-VAR2 CSA ANTIBODIES IN HYPOENDEMIC ZONE

Khadime Sylla[1], Ibrahima Diouf[2], Babacar Faye[2], Jean Louis Ndiaye[2], Roger C. Tine[2], Magatte Ndiaye[2], Oumar Gaye[2]

[1]Dakar University Cheikh Anta Diop, Dakar, Senegal, [2]Dakar University Cheikh Anta Diop, Laboratory of Parasitology, Dakar, Senegal

The susceptibility of pregnant women to malaria is mainly related to an immuno-modulation related to pregnancy, an adhesion of *Plasmodium* strains to chondroitin-A sulfate of syncytiotrophoblast and the selection of strains resulting it. In an endemic zone, studies showed that susceptibility to malaria depended on gravidity.In a hypo-endemic zone this placental antimalaral immunity is very slow in taking shape because transmission is not a continuous process and susceptibility seems to be more related to age than parity. Senegal adopted in June 2003, on the recommendations of WHO, the (IPT) through the sulfadoxine-pyrimethamine (SP) combination. However, IPT is somewhat limited because of some factors which could be related to the develoment of *P. falciparum* resistance to SP and to the low percentage of woment who take 2 SP doses. The general objective of this study is to determine the impact of TPI on the acquisition of antimalarial antibodies. 101 women were recruited between the first and the second term of pregnancy from September to December 2008. For each woman, blood sample collections were performed at inclusion and delivery as well as following each fever attack. After centrifugation, IgGs Anti-P MSP1, GLURP and Var2CSA (DBL5) were proportioned in the serums through ELISA test. Among the 101 women included in the study, 18 disappeared as they did not deliver at the hospital maternity. The anti-MSP1 and anti-GLURP antibodies were determined in 101 women at inclusion and 83 women at delivery. Specific Anti-P VAR2CSA were proportioned in 82 women both inclusion and delivery. During all the follow-up exercise, only one woman presented a thick drop positive. The IgGs anti MSP1 and anti GLUR did not undergo any significant variations between inclusion and delivery. However, a reduction in the percentage of women presenting these IgGs at delivery was observed. With regard to anti DBL5 IgGs, no significant difference between the primigravida and the multigravida was noted both at inclusion and delivery. On the other hand, a significant reduction of these IgGs was noted between inclusion and delivery. In conclusion, these results confirm that IPT reduces malaria incidence in pregnant women. The reduction in the rate of the anti CSA antibodies at delivery and the lack of significant difference between multigravida and primigravida seem to delay immunity acquisition against malaria during pregnancy.

280

THERAPEUTIC TARGETING OF NUCLEIC ACID-SENSING TOLL-LIKE RECEPTORS PREVENTS CEREBRAL MALARIA

Bernardo S. Franklin[1], Sally T. Ishizaka[2], Marc Lamphier[2], Fabian Gusovsky[2], Hans Hansen[2], Jeffrey Rose[2], Wanjun Zheng[2], Marco Antônio Ataíde[1], Rosane B. Rosane B. Oliveira[3], Douglas T. Douglas[3], Ricardo T. Gazzinelli[1]

[1]Laboratory of Immunopathology, René Rachou Institute/Oswaldo Cruz Foundation/Ministy of Health, Belo Horizonte, Brazil, [2]Eisai Research Institute, Andover, MA, United States, [3]Department of Medicine, Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, MA, United States

Toll-like receptors (TLRs) and their outputs, pro-inflammatory cytokines, f pro-inflammatory mediators. With this in mind we assessed E6446, a small moleculeantagonist for TLRs 7 and 9. Herein, we describe the protective effect and mechanism of action of E6446 on *Plasmodium berghei* ANKA induced cerebral malaria (CM). *In vitro*, E6446 inhibited the activation of human and mouse E6446 in a dose dependent manner. Furthermore, therapy with E6446 diminished the *in vivo* cytokine responses of dendritic cells to TLR9 ligands or *Plasmodium* infection and prevented severe signs of CM, such as limb paralysis, brain vascular leak and death. Therefore, we provide novel insights into how TLRs are involved in malaria pathogenesis and show that interference with nucleic acid sensing TLRs is a promising strategy to prevent deleterious pro-inflammatory responses mediating malaria severity. have been implicated in the pathogenic basis of malaria. We had previously shown that the nucleic acid sensing TLR9 is a key receptor that initiates pro-inflammatory responses during malaria leading to septic shock symptoms. We, thus, believe that interference with TLR function will, in all likelihood, render better clinical outcomes by preventing excessive release

281

ANTIBODIES TO *PLASMODIUM FALCIPARUM* BLOOD-STAGE ANTIGENS BUT NOT CIRCUMSPOROZOITE PROTEIN PERSIST IN THE ABSENCE OF MALARIA TRANSMISSION

Bartholomew O. Nyangahu[1], Gregory Park[2], Gideon Magak[3], Gregory Noland[2], Ayub Ofulla[1], Chandy John[2]

[1]Maseno University, Kisumu, Kenya, [2]University of Minnesota, Minneapolis, MN, United States, [3]Moi University, Eldoret, Kenya

As the campaign for malaria eradication widens, more areas will achieve low or absent transmission of *Plasmodium falciparum*. However, little data exists on how absence of transmission affects the immune responses to malaria. Such data are important for consideration of a population's epidemic risk after successful interventions and for assessment of potential differences in vaccine immunogenicity and efficacy. We documented possible interruption of malaria transmission in two highland areas of Kenya from 2007-2008. To characterize changes in immunity in this population, we measured antibody frequencies to eight *P. falciparum* vaccine candidate antigens, just before interruption of transmission and one year later. Testing for immunoglobulin G (IgG) antibodies was performed by multiplex cytometric bead assay (CBA) and ELISA in 1000 randomly selected individuals from the two sites (Kipsamoite, n = 457, Kapisisiywa, n = 543) in May, 2007 and July, 2008. Antigens tested included AMA-1, CSP, EBA-175, GLURP, LSA-1, MSP-1, MSP-3, and TRAP. None of the 1000 individuals had an episode of clinical malaria during this time period. For all antigens, antibody frequencies increased with age and were higher in the area of historically higher malaria transmission (Kapsisiywa). In both areas, frequencies of IgG antibodies to antigens other than CSP showed minimal decreases over the one-year period of absent transmission, but frequencies of IgG antibodies to CSP decreased significantly (CSP, Kipsamoite, 30.6% vs. 22.1%; Kapsisiywa, 38.9 % vs. 24.3 %, $P < 0.0034$, $P < 0.0001$ respectively). In conclusion, interventions that dramatically reduce or eliminate malaria transmission have differential effects on IgG antibodies to *P. falciparum* antigens. IgG antibodies to blood-stage antigens persist in the absence of malaria transmission, but antibodies to the pre-erythrocytic antigen CSP, the antigen used in the most successful malaria vaccine to date, wane rapidly.

282

THE EFFECT OF INTERMITTENT PREVENTIVE TREATMENT (IPT) DURING PREGNANCY WITH SULPHADOXINE-PYRIMETHAMINE (SP) ON *PLASMODIUM FALCIPARUM*-SPECIFIC IGG ISOTYPIC ANTIBODY LEVELS IN PAIRED MATERNAL-CORD BLOOD

Judith K. Anchang-Kimbi[1], Eric A. Achidi[2], Eva Sverremark-Ekström[3], Marita Troye-Blomberg[3]

[1]Faculty of Science, University of Buea, Buea, Cameroon, [2]Faculty of Health Sciences, University of Buea, Buea, Cameroon, [3]Department of Immunology, Wenner-Gren Institute, Stockholm University, Stockholm, Sweden

A successful regime of IPT could decrease exposure to malaria during pregnancy and antibody titres to malarial antigens could decline, leaving women more susceptible to malaria and consequently decrease transplacental transfer of immunity to malaria in their newborns. We

investigated the influence of IPTp/SP on the levels of *Plasmodium falciparum* specific IgG and its subclasses (1 - 4) in mothers and their newborn babies. IgG levels to *P. falciparum* crude blood stage antigens were determined using Enzyme-linked Immunosorbent Assay (ELISA) in 270 paired maternal-cord blood samples collected at delivery from women who attended the Mutengene medical centre, Cameroon from March-October, 2007.The use of SP/dosage were documented. All four IgG subclasses were transferred across the placenta. The mean values and hierarchy of cord/maternal concentration ratios of IgG subclasses were as follows: IgG1 (1.03) > IgG3 (0.98) > IgG4 (0.92) > IgG2 (0.81) indicating a preferential high transfer rate for IgG1 and a low for IgG2. Also, IgG1 levels were significantly higher (t = -7.223; $p < 0.001$) in cord (3.62 ± 0.38) than its corresponding maternal blood (3.53 ± 0.40).Women who took two or more SP doses (3.42 ± 0.55) had lower (t= 2.791; p = 0.006) plasma levels of *P. falciparum* specific IgG compared to those who had taken one dose (3.60 ± 0.40) during pregnancy. Similarly, neonates from women who had two or more SP doses (3.40 ± 0.56) had lower (t= 2.428; p = 0.015) plasma levels of IgG compared to those from who had taken one dose (3.56 ± 0.40) during pregnancy. In addition, IgG1 levels were significantly lower (t = 2.596; p = 0.01) in cord blood of neonates born to mothers who had taken two or more doses (3.54 ± 0.49) compared to those whose mothers had taken one dose(3.68 ± 0.35). Two or more doses of SP taken during pregnancy is associated with lower anti-malarial IgG levels in the mother and IgG l levels in newborns are particularly affected. Future studies are needed to evaluate the impact of IPTp/SP on development of maternal and infant immunity in malaria endemic areas.

283

CYTOKINE PROFILES AND HEMATOLOGICAL CHANGES ACCOMPANYING CLINICAL DISEASE IN *PLASMODIUM VIVAX* AND *P. FALCIPARUM* UNCOMPLICATED MALARIA

Rodrigo N. Rodrigues-Silva[1], Paulo R. Antas[1], Josué Costa Lima Junior[1], Bruna Fonseca[2], Fabio Storer[3], Fátima Santos[3], Arlete Baldez[4], Dalma Maria Banic[1], Joseli Oliveira-Ferreira[1]

[1]Oswaldo Cruz Institute, Rio de Janeiro, Brazil, [2]Bio-manguinhos, Rio de Janeiro, Brazil, [3]Central Laboratory, Porto Velho, Brazil, [4]National Health Foundation, Porto Velho, Brazil

The balance between pro- and anti-inflammatory cytokines may be important in malaria presentation and outcome. Over the past few decades, a literature has emerged that argues for most of the pathology seen in malaria being explained by activation of the inflammatory system, with the balance between the pro and anti-inflammatory cytokines being tipped towards the onset of systemic inflammation. However, the respective roles played by the different cytokines in humans during acute malaria episodes remain unclear. The aim of this study was to investigate the hematological changes and cytokine profiles in a group of patients infected with *Plasmodium vivax* and *P. falciparum* at the day of diagnosis before treatment (D0) and 2 weeks later (D15). As a result, a complete blood count and plasma cytokines levels were measured in patients suffering from an uncomplicated *P. falciparum* (n=24) and *P. vivax* (n=45) malaria and uninfected individuals (n=12). In our study, at the day of diagnosis patients with *P. falciparum* and *P. vivax* had thrombocytopenia, leucopenia and an increased number of band cells returning to normal levels at D15. The parasitemia was similar in *P. falciparum* (4604±4630 parasite/ul) and *P. vivax* (3020±3411) infections. The cytokines IL5, IL7, IL13, MCP-1 and GM-CSF was absent from most plasma samples at the day of diagnosis. In contrast, high levels of IL6, IL8, IL17, TNFα, IFNγ and Mip-1b were present in nearly all individuals. In *P. falciparum* plasma levels of IL12 and IL1β was elevated at D0 and IL4 at D15. Interesting IL-10 levels were high at D0 in both *P. falciparum* and P. vivax and decreased at D15. Our preliminary data shows that the cytokine profile in P. *falciparum* and P. vivax uncomplicated malaria is similar with elevated plasma levels of both pro and antinflamatory cytokines. Analysis on the levels of nitric oxide and inflammatory markers such as acute phase proteins and their correlation with the cytokine profile are in progress.

284

GENETIC MARKERS AND RISK OF MALARIA INFECTIONS: GENETIC-EPIDEMIOLOGY STUDY IN A LOW MALARIA ENDEMIC AREA OF SRI LANKA

Rajika L. Dewasurendra[1], Prapat Suriyaphol[2], Deepika Fernando[1], Patcharaporn Sereejaitham[2], **Nadira D. Karunaweera**[1]

[1]University of Colombo, Colombo 8, Sri Lanka, [2]Mahidol University, Bangkok, Thailand

Malaria transmission is seasonal and unstable in the dry zone of Sri Lanka and the levels have steadily declined over the past 15 years. This is a follow up of an immuno-epidemiological study conducted in 8 villages in the district of Moneragala, a known endemic area of Sri Lanka with transmission of predominantly P.vivax (>80%) and P.*falciparum*. The original study was a cohort study with active case detection of 1,951 individuals during 1992/93. All clinical data including malaria attacks and parasite densities were recorded during that period. In year 2006, 1133 of these individuals were traced, blood collected and past history of malaria during last 15 years recorded. DNA extracted from whole blood and SNPs in selected genes related to humoral immune-response investigated. Serum separated for serological investigations and titers of antibodies against; AMA1, MSP1, MSP2, NANP, Pv_AMA1 and Pv_MSP1 together with total IgE determined. SNP data analyzed in relation to past history of malaria attacks and serum antibody levels. A total of 169 SNPs were typed in 1008 study subjects. After sample and genotype quality control, 118 SNPs in all subjects were analyzed. Allele frequencies in 2 SNPs in 2 genes found to be significantly different between those who have experienced repeated malaria attacks and those with apparent protection ($p<0.05$; Chi-square test). When antibody levels were classified into low-high binary trait, significant association was found in 4 SNPs for AMA1; 2 for MSP1 (none for MSP2); 8 for NANP; 3 for Pv-AMA1; 7 for Pv-MSP1; and 9 for IgE. None of the SNPs had any significant association with all tested antibodies. Preliminary evidence is in favour of a genetic basis for susceptibility to or protection against malaria infection in this population, which may or may not have links with the generation and/or maintenance of anti-malarial antibodies, the levels of which appear to be maintained in spite of low malaria transmission levels.

285

CELLULAR IMMUNOLOGICAL RESPONSES IN PREGNANCY-ASSOCIATED MALARIA

Mayke Oesterholt[1], Stéphanie Boström[2], Nadine Fievet[3], Marita Troye-Blomberg[2], Ali Salanti[4], Christentze Schmiegelow[4], Daniel Minja[5], John Lusingu[5], Achille Massougbodji[3], Adrian J. Luty[1]

[1]Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, [2]Wenner-Gren Institute, Stockholm University, Stockholm, Sweden, [3]Development Research Institute, Cotonou, Benin, [4]Centre for Medical Parasitology at the Institute of International Health, Immunology, and Microbiology, University of Copenhagen, Copenhagen, Denmark, [5]National Institute for Medical Research, Tanga Medical Research Centre, Tanga, United Republic of Tanzania

Pregnancy-associated malaria (PAM) due to *Plasmodium falciparum* is detrimental to both mother and child. Ongoing anti-PAM vaccine development focuses on the induction of antibodies targeting VAR2CSA, a parasite-derived protein expressed on the surface of infected erythrocytes that sequester in the placenta, since naturally-acquired anti-VAR2CSA IgG titres increase in a gender-specific and parity-related way, and PAM shows a concomitant parity-related decrease in incidence. These findings imply a protective function for antibody responses. In contrast, a defined role for VAR2CSA-specific T cell responses is unclear and remains largely unexplored. We are conducting a longitudinal, prospective study of 1000 pregnant mothers in Korogwe, north-eastern Tanzania. For a subgroup of mothers with and without evidence of *P. falciparum* infection, *ex vivo* frequencies of the T cell, B cell, monocyte, regulatory T cell and dendritic cell populations are being measured at inclusion and at delivery.

Cytokine activity of isolated peripheral blood mononuclear cells is assessed following short-term stimulation *in vitro* with either VAR2CSA-specific reagents or *P. falciparum*-infected red blood cells. Cord blood mononuclear cells isolated at delivery are assessed in a similar way in order to determine the extent of sensitization to *P. falciparum* antigens *in utero*. For comparative purposes, *P. falciparum*-infected women are matched to uninfected women based on age, gestational age and gravidity. We have completed assays on samples collected at inclusion and data analysis is ongoing. The collection of samples at delivery is still ongoing. The focus of the results presented will be on the *ex vivo* phenotyping of T regulatory cells and the *in vitro* T cell responses to VAR2CSA-specific reagents. We will compare and contrast our cellular immunological findings with those from an identical study that is being conducted in parallel in southern Benin, in an area where malaria transmission is both more intense and perennial rather than seasonal.

286

EFFECT OF ARTEMETHER ON THE EXPRESSION OF GENES INVOLVED IN THE MOSQUITO IMMUNE RESPONSE TO *PLASMODIUM* INFECTION

Yanyan Wang, Jian Zhang, Fusheng Huang

Third Military Medical University, Chongqing, China

Malaria is a vector-borne disease that still remains, to our days, as one of the major causes of mortality worldwide. At present, there is still no effective prevention and control measure, so it is necessary to develop new strategies for controlling Malaria transmission.We are trying to find out the effective drug, which can not only kill the malaria parasite in the human host, but also block the development of malaria parasite in the mosquito. Recently, Anti-malarial drugs have played a key role in controlling the spread of malaria. Although the effect of anti-malarial drugs on mosquito immunity has been recently improved, nothing is known about the impact of artemether, one of artemisinin derivatives, on mosquito immunity. Artemether which is the recommended first-line treatment is a potent and quick acting anti-malarial, used for treating chloroquine resistant *falciparum* malaria, including cerebral malaria. In order to characterize the influence of artemether on the mosquito immune system, we have analyzed the effect of artemether on *Anopheles stephensi* six important immune-related genes expression using semiquantitative PCR, and the activity of PO enzyme in uninfected and *Plasmodium yoelii* infected *Anopheles stephensi*. we have demonstrated for the first time that fed on *Anopheles stephensi* 632ng/ml artemether, according to pharmacokinetic study, we chose the highest the plasma concentration of artemether in human bodies. Our results showed artemether significantly down-regulated the expression of serine protease1 (AsSP1), serine protease2 (AsSP2), serine protease inhibitor(AsSNP), nitric-oxide synthase(AsNOS), thioester-containing protein 1(AsTEP1), Prophenoloxidase(AsPPO),and PO enzyme activity, which are necessary for interrupting *Plasmodium* development during infection *Anopheles stephensi*, in different degrees. We found that artemether could increase *Plasmodium* oocyst counts 1-3 times to untreated *Anopheles stephensi*. These results suggest that artemether might act on *Anopheles* serine proteases cascade and synthesis of nitric oxide at the transcriptional level. Understanding the mechanism artemether of action in mosquito vector and vertebrate hosts will reveal biological details that can be fruitful for novel malaria control strategies such as those based in transmission-blocking vaccines.

287

EVALUATION OF MALARIA PREVENTION STRATEGY DURING PREGNANCY IN NDOLA, ZAMBIA

Seraphin M. Mulamba

Maongo Medical Services, Ndola, Zambia

Malaria in pregnancy is associated with many negative outcomes for the pregnant woman, fetus and neonate. Intermittent Preventive Treatment during pregnancy (IPTp) using 3 doses of Sulfadoxine-Pyrimethamine (SP), Insecticide Treated mosquito Nets (ITN) and Indoor Residual Spraying (IRS) are the main strategies used to prevent malaria. The aim of this study was to evaluate the effectiveness of these strategies on the reduction of malaria prevalence in pregnant women, five years after their implementation in Ndola, Zambia and to make recommendations on how prevention can be improved. We had ethical approval from Tropical Disease Research Centre and Stellenbosch University's Human Research Ethics Committee. A questionnaire on socio-demographic information, history of malaria during current pregnancy and malaria prevention strategies used, was administered to 450 consecutive patients admitted in the labour ward of 3 local clinics. Information was collected from the antenatal cards concerning the last menstrual period, date of taking each dose of SP, gravidity, and HIV status. A blood slide to detect *Plasmodium* was collected from each woman in labour ward. 2.4% of participants had a positive blood slide at term and 15.8% reported malaria during pregnancy. All the participants took at least one dose of SP, 87.6% compled the stipulated three doses. The mean gestational age for delivery of each dose was 22.1 (SD 4.6), 29.1 (SD 4.4) and 34.4 (SD 3.9) weeks for the first, 2nd and 3rd dose respectively. 79.5% had an ITN, but only 74.1% used it regularly. Only 23.4% used commercial insecticide. In conclusion, the measured malaria prevalence was remarkably low although, the self-reported malaria rate was still high. The national target for IPTp access was exceeded, but the timing of delivery of each dose of SP needs improvement and so is the utilization rate of ITN with more sensitization of health workers and the community.The national policy on use of quinine in pregnancy may need revision with view of using it throughout pregnancy due to current SP resistance level. IRS had been complted in all the 3 clinics catchment areas.

288

IMPLEMENTING A CAMPAIGN TO DISTRIBUTE NINE MILLION FREE LLINS TO CHILDREN UNDER FIVE YEARS IN TANZANIA

Kimberly Bonner[1], Alex Mwita[1], Peter McElroy[2], Rose Nathan[3], Naomi Kaspar[4], Susan Omari[5], Ally Mnzava[5], Nick Brown[5]

[1]Tanzania National Malaria Control Program, Dar es Salaam, United Republic of Tanzania, [2]President's Malaria Initiative/Centers for Disease Control and Prevention, Dar es Salaam, United Republic of Tanzania, [3]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [4]President's Malaria Initiative, Washington, DC, United States, [5]Swiss Tropical and Public Health Institute, Basel, Switzerland

Tanzania launched a national voucher program in 2004 to provide pregnant women and infants with subsidized insecticide-treated nets (ITNs). Three years later, 24.8% of Tanzanian children <5 years of age were sleeping under an ITN (only 12.9% of the lowest wealth quintile). In 2008, the Ministry of Health and Social Welfare (MoHSW) initiated a national campaign to rapidly and equitably deliver a free long-lasting insecticidal net (LLIN) to every child <5 years of age in Tanzania. The ITN Cell, a Swiss-funded unit within the MoHSW's National Malaria Control Program (NMCP), coordinated the campaign. Government contractors trained and facilitated local government officials to supervise village-level volunteers to conduct a house-to-house registration of all children under 5 years. The registration formed the basis for the LLIN factory order and delivery to village level. Caregivers brought their registration coupons to LLIN issuing posts during a 3-day period. Five district-representative rapid household surveys (two-stage cluster sampling) assessed household ownership of an ITN and ITN use among children <5 approximately one month following a hang-up campaign. Nine donors contributed to the national campaign, purchasing 9.2 million Olyset LLINs (4x6x7 ft) at a cost of $7.51/LLIN, including delivery and all campaign-associated activities. The campaign started March '09 and ended May '10. Household (n=1,483) surveys found ITN ownership of at least one ITN ranged from 61-82%. Overall, use among children <5 was 48.0% and 62.2% in the first and second zones, respectively. ITN use generally increased across all wealth quintiles, but regional variation was detected. Despite providing free LLINs to all children <5 years of age and substantially increasing household ownership,

use did not rise as high as anticipated. The campaign addressed issues of equity, but no across all regions. Additional strategies will be needed to address the gap between ITN ownership and use.

289

A SIMPLE COLORIMETRIC TEST FOR THE RAPID DETECTION OF TYPE 2-PYRETHROIDS ON BED NETS AND ON SPRAYED WALLS

Harparkash Kaur[1], Teunis A. Eggelte[2]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Academic Medical Center, Division of Infectious Diseases, Amsterdam, The Netherlands

Insecticide treated nets and indoor residual spraying of insecticides (IRS) are used as the major modes of intervention in the fight against malaria. Measuring the actual amount of deposits of insecticides on nets and on walls is essential for evaluation of quality control of the applied intervention as per instruction. Currently such information can only be provided by costly, chromatography techniques or through technically demanding bioassays, both requiring sophisticated laboratory facilities. We have developed a rapid, field friendly / cost effective colorimetric test that can be carried out by individuals without specialised scientific training to estimate the amount of type 2 pyrethroids on the bed nets as well as to check for the compliance of IRS, as reported previously. These tests rely on the detection of cyanide using three inexpensive reagents. The tests are equallly sensitive for deltamethrin, α-cypermethrin and λ-cyhalothrin. Various types of the tests can be developed depending on choice of reagents and assay format e.g. microtitre plate, test tube, dipstick. Our simple test is performed in situ and leads to the formation of an orange-red colour whose depth will indicate semi quantitatively the amount of type 2 pyrethroid on the bed net and has been validated by measuring the amount the extracted insecticide from parts of bed nets with HPLC, as reported previously. No interference to the formation of this colour has been found from soaps, possible degradation products of deltamethrin, insecticide binders, non-fast colour bleaching off the nets or charcoal. Prototype KITs of our test have recently undergone field evaluation in Rwanda and Tanzania.The final KIT will be widely available in the near future.

290

PERCEPTIONS ON THE USE OF INSECTICIDE TREATED NETS IN PARTS OF THE IMO RIVER BASIN OF NIGERIA: IMPLICATIONS FOR PREVENTING MALARIA IN PREGNANCY

Uchechukwu M. Chukwuocha, Ikechukwu N. Dozie, Celestine O. Onwuliri

Federal University of Technology, Owerri, Nigeria

This study aimed at assessing perceptions on use of Insecticide Treated Nets(ITNs) in parts of the Imo River Basin,Nigeria and its implications in preventing malaria in pregnancy. Data was collected using focus group discussions, key informant interviews and structured questionnaires. Results showed high awareness on the benefits of ITNs. Factors affecting use of ITNs included its high cost, perceptions of chemicals used to treat them as having dangerous effects on pregnancy, low utilization of antenatal care, husband's lack of interest in malaria prevention and perceptions that adolescent girls are at low risk of getting malaria. The implications of these findings include demystifying the negative perceptions on the chemicals used for net treatment and subsidizing the cost of ITNs to increase access. These findings provide important lessons for malaria programmes that aim at increasing access to ITNs by pregnant women in developing countries.

291

EVALUATION OF SENEGAL'S FIRST NATIONWIDE CAMPAIGN TO DISTRIBUTE LONG-LASTING INSECTICIDE-TREATED NETS (LLINS)

Julie I. Thwing[1], David Townes[1], Salif Ndiaye[2], Robert Perry[1], Mame Birame Diouf[3]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Centre de researche et developpement humaine, Dakar, Senegal, [3]National Malaria Control Program Senegal, Dakar, Senegal

In 2009, the first national LLIN distribution campaign in Senegal resulted in the distribution of 2.3 million LLINs in two phases. Door-to-door teams visited all households to administer vitamin A and mebendazole, and to give a coupon to children <5 to later redeem for an LLIN. We conducted a nationwide two-stage cluster survey, with clusters selected within regions by probability proportional to size sampling, followed by GPS-assisted mapping, simple random selection of households in each cluster, and administration of a questionnaire on PDA. The questionnaire followed the Malaria Indicator Survey format, with rosters of household members and bednets, and questions on campaign participation. We surveyed 3,302 households representing 33,222 people. At least one insecticide-treated net (ITN) was present in 82% of all households, 89% of households with a child < 5 years and 57% of households without a child < 5 years. Just over half (53%) of ITNs had been received during the campaign. In 60% of households at least one ITN was hanging the previous night. Considering possible indicators of universal coverage, 40% of households had at least one ITN per two people, 22% had at least one ITN per sleeping space and 34% of the general population slept under an ITN the night before the survey. In addition, 45% of children < 5 years, and 49% of pregnant women had slept under an ITN. Most (92%) of guardians of eligible children had heard about the campaign, 34% from a health agent, 26% from a neighbor and 22% by radio. Campaign coverage was 93% for mebendazole, 95% for vitamin A, and 83% for LLINs. Almost all (91%) LLINs received during the campaign remained in the household; of those not remaining 74% had been given away and none were reported sold. The nationwide integrated LLIN distribution campaign successfully reached its target population. It allowed household ITN ownership to surpass the RBM target of 80% set for 2010 and contributed substantially to universal coverage, though work remains to reach Senegal's goal of 80% utilization in the general population.

292

MODELING AND SIMULATION TO EXPLORE THE FACTORS INFLUENCING THE IMPACT OF TREATMENT OF *PLASMODIUM FALCIPARUM* CARRIERS WITH ARTEMETHER-LUMEFANTRINE ON DISEASE TRANSMISSION

Steven E. Kern[1], Olinto Linares[1], Alfred B. Tiono[2], Michael Makanga[3], Adama Dodji Gbadoe[4], Issaka Sagara[5], Bernhards Ogutu[6], David Ubben[7], Anne Claire Marrast[8], Marc Cousin[8], Fiyinfolu Oladiran[9], Oliver Sander[8], Zulfiqarali Premji[10]

[1]University of Utah, Salt Lake City, UT, United States, [2]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [3]European and Developing Countries Clinical Trials Partnership, Cape Town, South Africa, [4]University of Lome, Lome, Togo, [5]Malaria Research and Training Center, University of Bamako, Bamako, Mali, [6]Centre for Clinical Research-Kenya Medical Research Institute and Malaria Clinical Trials Alliance – INDEPTH Network, Nairobi, Kenya, [7]Medicines for Malaria Venture, Geneva, Switzerland, [8]Novartis Pharma, Basel, Switzerland, [9]Novartis Pharma, Kempton Park, South Africa, [10]Muhimbili University of Health and Allied Sciences, Dar-es-Salaam, United Republic of Tanzania

Computer modeling of malaria is important for assessing key factors that may impact the effectiveness of a proposed intervention strategy. Though the range of model complexity can be quite wide, relatively simple models that can run on laptop computers are informative and provide insights that

were not intuitively apparent. This is highlighted for the evaluation of a study to assess the impact of detecting and treating asymptomatic carriers (AC) of *Plasmodium falciparum* with artemisinin combination therapies (ACT) through scheduled community screening campaigns (CSC). A deterministic model of parasite vector and host populations dynamics developed by Okell et al (2008) in order to explore the impact of ACTs on malaria prevalence, was coded in Matlab (Newton, USA). The model was modified to represent different settings of malaria transmission (intensity and seasonality) and use of AL as treatment of AC and of clinical malaria episodes. Simulations of CSC were assessed for number and interval that showed the greatest impact on malaria reduction in these different settings. Conditions that significantly extend any effect of this intervention were assessed. The transmission intensity in the simulated region was the most important factor affecting reduction in malaria incidence after the intervention. The timing of CSCs and the interval between them were also important criteria. Short intervals between CSCs allowed the capture of cases that would have been missed earlier due to disease latency. If transmission intensity is low and markedly seasonal, a single round of intervention can show persistent effects for multiple years, which gradually taper off. Perennial transmission would not allow a sustained effect. The simulation results identified factors that have the greatest impact on study results in a study of treating asymptomatic carriers. The timing of CSCs relative to the pattern of malaria transmission is important for maximizing the intervention impact. The intervention will have immediate effect in regions with marked seasonality and moderate transmission intensity.

293

SYSTEMATIC SCREENING FOR AND TREATMENT OF ASYMPTOMATIC CARRIERS OF *PLASMODIUM FALCIPARUM* MALARIA WITH ARTEMETHER-LUMEFANTRINE (AL) IN A COMMUNITY SETTING TO REDUCE DISEASE TRANSMISSION: A CLUSTER RANDOMIZED, SINGLE-CENTER, CONTROLLED, 12-MONTH PROSPECTIVE STUDY IN AFRICA

Bernhards Ogutu[1], Alfred B. Tiono[2], Michael Makanga[3], Adama Dodji Gbadoé[4], Zulfiqarali Premji[5], Issaka Sagara[6], David Ubben[7], Marc Cousin[8], Verena Walter[8], Fiyinfolu Oladiran[9], Anne Claire Marrast[8]

[1]Centre for Clinical Research-Kenya Medical Research Institute and Malaria Clinical Trials Alliance – INDEPTH Network, Nairobi, Kenya, [2]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [3]European and Developing Countries Clinical Trials Partnership, Cape Town, South Africa, [4]University of Lome, Lome, Togo, [5]Muhimbili University of Health and Allied Sciences, Dar-es-Salaam, United Republic of Tanzania, [6]Malaria Research and Training Center, University of Bamako, Bamako, Mali, [7]Medicines for Malaria Venture, Geneva, Switzerland, [8]Novartis Pharma AG, Basel, Switzerland, [9]Novartis Pharma, Kempton Park, South Africa

Treatment of asymptomatic carriers (AC) of *Plasmodium falciparum* with artemisinin combination therapies should reduce the parasite reservoir and impact transmission. A 12-month follow-up, open label, controlled, prospective, cluster randomized study was designed to evaluate the effect of such an intervention at community level on the malaria pattern in a Sahelian country with a 6 month transmission season. The study population will consist of all consenting inhabitants of randomized clusters. A total of 18 clusters (ca 8000 subjects) will be randomized (1:1) to an intervention and a control arm. Community screening campaigns (CSCs) will be conducted: Systematic screening of the study population by RDT and microscopy (MC), followed by AL treatment (intervention) or screening of a population subset by MC alone (delayed reading), without treatment (control). Three CSCs will be performed at around bi-monthly intervals before the malaria transmission season, with a final CSC at study end (no treatment). Universal bed-net coverage will be provided. RDT confirmed malaria episodes occurring at any time during the study (in either arm) will be treated with AL (or alternative treatment). Primary objectives are to assess (1) the number of confirmed malaria episodes per person-year in pediatrics (i.e. <5 years of age) over 12 months and in intervention vs. control arm (2) the direct benefit in ACs by measuring their hemoglobin level change from baseline to D 28 in treated (intervention) vs. not-treated (control) AC diagnosed at first CSC. Secondary objectives: assessment of incidence of confirmed malaria episodes in the whole population and in individuals diagnosed as AC; mortality and hospitalization (all-cause and for malaria); severe malaria, proportion of asymptomatic carriers and gametocyte carriers (confirmed by MC) over time, adverse events, serious adverse events. If the strategy is proved effective, it may be considered by public health policymakers to make a significant contribution to the multifaceted approaches of surveillance strategies being implemented by malaria control programs across Africa.

294

THE EFFECT OF ARTEMETHER-LUMEFANTRINE ON GAMETOCYTE CARRIAGE IN *PLASMODIUM FALCIPARUM* MALARIA

Zulfiqarali Premji[1], Quique Bassat[2], Abdoulaye A. Djimde[3], Catherine O. Falade[4], Raquel González[2], Marc Cousin[5], Philip Hunt[6], Fiyinfolu Oladiran[7], Verena Walter[5], Bernhards Ogutu[8]

[1]Muhimbili University of Health and Allied Sciences, Dar- es- Salaam, United Republic of Tanzania, [2]Barcelona Centre for International Health Research (CRESIB), Hospital Clínic, Universitat de Barcelona, Barcelona, Spain, [3]Malaria Research and Training Center, University of Bamako, Bamako, Mali, [4]Department of Pharmacology & Therapeutics, University of Ibadan, Ibadan, Nigeria, [5]Novartis Pharma AG, Basel, Switzerland, [6]Novartis Pharmaceuticals UK Limited, Horsham, United Kingdom, [7]Novartis Pharma, Kempton Park, South Africa, [8]Centre for Clinical Research-Kenya Medical Research Institute, & Malaria Clinical Trials Alliance – INDEPTH Network, Nairobi, Kenya

Plasmodium falciparum gametocytes are not affected by most antimalarials, except the amino-8-quinolines. The artemisinin-based combination therapies (ACTs) have been shown to reduce gametocyte carriage and transmission in areas of low endemicity. The effect of artemether-lumefantrine (AL) as per current dosing recommendation on gametocyte carriage was analysed in data pooled from 7 studies (2 in Africa, 3 in South-Asia, 1 in South America and 1 in non-immune travellers from Europe and Colombia). In all studies there was a marked reduction of the gametocyte carriage in patients treated with AL. This can be explained by a direct gametocytocidal effect of artemether and its metabolite as well as the rapid killing of asexual stages of *P. falciparum*. We will present an analysis of published data, comparing the effect on gametocyte carriage of AL with other ACTs such as dihydroartemisinin-piperaquine, artesunate-amodiaquine and artesunate mefloquine. We will also describe the potential factors (age, gender, baseline parasite count, area, anaemia etc) associated with gametocyte carriage following treatment with these ACTs.

295

MALARIA VECTOR CONTROL USING INDOOR RESIDUAL SPRAYING WITH DDT IN ARUSHA REGION, TANZANIA: A COMPARISON OF COMMUNITY AND GOVERNMENTAL VIEWS ON THE PERCEIVED BARRIERS PREVENTING A HIGH LEVEL OF COMMUNITY UPTAKE AND WIDER IMPLEMENTATION

James A. Cameron

Nuffield Centre for International Health and Development, Leeds, United Kingdom

Malaria causes over a million deaths worldwide annually. The World Health Organisation (WHO) estimates that 91% of these deaths occur in Africa. In Tanzania, malaria accounts for 30% of the national diseases burden posing a significant impediment to social and economic development. Indoor residual spraying (IRS) with dichloro-diphenyl-trichloroethane (DDT) was successfully used worldwide to reduce malaria transmission from

1940-70. Perceived health worries about DDT led to its decline in use. The Government of Tanzania banned DDT (1991) and severe restrictions were placed on DDT by the Stockholm Convention on Persistent Organic Pollutants (2001). In 2006, the WHO realised the potential of IRS with DDT to combat malaria and endorsed/actively promoted its use. Despite WHO recommendations, the Government of Tanzania has failed to reintroduce IRS with DDT, even though advocated in the National Guidelines for Integrated Malaria Vector Control (NGIMVC) (2008). The aims and objectives of this study were to assess the public's current knowledge of malaria, the community and governmental perceived barriers to the reintroduction of IRS with DDT, and community ideas of how to reintroduce IRS with DDT to Arusha Region. Qualitative research, including 16 interviews with community members, 1 focus group discussion and 5 interviews with government officials was undertaken. Data was analysed using thematic analysis. Community members had good knowledge about malaria transmission and vector control. All interviewees claimed that IRS was not used but believed the government should reintroduce IRS with DDT with adequate education and precautions. Several government officials claimed IRS was being performed but that before DDT could be used it needed to be approved by the Ministry of Health, despite the strong advocacy of DDT use in the NGIMVC. With the use of IRS with DDT in conjunction with other techniques (insecticide treated nets, public education) provided through a Malaria Control Unit, Tanzania could reduce the burden of malaria.

296

TRACKING WEEKLY NET USE IN KONGWA, TANZANIA

Hannah Koenker[1], Marc Boulay[1], Anna McCartney-Melstad[1], Beatriz Munoz[2], Sheila West[2], Matthew Lynch[1]

[1]Johns Hopkins University Center for Communication Programs, Baltimore, MD, United States, [2]Dana Center for Preventive Ophthalmology, The Wilmer Institute, The Johns Hopkins University, Baltimore, MD, United States

The use of long-lasting insecticidal nets (LLINs) is one of the principal interventions to prevent malaria in young children. Prioritizing young children for net use is important to achieve mortality reductions, particularly during transmission seasons. The aim of this study was to measure patterns of net use within households before, during and after the rainy season in a rural area of Tanzania. Data collection was carried out from January to July 2009 as part of the PRET+ antibiotic study, a randomized, community-based trial to determine the effect of a single mass administration of Azithromycin on community prevalence of malaria, diarrheal diseases, and other diseases, including longitudinal surveillance of 1040 households on a weekly basis. Households were asked to list all children 6 years of age or younger and their mothers who had slept under a net the previous night. Weekly data was compiled for each member of the household and analyzed by age and for correlation with the rainy season, which lasted from mid-January through April. In July, an exit survey was conducted with each household, in which behavioral questions and more detailed questions about net shape and size were asked. Net ownership among households was 51.5%, and among these, net use was very high. Reported use of nets rose quickly as the rainy season began and remained high through the end of the study period in July. Younger children (0-2 years) were prioritized, reaching a steady rate of 93% use throughout the study period. Children 3 and 4 years old had use rates in the 80% range, while 5 and 6 year olds reached only 50-60% use. Net use was not affected by the net's shape, by the number of nets in the household, or the presence of holes in the net. Ninety-three percent of net-owning households reported noticing fewer fevers among their children since they obtained a net, and 94.5% said they used nets in order to prevent malaria. In this area of Tanzania, net use is very high among net-owning households, especially during the rainy season and continuing through the post-rainy season high transmission period. The youngest children are prioritized for sleeping under the net.

297

DO INSECTICIDE TREATED NETS PROTECT AGAINST MALARIA INFECTION IF THEY HAVE HOLES?

Immo Kleinschmidt[1], Cindy Aragon[2], Andrea Rehman[1], Abrahan Mathias[2], Gloria Nseng[3], Michel Slotman[4], Michael Reddy[5], Christopher Schwabe[2]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]Medical Care Development International, Silver Spring, MD, United States, [3]Ministry of Health and Social Welfare, Malabo, Equatorial Guinea, [4]Texas A&M University, College Station, TX, United States, [5]Yale University, New Haven, CT, United States

Pyrethroid insecticide treated nets (ITN) are one of the most effective and widely used means of malaria prevention. In areas where *Anopheles* mosquitoes are no longer susceptible to pyrethroid insecticide the effectiveness of ITNs may be compromised. Mass distribution of ITNs was carried out in Equatorial Guinea in 2007 as part of comprehensive malaria control activities. High frequencies of the resistance associated *kdr* gene in *An.gambiae* populations have been observed in both the continental and island regions of Equatorial Guinea. The ownership, use and condition of ITNs, and the prevalence of infection with malarial parasites in children were monitored through annual malaria indicator surveys. The condition of nets was classified with respect to whether they were long lasting, treated or untreated, and whether they were intact, with small holes or with large holes. Infection in children was analysed in relation to whether the child slept under a net and the condition of the net. Results show that prevalence of infection is associated with net condition, with children who slept under treated nets with holes having a higher risk of infection than those who slept under treated nets that were intact. If confirmed, this finding may be an indication of the epidemiological impact of insecticide resistance on the effectiveness of pyrethroid based vector control.

298

PLACENTAL MALARIA IN PREGNANT WOMEN USING ITN/ LLIN AND IPT AS CONTROL MEASURES IN THREE SELECTED TOWNS OF SOUTHEAST NIGERIA

Florence Nduka[1], Eugene Nwosu[1], Ndukwe Ukoha[2]

[1]Abia State University, Uturu, Nigeria, [2]John Snow Incoporated, Abuja, Nigeria

In recent years ITN/LLIN and IPT have been considered appropriate measures to help reduce or prevent Malaria infections in pregnant women. This study was carried out on 844 pregnant women in Afikpo, Okigwe and Umuahia towns of Southeast Nigeria to evaluate the role of ITN/ LLIN and IPT in Malaria control. The Placentas of consenting women were obtained post delivery (following Ethical clearance by relevant authorities) and histological sections were prepared, stained and observed under the microscope for *Plasmodium* parasites. Of the 844 women examined, 225 (26.7%) used ITN/LLIN, 276 (32.7%) used IPT while 343 (40.6%) used other measures. The ITN/LLIN group had 36.9% infection with 83 of 225 infected. The IPT group had 39.1% infection with 108 of 276 infected while those who used other measures had 216 0f 343 (63%) infected. The difference between the ITN/IPT group and the other measures group was statistically significant ($P<0.05$). There was also variations between the towns with Okigwe having the lowest infection of 27.3% among those using ITN/LLIN and IPT while Umuahia had the highest, 48.3% with ITN/ LLIN and 70.8% with IPT. The significance of these results was discussed in relation to Malaria in pregnancy.

299

EFFECT OF INCENTIVES ON INSECTICIDE-TREATED BED NET USE IN SUB-SAHARAN AFRICA: A CLUSTER RANDOMIZED TRIAL IN MADAGASCAR

Paul J. Krezanoski

Massachusetts General Hospital, Boston, MA, United States

Insecticide-treated bed nets (ITNs) have been shown to reduce morbidity and mortality due to malaria in sub-Saharan Africa. Traditional ITN distribution campaigns have focused on education as a means of driving demand for ITNs, but behavioral incentives for ITN use could be more efficient. To date, behavioral incentives have been studied mostly in developed countries, and no study has yet looked at the effect of incentives on the use of ITNs. Reported here are the results of a cluster randomized controlled trial testing household-level incentives for ITN use following a free ITN distribution campaign in Madagascar. The study took place from July 2007 until February 2008. Twenty-one villages were randomized to either intervention or control clusters. Households in both clusters received a coupon redeemable for one ITN. After one month, intervention households received a bonus for ITN use, determined by visual confirmation of a mounted ITN. Data were collected at baseline, one month and six months. Both unadjusted and adjusted results, using cluster specific methods, are presented. At baseline, 8.5% of households owned an ITN and 6% were observed to have a net mounted over a bed in the household. At one month, there were no differences in ownership between the intervention and control groups (99.5% vs. 99.4%), but net use was substantially higher in the intervention group (99% vs. 78%), with an adjusted risk ratio of 1.24 (95% CI: 1.10 to 1.40; $p<0.001$). After six months, net ownership had decreased in the intervention compared to the control group (96.7% vs. 99.7%), with an adjusted risk ratio of 0.97 ($p<0.01$). There was no difference between the groups in terms of ITN use at six months; however, intervention households were more likely to use a net that they owned (96% vs. 90%; $p<0.001$). In conclusion, household-level incentives have the potential to significantly increase the use of ITNs in households in the short-term, but, over time, the use of ITNs is similar to households that did not receive incentives. Using incentives to target vulnerable populations may be even more cost-effective. Providing incentives for behavior change is a promising tool that can complement traditional ITN distribution programs and improve the effectiveness of ITN programs in protecting vulnerable populations in the short-term. Further study of the cost-effectiveness of these incentives and their longer term effects is warranted.

300

IDENTIFICATION OF NOVEL COLOR VARIANT IN THE *ANOPHELES ARABIENSIS* PATTON (DIPTERA: CULICIDAE)

Mulenga Musapa[1], Sungano Mharakurwa[1], Douglas E. Norris[2]

[1]Malaria Institute at Macha, Choma, Zambia, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Sufficient malaria vector control has proved difficult over the past years. The traditional first line of attack on malaria, killing the mosquitoes, initially proved effective, but was soon hampered by development of insecticide resistance. To circumvent this, a new genetic strategy is being developed which involves vector replacement with genetically modified Vectors (GMV) incapable of supporting development of malaria parasites. Transgenic technologies make use of markers for genetic mapping and manipulation. We have isolated color variants in *Anopheles* arabiensis Patton. The aberration is apparently sex-limited to females. To determine inheritance pattern, we attempted to purify a stock homozygous for black spot (Bs) by selection of spotted females and crossing them to sibling males but the attempt was unsuccessful. When we crossed Bs females to sibling males, serendipitously, a surge of 10% lethal mutation resulted from these crosses. Mutant larvae were characterized by black pigmentation of entire body and larval development progressed normally but died at larval-pupal ecdysis. Among the F1 progeny resulting from the crosses between Bs females and wild-type males, melanotic lethal mutants were completely absent and penetrance of 44% was observed. Our preliminary data from the above crosses superficially indicates that Bs is incomplete dominant and the aberration is lethal when homozygous. Further investigation involving genetic crosses to describe genotypes of individuals and inheritance pattern are in progress.

301

SITE-SPECIFIC INTEGRATION AND EXPRESSION OF A *PLASMODIUM FALCIPARUM* RESISTANCE TRANSGENE IN *ANOPHELES STEPHENSI*

Alison T. Isaacs[1], Nijole Jasinskiene[1], Fengwu Li[2], Joseph M. Vinetz[2], Anthony A. James[1]

[1]University of California Irvine, Irvine, CA, United States, [2]University of California San Diego, La Jolla, CA, United States

We used the *phi C31* site-specific integration system to produce transgenic *Anopheles stephensi* lines that express two effector molecules designed to target the human malaria parasite *Plasmodium falciparum*. These effector molecules are composed of an antimicrobial peptide, *An. gambiae* Cecropin A, joined to a single-chain antibody (scFv) derived from a *P. falciparum*-specific monoclonal antibody. The M4B7 immunotoxin contains an scFv designed to recognize Pfs25, a surface protein expressed by ookinetes, while the M2A10 immunotoxin contains an scFv designed to recognize circumsporozoite protein, a protein expressed on the surface of sporozoites. Previously characterized *Anopheles cis*-acting DNA regulatory elements were included in the transgene to coordinate immunotoxin production with parasite development. While the *An. gambiae* carboxypeptidase gene regulatory elements stimulate M4B7 expression in females within the first 12 hours post blood meal (hPBM), the *An. stephensi* vitellogenin gene regulatory elements direct expression of M2A10 in females ~12-24 hPBM. Through Southern blot, fluorescent hybridization *in situ*, RT-PCR, and western blot analyses, we confirmed transgene integration and expression. Having produced four transgenic lines that each contain a single copy of the M4B7/M2A10 transgene integrated into a different genomic location, we were able to observe the affect of flanking genomic DNA upon expression of these two immunotoxin genes.

302

CLONING OF THE BREAKPOINTS OF FIXED 2RO AND 2RP INVERSIONS IN THE *ANOPHELES GAMBIAE* COMPLEX

Maryam Kamali, Ai Xia, Zhijian Tu, Igor V. Sharakhov

Virginia Polytechnic Institute and State University, Blacksburg, VA, United States

An. gambiae is a major vector of malaria and it belongs to a complex of seven sibling species which are morphologically indistinguishable. However, their behavior, ecological adaptation, vectorial capacity and geographical distribution differ. Studying the phylogenetic relationships and comparative genomics among the members of the complex is crucial to understand the genetic changes of evolving traits. This can help us identify the evolutionary changes that can be related to the gain or loss of human blood choice during the evolution. It has been confirmed that the structure of breakpoints can clarify the direction of evolution. *Anopheles gambiae* and *An. merus* can be distinguished based on two overlapping inversion, 2Ro and 2Rp. In this study, the inversion breakpoints of 2Ro and 2Rp in *An. merus* and their homologous sequence in the outgroup species *An. stephensi* have been analyzed. Genes adjacent to inversion breakpoints had been identified. Four genes from the *An. gambiae* 2Ro inversion breakpoints, and four genes from *An. gambiae* 2Rp inversion breakpoints were labeled and used as probes to screen the *An. merus* phage library. The same genes were also used to screen the BAC library of the outgroup species *An. stephensi*. Positive phages and BAC clones were obtained from the proximal 2Ro and 2Rp breakpoints. Twelve phages and BAC clones have been isolated and sequenced. A phage

clone from the proximal 2Ro breakpoint was used for Fluorescent *In Situ* Hybridization (FISH) with *An. gambiae*, *An. merus* and *An. stephensi* polytene chromosomes. Our results from FISH analysis of the 2Ro breakpoint showed that the phage DNA hybridizes to both breakpoints in *An. gambiae* and to one breakpoint in *An. merus* and an outgroup species *An. stephensi.* The results demonstrated the common organization of the 2Ro breakpoint in *An. merus* and *An. stephensi*. Since the gene order is the same in the inversion breakpoint within outgroup species, we can conclude that the 2Ro inversion can be considered closest to ancestral in *An. merus* or the inversion have originated independently in *An. merus* and *An. stephensi*

303

ALLELIC GENE STRUCTURE VARIATIONS IN HUMAN MALARIA VECTOR *ANOPHELES GAMBIAE*

Jun Li

University of Minnesota, St. Paul, MN, United States

Malaria, transmitted by anopheline mosquitoes, kills more than one million people annually. *Anopheles gambiae* is the major malaria vector. Vector control is an efficient approach for malaria control. Malaria resistant or insecticide resistant mosquitoes have been observed in nature, and genetic variations underlie these phenotypes. This study focuses on allelic gene structure variations that change protein sequences, functions or regulation. By analyzing 235,971 *A. gambiae* ESTs, we found about 2,340 transcript structure variation events in 1,490 genes. About 78% of transcript structure variations were located within the coding sequence (CDS) regions, and >65% of variations at the CDS regions have the same open-reading-frame, which indicated that most transcript structure variations just insert or delete some amino acids or functional motifs without changing the whole protein structure. From the same set of ESTs, we detected 113,367 single nucleotide polymorphisms (SNPs) that were present in more than one EST. Using these multi-hit SNPs as tags, we discovered that more than 28% of transcript structure variation events were contributed by different gene alleles in *A. gambiae*. Furthermore, genome sequences from two dozen individual wild *A. gambiae* mosquitoes from Kenya confirmed that allelic gene structure variation plays a major role in transcript diversity in this important human malaria vector. The genes with allelic gene structure variations will be novel genetic markers for genome-wide direct-association studies of malaria resistance and insecticide resistance.

304

GENOME-BASED MICROSATELLITE DEVELOPMENT IN *CULEX QUINQUEFASCIATUS* WITH BROAD APPLICATION TO THE *CX. PIPIENS* COMPLEX

Paul V. Hickner[1], Becky deBruyn[1], Diane D. Lovin[1], Akio Mori[1], Robert Pinger[2], David W. Severson[1]

[1]Eck Institute for Global Health, University of Notre Dame, Notre Dame, IN, United States, [2]Department of Physiology and Health Science, Ball State University, Muncie, IN, United States

Mosquitoes in the *Culex pipiens* complex are among the most medically important vectors for human disease worldwide and include major vectors for lymphatic filariasis and West Nile virus transmission. However, detailed genetic studies in the complex are limited by the number of genetic markers available. Here we describe methods for the rapid and efficient identification and development of single locus, highly polymorphic microsatellite markers for *Cx. pipiens* complex mosquitoes via *in silico* screening of the *Cx. quinquefasciatus* genome sequence. Six laboratory colonies representing four *Cx. pipiens* and two *Cx. quinquefasciatus* populations were utilized in the preliminary assessment of 35 putative loci identified within 16 *Cx. quinquefasciatus* supercontig assemblies (CpipJ1) containing previously mapped restriction fragment length polymorphism (RFLP) genetic marker sequences. We identified and validated 12 new microsatellite markers distributed across all three linkage groups that amplify consistently in *Cx. pipiens* strains from Japan, Johannesburg, Mozambique, and North America. To increase genotyping efficiency we developed groups of 3-5 microsatellite loci each for multiplex-ready PCR. Field collections from three cities in Indiana were used to assess ten microsatellite loci for their application to natural populations. All were highly polymorphic with 7 to 24 alleles (Mean 13.4) per locus and polymorphism information content (PIC) ranging from 0.654 to 0.882 (Mean = 0.765). Results of AMOVA indicated that most of the genetic variation was within individuals (89.20%) and within populations (10.57%) while only 0.23% was among populations. Pairwise FST values were low (0.0003-0.0043) among all three cities suggesting little population structuring at distances ranging from 110 to 260 km.

305

GENE-OF-INTEREST EXPRESSION IN TRANSGENIC *AEDES AEGYPTI* AFTER TRANSFORMATION WITH A TRANSPOSABLE ELEMENT OR THE PHIC31 SITE-SPECIFIC RECOMBINATION SYSTEM

Alexander W. Franz[1], Irma Sanchez-Vargas[1], Nijole Jasinskiene[2], Mark R. Smith[1], Cynthia C. Khoo[1], Joseph Piper[1], Anthony A. James[2], Ken E. Olson[1]

[1]Colorado State University, Fort Collins, CO, United States, [2]University of California, Irvine, CA, United States

During the last few years germline transformation of mosquitoes has become more widely applied to study gene function or to express anti-pathogen effector genes. In most cases germline transformation has been achieved by using a non-autonomous transposable element (TE) such as mariner *Mos1*, *piggyBac* or *Minos* as an insertion vector for the transgene. The gene-of-interest is inserted into the plasmid DNA of the TE, which is then co-injected into the mosquito embryo along with a helper plasmid expressing the TE transposase. One major caveat when using a TE is the fact that the integration site of the transgene into the host genome is unpredictable and uncontrollable. This often leads to position effects causing poor gene-of-interest expression. The PhiC31 system has been described as an elegant alternative to avoid such position effects. The basic components of the system derived from bacterio-phage PhiC31 are a 'phage' attachment site (attP), a 'bacterium' attachment site (attB) and the integrase, which catalyzes recombination between the two sites. The attP site is inserted into the host genome via a TE. In a subsequent experiment the resulting 'docking strain' is then 'super-transformed' with a donor plasmid encoding the corresponding attB site and the gene-of-interest. We transformed *Aedes aegypti* with plasmid DNA encoding EGFP under control of the bloodmeal-inducible, midgut-specific carboxypeptidase A promoter using the *Mariner Mos1* TE or the PhiC31 system. Here we compare gene-of-interest expression patterns between TE and PhiC31 generated mosquitoes to validate the efficacy of either approach. We also describe integration patterns and integration loci of the transgenes in both systems.

306

FUNCTIONAL ANALYSIS OF ELMO AND F-BOX/LRR IN THE MOSQUITO, *AEDES AEGYPTI*

Shin-Hong Shiao

National Taiwan University, Taipei, Taiwan

It has been widely accepted that invertebrates harbor only innate immunity. The innate immunity includes phagocytosis, encapsulation, melanization and secretion of antimicrobial peptides (AMPs). Five AMPs, named Attacin, Cecropin, Defencin, Diptericin and Gambicin have been identified in the mosquito *Aedes aegypti*. ELMO was shown to be involved in *D. melanogaster* development and cytoskeleton stability. It was demonstrated to affect phagocytosis in mammals. Previous research showed that F-box/LRR play important role in protein-protein interaction and in ubiquitylation. Therefore, we ought to explore the functions of ELMO and F-box/LRR in the mosquito *A. aegypti*. We made use of RNA

interference (RNAi) technique to silence the mRNA expression of ELMO and F-box/LRR in *A. aegypti*, followed by the challenge of *Staphylococcus aureus* or *Escherichia coli.* The survival assay was performed to analyze the mosquito resistance to these bacteria. Our results revealed that *Aedes aegypti* showed resistance to *S. aureus* in the absence of ELMO and F-Box. Therefore, we speculated that ELMO and F-box may serve as negative regulators in Toll pathway. Next, the expression of Cecropin A, a downstream target of Toll pathway, was examined. The results showed that silencing of ELMO resulted in the over-expression of Cecropin A upon *S. aureus* challenge, suggesting that ELMO negatively regulate the expression of Cecropin A. Finally we made use of FITC-labeled bacteria to observe the effect of phagocytosis in *A. aegypti*. The result showed that silencing of ELMO can increase the phagocytic ability to *S. aureus* in the mosquito. It's suggested that ELMO played a negative role in phagocytosis to Gram positive bacteria. Interestingly, silencing of F-box/LRR in the early pupal stage revealed a significant reduction of emerging adults. Our findings showed novel role of ELMO and F-box/LRR in the mosquito *Aedes aegypti*.

307

THE WEST-SIDE STORY OF *ANOPHELES GAMBIAE* MOLECULAR FORM SPECIATION

Beniamino Caputo[1], Davis Nwakanama[2], Eniyou C. Oriero[2], Mussa Jawara[2], Ibrahima Dia[3], Lassana Konate[4], Daniele Canestrelli[1], Vincenzo Petrarca[1], David Conway[2], **Alessandra della Torre**[1]

[1]University of Rome "Sapienza", Rome, Italy, [2]Medical Research Council, Banjul, Gambia, [3]Institut Pasteur Dakar, Dakar, Senegal, [4]University of Dakar, Dakar, Senegal

Throughout west and central Africa, *Anopheles gambiae* M and S molecular forms are characterised by largely overlapping geographical/temporal distributions, high levels of gene-flow restriction, low degree of inter-form genetic differentiation. Floating paracentric inversions on chromosome-2, probably involved in ecological adaptation to marginal sub-niches, are shared by the 2 forms, although with different frequencies of alternative inverted arrangements. In fact, while in forested/humid areas of west and west-central Africa M and S are both characterised by a standard homokaryotype, in northern savannah areas they show a very high level of chromosomal differentiation. We here report the first data on M and S population structure in the area along the Gambia river, where a frequency of 3-7% M/S hybrid has been observed. The results show that in the western part of the study area the M-form presents a very unusual chromosomal constitution, undistinguishable from that of sympatric S-form (i.e. high frequencies of 2Rb, 2Rd and 2La arrangements). The resulting karyotypes - never observed before at high frequencies in M-form - are also found in M-populations from the rice-cultivated central area of the transect, suggesting that M-form is able to adapt to this peculiar environment even in the absence of high frequencies of inversions 2Rbc and 2Ru, usually associated to comparable ecosystems in Mali and Burkina Faso. On the other hand, the 2Rbc and 2Ru inverted arrangements are observed in the few M-specimens found at the eastern part of the transect, where sympatric S-populations are prevailing and are characterized by increased chromosomal complexity, based on 2Rj, 2Rbk and 2Rcu arrangements typical of S-form in eastward geographic areas. These observations, coupled with the results from the analysis of 20 microsatellite loci on chromosome-X and -3, allow to speculate on the peculiar status of M and S forms at the western extreme of their range and on the multiplicity of genetic adaptive mechanisms allowing the great ecological flexibility of *A.gambiae* along its range.

308

GLOBAL CROSS-TALK OF GENES IN RESPONSE TO DENGUE VIRUS INFECTION IN THE MOSQUITO *AEDES AEGYPTI*

Susanta K. Behura, David W. Severson

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

The mosquito *Aedes aegypti* is the primary global vector of dengue virus (DENV). The early time periods after infection with DENV define critical cellular processes that determine success or failure of the virus to establish infection in the mosquito. To identify genes involved in these processes, we performed genome-wide transcriptome profiling between susceptible and refractory *Ae. aegypti* mosquitoes at two early periods after challenging them with DENV. Our data reveal extensive transcriptional networks of mosquito genes that are expressed in modular components in response to DENV infection. The genes that responded coordinately to DENV infection in the susceptible strain were clustered in one specific expression module whereas in the refractory strain they were distributed in four distinct modules. The susceptible- and refractory-specific differential expression of five randomly chosen genes from the array data were validated by qRT-PCR. The specificity of expression was also observed in two additional *Ae. aegypti* strains, DS3 and Moyo-In-Dry, in response to DENV infection. The susceptible response module in the global transcriptional network showed enrichment with genes related to energy metabolism and DNA replication whereas the refractory response modules showed enrichment with different metabolism pathway genes including cytochrome P450 and DDT degradation genes, and also genes associated with cell division and apoptosis. Two additional modules were identified that represented a common core set of coordinately expressed genes in both the susceptible and refractory mosquitoes. These core response modules are enriched mostly with genes related to different signal transduction pathways including the wnt, MAPK, mTOR and JAK-STAT pathways. These pathways may have important roles in the global-cross talk among the host factors during the early infection period that could trigger the appropriate host action in susceptible and refractory mosquitoes.

309

AEDES AEGYPTI: AN EMERGING MODEL FOR THE STUDY OF DEVELOPMENTAL BIOLOGY IN VECTOR MOSQUITOES

Ellen Flannery[1], Anthony Clemons[1], Morgan Haugen[1], Kristopher Kast[1], David Severson[1], Molly Duman-Scheel[2]

[1]University of Notre Dame, South Bend, IN, United States, [2]Indiana University School of Medicine-South Bend at Notre Dame, South Bend, IN, United States

Mosquito genome projects have stimulated increased interest in the potential for arthropod-borne disease control by genetic manipulation of vector insects. Although targets of particular interest include developmental regulatory genes, extremely little is known about the genetic regulation of vector mosquito development. We have recently developed methodology for analysis of gene and protein expression during embryonic, larval, and pupal development of the vector mosquito *Aedes aegypti.* We have also achieved knockdown of developmental genes through microinjection of siRNAs into *Aedes aegypti* embryos. This methodology is permitting detailed analyses of the functions of developmental regulatory genes and the selective inhibition of such genes during *Aedes aegypti* development. We are presently using these techniques to characterize the *Aedes aegypti semaphorin* and *plexin* homologs, genes which are known to regulate numerous developmental processes in *Drosophila melanogaster*, including development of the olfactory system. Expression data support the hypothesis that the roles of the *sema1a* and *plexin* genes are generally conserved between fruit fly and mosquito development. This hypothesis is currently being tested by siRNA-mediated knockdown of *sema1a.* Our investigation, in combination

with our ongoing functional analyses of additional developmental genes of vector importance, is helping to establish *Aedes aegypti* as an emerging model for vector mosquito development.

310

MICROGEOGRAPHIC GENETIC DIVERSITY OF *ANOPHELES NUNEZTOVARI* S.L. FROM CORDOBA AND ANTIOQUIA, COLOMBIA

Luz M. Jaramillo[1], Giovan F. Gómez[1], Astrid V. Cienfuegos[1], Lina A. Gutiérrez[1], Shirley Luckhart[2], Jan E. Conn[3], **Margarita M. Correa**[1]

[1]Grupo de Microbiología Molecular, Escuela de Microbiología, Universidad de Antioquia, Medellin, Colombia, [2]Department of Medical Microbiology and Immunology, University of California, Davis, CA, United States, [3]Griffin Laboratory, Wadsworth Center, New York State Department of Health, Albany, NY, United States

Anopheles nuneztovari s.l. has a wide distribution in northern South America and is an important vector of malaria in Colombia and Venezuela. To test genetic diversity of *An. nuneztovari* s.l, at a microgeographic scale (approximately 150 km), mtDNA *COI* gene sequences were analyzed from 145 specimens collected in four Colombian localities: Montelibano and Puerto Libertador in Cordoba department/state, and El Bagre and San Pedro de Urabá in Antioquia department, July 2007- February 2010. Nucleotide and haplotype diversity values were higher in the populations of Antioquia. There were 20 unique haplotypes, 4 shared among all the localities and a few (13) from both states were tip alleles, suggesting high demographic stability in the populations. A statistical parsimony *COI* gene network showed the most common interior haplotype (38% of all sequences analyzed) was represented in all collection sites. Overall, different analyses indicated low to moderate genetic differentiation and high gene flow among all populations tested from Córdoba and Antioquia; neutrality tests also supported demographic equilibrium. Despite the fact that *An. nuneztovari* s.l. is a species complex, the four populations in this study comprise a single mtDNA evolutionary unit. Continuation of this study with the analyses of additional markers will further elucidate the genetic structure of this important Colombian malaria vector and the understanding of its population genetics will contribute to the improvement of local malaria control strategies.

311

SUPPRESSION OF DENGUE VIRUS REPLICATION IN THE SALIVARY GLANDS OF TRANSGENIC *AEDES AEGYPTI*

Geetika Mathur[1], Irma Sanchez-Vargas[2], Danielle Alvarez[1], Ken E. Olson[2], Osvaldo Marinotti[1], Anthony A. James[1]

[1]University of California Irvine, Irvine, CA, United States, [2]Colorado State University, Fort Collins, CO, United States

Genetic control strategies for vector-borne diseases based on population replacement require development of genetically-modified mosquitoes that provide resistance against the target pathogen. In order to achieve this, regulated expression of anti-pathogen effector molecules in a sex- and tissue-specific manner by using *cis*-regulatory DNA sequences is essential. Salivary glands of the female *Aedes aegypti* play an important role in the transmission of the dengue viruses and therefore are ideal sites for the expression of effector molecules. The *Aedes aegypti 30K a* and *30K b* genes are expressed exclusively in the distal-lateral lobes of the female salivary glands and are separated by a 263 bp intergenic region. The *cis*-regulatory sequences of the *30K a* and *30K b* genes were used to express EGFP reporter and an anti-dengue effector gene in the salivary glands of female mosquitoes. The anti-dengue molecule, *Mnp*, consists of an inverted repeat sequence derived from the coding region for the membrane precursor region of the DENV-2 genome. Transgenic mosquitoes expressing *Mnp* fed on blood infected with DENV-2 showed reduced prevalence and mean intensities of infection of the virus in the salivary glands compared to control mosquitoes. The DENV-2 transmission potential also was reduced significantly in the mosquitoes carrying the *Mnp* transgene compared to the controls. Work is in progress to achieve complete resistance against the virus by expressing the anti-effector gene in multiple tissues simultaneously.

312

PREVALENCE OF INTESTINAL PARASITES, ANAEMIA AND ANTHROPOMETRIC STATUS AMONG CHILDREN UNDER FIVE YEARS OF AGE IN LAMARAME (SENEGAL)

Roger C. Tine[1], B. Faye[1], J.L. Ndiaye[1], C.T. Ndour[2], P. Magnussen[3], Ib C. Bygbjerb[4], O. Gaye[1]

[1]Service de Parasitologie, Faculté de Médecine et Pharmacie, UCAD, Dakar, Senegal, [2]Clinique des Maladies Infectieuses, Centre Hospitalier Universitaire de Fann, Dakar, Senegal, [3]DBL - Centre for Health Research and Development, Faculty of Life Sciences, University of Copenhagen, Copenhagen, Denmark, [4]University of Copenhagen Faculty of Health Sciences Department of International Health, Immunology and Microbiology, Copenhagen, Denmark

In order to target interventions against malaria and other tropical diseases, we conducted a baseline study at the new Health Demographic Surveillance System (HDSS) of Lamarame (Senegal) where malaria is seasonal. Our aim was to assess the prevalence of intestinal parasite (IP), anaemia and malnutrition among children under 5 years. A cross sectional survey was carried in January 2010. A two level random cluster sampling technique was used. A total of 30 clusters (villages) covered by Lamarame health post were randomly selected based on probability proportional to population size. Children were examined by a study physician after parents had given informed consent. For each child, anthropometric measures (weight, height, age) were taken. Height for age and weight for age z-scores were calculated by Epi info software using the NCHS/ WHO international reference values. Haemoglobin rate was measured with HemoCue®; stool samples were collected and examined using the Ritchie technique. The study was approved by the Senegalese national ethical committee. For 722 examined children the average prevalence of IP was 26.2% [CI95% 22.9-29.5]. *Giardia intestinalis* was found in 15.6%[CI95%13-18.5], *Entamoeba coli*:10.9% [CI95% 8.7-13.4], *Hymenolepis nana*: 1.8% [CI95% 0.9-3], *Ascaris lumbricoides* :0.42% [CI95% 0.08-1.2] and *Enterobius vermicularis*: 0.28%[CI95% 0.03-0.9]. IP prevalence was significantly higher in villages located at a distance ≥ 1 km from the health post (73.9% versus 26% RR: 1.7 [CI95% 1.2 - 2.6]). Prevalence of anaemia (Hb <11g/dl) was 66.4%. Severe anaemia (Hb < 8g/ dl) and moderate anaemia (8<Hb<11 g/dl) was found in 12.7% [CI95% 10.4-15.4], and 53.7%[CI95% 49.9-57.3], respectively. Stunting (HAZ<-2SD) was found in 21.6% [CI95% 18.6-24.8] and underweight (WAZ<-2SD) in 16.5% [CI95% 13.8-19.4]. In a logistic regression model, stunting was significantly associated with: severe anaemia (aOR 3.65 [2.01-6.64]) moderate anaemia (aOR 2.03 [1.27-3.26], living at a distance > 1 Km from the health post (aOR: 3.74 [1.62-8.64] and number of children in the household (aOR1.5 [1.15-1.95]. Anaemia and stunting constitute a public health problem in Lamarame despite periodical mass administration of vitamine A and mebendazole treatment for STH. Protozoan infections such *Giardia* and *E. coli* are frequent in the area. Additional interventions are needed to target these parasitic diseases.

313

RECURRENT AMEBIC LIVER ABSCESS

Subramanian Swaminathan, Pranatharthi Chandrasekar

Wayne State/Detroit Medical Center, Troy, MI, United States

A 44 year old man of Iraqi origin presented to our institution with a 5 day history of fever, chills and vomiting. He was treated for amebic liver abscess (proven by aspiration) in 2001 and 2003. The patient was treated with a combination of aspiration of the pus and metronidazole. Since his arrival from Iraq in 2000, the patient had not traveled outside of Michigan. On examination, he was febrile (39°C), with tachycardia (120/min); the rest of the exam was normal. Pertinent laboratory findings were an

elevated white blood cell count (15,500/µl), serum alkaline phosphatase (684 IU/ml) with negative hepatitis serologies. Ultrasound of abdomen showed an 8x7x7 cm abscess in the right lobe of the liver. Aspiration showed "anchovy paste", consistent with amoebic liver abscess. Antibody to Entamoeba was elevated. The patient was successfully treated with aspiration of the pus and metronidazole, along with oral iodoquinol as a luminal cysticidal agent. It is likely that the lack of use of a cysticidal drug for the previous episodes resulted in persistent colonization of the colon, with subsequent reactivation causing invasive liver disease. There have been few reports of recurrent amebic liver abscess; it occurs in about 0.04% of cases. Most recurrent abscesses are seen within a year of the first episode. Our patient represents the first documented instance of such a late recurrence. Available data show that metronidazole is a poor luminal cysticidal agent. The case history of our patient highlights the risk of recurrence of liver abscess if intestinal amebic cysts are not eradicated during treatment.

314

PREVALENCE OF INTESTINAL PARASITIC PATHOGENS IN DIARRHEAL AND NON-DIARRHEAL HUMAN STOOL SAMPLES IN TURKEY, 2001-2010

Ozgur Koru[1], Mehmet Tanyuksel[1], Remzi Engin Araz[1], Suzan Esin[1], Fatma Karakaya[1], Ahmet Celal Basustaoglu[2]

[1]Gulhane Military Medical Academy, Department of Microbiology, Division of Medical Parasitology, Ankara, Turkey, [2]Gulhane Military Medical Academy, Department of Microbiology, Ankara, Turkey

Intestinal parasites are two major groups including protozoa and helminths that inhabit the gastro-intestinal tract in humans. They are one of the major health problems of especially poor and under-developed countries. This study was undertaken to determine the prevalence of intestinal parasitic infection in patients with diarrhea and non-diarrhea at the Gulhane Military Medical Academy, Military Hospital in Turkey. This retrospective study reviewed the hospital records of 26842 patients admitted over a ten-year period from 2001 to 2010. We used a standardized data collection form to obtain data for sociodemographic characteristics and laboratory diagnosis. Stool samples were collected from 26842 patients who applied to Gulhane Military Medical Academy Parasitology Laboratory between 2001 and 2010. During the study period, 2.2% (1314/57375) of stool samples were tested positive for sixteen species of intestinal parasites, by using standard parasitological techniques. Multiple infections with 2-4 parasitic species constituted 0.1 % of 26842 infected cases. *Giardia intestinalis* (2% of the 26842 cases) was the most common parasitic cause of diarrhea among the patients. Its prevalence appears to be decreasing in recent years. Fifteen other species of intestinal parasites were identified. *Entamoeba coli* (0.8%) and *Blastocystis hominis* (0.3%) ranked second and third in prevalence, respectively. *Enterobius vermicularis* (0.2%) was more common in non-diarrheal samples. Prevalence of intestinal parasitic infection was lowest (14%) in winter, gradually increased during the spring, reached peaks of 56% between July and October, and gradually decreased to 2% in December. These data will help provide accurate estimates of the prevalence of intestinal parasites, which are crucial for the development of policies and strategies to enhance their effective control.The present study has demonstrated that *G. intestinalis*, *E.coli* and *B. hominis* are common parasitic causes of diarrhea in Turkey.

315

SUCCESSFUL TREATMENT AN IMMUNE-COMPETENT PATIENT WITH REFRACTORY GIARDIASIS USING NITAZOXANIDE AND GENETIC CHARACTERIZATION OF THE *GIARDIA INTESTINALIS* ISOLATE

Omer Coskun[1], **Ertan Altayli**[2], Ozgur Koru[3], Mehmet Tanyuksel[3], Can Polat Eyigun[1]

[1]Gulhane Military Medical Academy, Department of Infectious Disease, Ankara, Turkey, [2]Gulhane Military Medical Academy, Center for Research and Development, Division of Medical and Cancer Research, Ankara, Turkey, [3]Gulhane Military Medical Academy, Department of Microbiology, Division of Medical Parasitology, Ankara, Turkey

Giardia intestinalis is a most common protozoan infection in the world. It is the cause of both epidemic and endemic diarrhea and upset of the gastrointestinal system. There are a number of drugs whose efficacies are well studied and accepted for the treatment of patients with this infection. However, some individuals experience treatment failure, despite having received successive courses of treatment that have been documented to result in a cure for most patients. In humans, nitazoxanide has been reported to be effective against a broad range of parasites, including *G. intestinalis*. We report the case of a 21 year old male immuno-competent patient admitted to Gulhane Military Medicine Academy Hospital in February 2008. He had nausea, vomiting and fever. The results of an extensive evaluation including fibrinogen, immunglobuline G, A, and M tests were initially negative. He had a pain in region of epigastric and upper gastrointestinal endoscopic examination was performed on this patient. Chronic gastritis was diagnosed through endoscopic examination and a plenty of *G. intestinalis* trophozoites was detected in samples obtained from stomach and bulbus. And he received oral metronidazole, 500 mg given t.i.d. for five days. After the patient received the treatment, *Giardia* cysts were still present in stool samples. He complained of watery diarrhea with 7-8 times a day. Subsequently, the patient underwent two sequential treatment regimens that consisted of secnidazole, 2 g given once and albendazole, 400 mg given b.i.d., for 5 days. Stool samples tested negative for *Giardia* during therapy but revert to positive after the treatments. Successful treatment with nitazoxanide, 1 g given b.i.d. for 15 days, resulted in stool samples that tested negative by microscopy. He reported no side effects and the results of microscopic evaluation of his stool samples have remained repeatedly negative for *Giardia*. Genetic analyses of *Giardia* cysts isolated from patient revealed that they were from Assemblage B. In this report, nitazoxanide was found to be active against a metronidazole and albendazole-resistant *G. intestinalis* isolate from an immune-competent patient. If its anti-giardial efficacy is confirmed in additional studies, nitazoxanide may be considered as an alternative therapy for resistant giardiasis.

316

PREVALENCE OF SPECIES AND SUB-TYPES OF *CRYPTOSPORIDIUM SPP.* AND *GIARDIA DUODENALIS* IN FOUR COMMUNITIES IN THE PERUVIAN AMAZON BASIN

Daniel Velasquez[1], Geoffrey Kahn[1], Juan Francisco Sanchez-Chiroque[2], Angela M. Bayer[2], Victor E. Gonzaga[2], Joel M. Montgomery[2], John W. Sanders[2], Paul C. Graff[2], Andres G. Lescano[2], **Vitaliano A. Cama**[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]United States Naval Medical Research Center Detachment, Lima, Peru

Childhood diarrhea is an important cause of morbidity and mortality in children less than 5-years of age. The prevalence and genetic diversity of *Cryptosporidium spp.* and *Giardia duodenalis,* pathogens associated with childhood diarrhea, were assessed in microscopy-confirmed samples from children ≤5 years in four riverside communities located in the Peruvian Amazon basin. Stool samples (one per child) and data on diarrhea in the previous 2 weeks were collected in 2009 from

453 children by the U.S. Naval Medical Research Center Detachment (Naval Medical Research Center Detachment) in collaboration with the Peruvian Ministry of Health. Participants' stool samples were first examined microscopically for parasites. Nineteen samples were positive by microscopy for *Cryptosporidium* and 7 samples were further genotyped by SSU rRNA PCR-RFLP. Five children had *C. hominis* and two had *C. canis*. A fragment of the gp60 locus was sequenced and sub-types for 4/5 of the *C. hominis* samples were obtained: Ib (2 samples), Ie, and a novel Ig sub-type. Eighty samples were microscopy positive for *Giardia;* 72 of those were confirmed positive by SSU rRNA-based real-time PCR. Forty-three samples were successfully genotyped by sequence analysis of the TPI locus. Two genotypes were detected: assemblage A in three samples, and assemblage B in 40. Within assemblage A, we detected two different sub-types. Subtype analysis of samples with assemblage B revealed 11 distinct subtypes, and the presence of mixed infections with B subtypes in 7 samples. There were no mixed infections with assemblages A and B. The genetic diversity of *Cryptosporidium* and *Giardia* in these communities Amazon basin is similar to that found in other endemic settings, where transmission may occur through direct contact, food- or waterborne routes. Although the geno- and sub-types of *Cryptosporidium* and *Giardia* detected in this study are associated with anthroponotic transmission, the presence of large number of samples with assemblage B of *G. duodenalis,* and *Cryptosporidium canis* in two children also suggest potential zonotic transmission.

317

ENTAMOEBA HISTOLYTICA AND *ENTAMOEBA DISPAR* IN CYST-POSITIVE FECAL SAMPLES FROM SMALL COMMUNITIES IN THE PERUVIAN AMAZON

Daniel Velasquez[1], Juan Francisco Sanchez-Chiroque[2], Geoffrey D. Kahn[1], Angela M. Bayer[2], Victor E. Gonzaga[2], Joel M. Montgomery[2], John W. Sanders[2], Paul C. Graf[2], Andres G. Lescano[2], **Vitaliano A. Cama**[3]

[1]Centers for Disease Control and Prevention-AREF, Atlanta, GA, United States, [2]United States Naval Medical Research Center Detachment, Lima, Peru, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

Cysts of pathogenic *Entamoeba histolytica* and non-pathogenic *E. dispar* have similar morphologic characteristics. The differentiation of these species is important because it leads to different therapeutic courses or additional diagnostic testing. We determined the prevalence of *E. histolytica* and *Entamoeba dispar* in children <5 years-old. Four-hundred fifty-six samples as well as data on diarrhea and fever were collected in 2009 from four riverside communities located in the Peruvian Amazon basin. Ova and parasite examination identified cysts with morphology of *Entamoeba* histolytica/dispar in 34/456 samples (7.5%). These samples were further analyzed by species-specific PCRs to identify *E. histolytica* and *E. dispar,* using two tests for each: conventional PCR and Real-Time TaqMan-PCR. These methods amplify fragments of the small-subunit rRNA gene. Conventional PCR detected 11 samples positive for *E. dispar*, while the TaqMan assay detected 7 additional positives (total = 18). In contrast, *Entamoeba histolytica* was not detected in any sample by either assay. The absence of *E. histolytica* in the samples microscopically diagnosed with *E. histolytica/dispar*-like cysts, and the detection of *E. dispar* in 18/34 samples suggest that non- pathogenic amoeba are quite frequent in these communities. These findings highlight the importance of species-specific tests for *E. histolytica* and further differential diagnosis among people with bloody dysentery, particularly those whose samples had microscopy positive results for *E. histolytica/dispar*.

318

EPIDEMIOLOGY OF *ENTAMOEBA HISTOLYTICA* INFECTIONS AMONG ABORIGINAL CHILDREN IN PERIPHERAL VILLAGES IN SELANGOR, MALAYSIA

Hesham M. Al-Mekhlafi[1], 'Azlin M. Yasin[2], Fatmah M. Salleh[2], Norhayati Moktar[2]

[1]Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, [2]Department of Parasitology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, Kuala Lumpur, Malaysia

Entamoeba histolytica infection is one of the most common parasitic infections in the world particularly in developing countries. A community-based study to determine the prevalence and predictors of this infection was carried out among Aboriginal children aged between 2 and 15 years in selected peripheral villages in Selangor, Malaysia. Socioeconomic data were collected using pre-tested questionnaires. Of 281 trichrome stained fecal smears examined, 25(8.9%) children were positive for E. histolytica. The prevalence of *E. histolytica* infections increased with age and associated with large family size and significant wasting (P<0.05). Binary logistic regression confirmed that large family size (>=8 members) was a significant predictor of *E. histolytica* infections (OR=3.34; 95%CI=1.31, 8.52). The subjects were asymptomatic or presented non-specific symptoms that could be attributed to amoebiasis. In conclusion, the predictors found in this study are known to be important determinants of *E. histolytica* infections. Further studies are needed and molecular approaches to distinguish the invasive *E. histolytica* from the non-invasive parasites such as *E. dispar* and *E. moshkovskii* may lead to a better understanding of the burden of this infection in Malaysia.

319

GIARDIA LAMBLIA AND *CRYPTOSPORIDIUM* SPP. GENOTYPING IN CHILDREN UNDER FIVE YEARS OLD FROM PANAMA, CENTRAL AMERICA

Vanessa Pineda, Dayra Alvarez, **Jose E. Calzada**, Azael Saldaña

Gorgas Institute, Panama, Panama

Giardia lamblia and *Cryptosporidium* spp are zoonotic parasites that can cause gastrointestinal disease and nutritional deficiency in children. In Panama, these two pathogenic protozoa are present with important prevalence in children, especially giardiasis. However, information on their genetic characteristics, distribution, and role in human disease is limited. We analyzed the genetic diversity and geographic distribution of both protozoa from infected children younger than five years. Stool samples were taken from 1,560 diarrheic and non-diarrheic children from eight different regions in Panama. Oocysts were microscopically detected using the formalin-acetate concentration procedure and Kinyoun stain. Of these samples, 201 presented *G. lamblia* and 79 presented *Cryptosporidium* spp. DNA was extracted from positive samples. Molecular diagnosis and characterization was possible in 120 *Giardia* and 24 *Cryptosporidium* positive samples. *G. lamblia* genotyping was performed using a PCR-RFLP analysis based on the polymorphisms of the *tpi* gene. For *Cryptosporidium* spp, the SSU rRNA gene was used as molecular marker. Genetic analysis revealed that 23.3% *Giardia* samples belonged to assemblage A, 69.0% belonged to assemblage B and 7.5% were mixed infections. Subtyping of assemblage A samples showed that type AII is nine times more frequent than AI. *Cryptosporidium g*enotyping showed that 62.5% were *C. hominis*, 21.0% *C. parvum*, 12.5% *C. meleagridis* and 0.04% *C. canis*. *Cryptosporidium* identification was confirmed by direct sequence analysis. Further genetic diversity within *C. parvum* samples was assessed by sequence analyses of the GP60 gene. This is the first report of *G. lamblia* and *Crytosporidium* spp genotype in human isolates from Panama. *G. lamblia* organisms belonging to assemblage B and *C. hominis* are the

predominant genotypes in different regions of Panama. Further studies are required to evaluate the molecular epidemiology and to develop prevention and control measures of both protozoa parasites in Panama.

320

ANTHROPOGENIC DISTURBANCE INCREASES PREVALENCE OF PATHOGENIC PROTOZOA IN WILD RODENT RESERVOIRS IN UGANDA

Thomas R. Gillespie, Stefanie Lang, Innocent B. Rwego, Johanna Salzer, Sandra Ockers, Amanda Williams

Emory University, Atlanta, GA, United States

Giardia and *Cryptosporidium* are zoonotic pathogens that cause widespread morbidity in the developing world. Despite the impact of these pathogens, little is known about their reservoirs in nature or how anthropogenic disturbance affects their patterns of infection. To better understand this interplay, we sampled rodents for these pathogens along a disturbance gradient traversing primary forest, logging concessions, small-scale and commercial agricultural plots and villages in Kabarole District, Uganda. Trapping webs were utilized to create accurate density and diversity estimates of the rodent community along each transect of this grid. Rodent fecal samples were screened for the presence of both protozoa via immunoflourescent antibody testing (IFA). Pathogen prevalence was calculated as number of individuals of a species expressing positive results divided by total number of individuals of that species sampled. Effects of rodent species and geographic location on pathogen prevalence were examined using parametric statistics. *T*he brush-furred rat (*Lophuromys* sp.) was found to have significantly higher prevalence of *Giardia* compared to other wild rodent species examined ($P < 0.01$). In addition, *Cryptosporidium* was only observed in *Lophuromys* sp. Neither *Cryptosporidium* nor *Giardia* were observed in the invasive black rat (*Rattus rattus*). Every rodent sample collected from the undisturbed forest was negative for both pathogens and rodent samples from the most disturbed site had significantly higher prevalence of both *Cryptosporidium* and *Giardia* compared to all other sites ($P < 0.01$). These results suggest that *Lophuromys* sp. may serve as a viable reservoir for these neglected tropical pathogens and that anthropogenic disturbance may be associated with their prevalence in rural, forested Africa. These results highlight the need for future research into the epidemiology, cross-species transmission ecology, and clinical consequences of *Giardia* and *Cryptosporidium* not only in humans and livestock, but also in the wild animals that share their environments.

321

COMMON DIAGNOSTIC METHODS FOR *ENTAMOEBA* SPECIES LEAD TO OVER-DIAGNOSIS OF THE PATHOGENIC *ENTAMOEBA HISTOLYTICA*, IN POPULATIONS OF EASTERN VENEZUELA

Hectorina Rodulfo[1], Marcos De Donato[1], María E. Rodriguez[1], Beatriz Ahmar[1], Leonor Mora[2]

[1]IIBCA, Universidad de Oriente, Cumana, Bolivarian Republic of Venezuela, [2]Departamento Bioanalisis, Universidad de Oriente, Cumana, Bolivarian Republic of Venezuela

In Venezuela, since 1990, infection frequencies are reported from 6.8 to 42% throughout the country. Between 2005 and 2008, 124,142 diarrhea cases were reported in children, 7,366 of these were reported as amebiasis, with Capital District, Zulia, Anzoategui and Sucre states showing highest prevalences. Even tough WHO since 1997 have recommended to differentiate the pathogenic species *Entamoeba histolytica* from the other non pathogenic species of the genus, in order to rationalize the treatment, few places carry out such differentiation. In Venezuela, the main diagnostic method is based on the microscopic observation of the trophozoites and cysts, which has a proven sensitibity of about 60% and cannot differenciate among *E. histolytica*, *E. dispar* and *E. moshkovskii* because of their identical morphology, except in cases where hematophagous trophozoites are seen. To study the real prevalence of these species in the states of Sucre and Anzoategui, we have diagnosed 1,045 fecal samples from symptomatic and asymptomatic individuals using nested-multiplex PCR for the detection of *E. histolytica*, *E. dispar* and *E. moshkovskii*. These samples were previously diagnosed by clinical laboratories using microscopic observation in saline physiologic solution and Lugol. The results show variations in the prevalence from 11 to 20% for the complex *E. histolytica/E. dispar* using microscopy, while PCR detected infection in symptomatic and asymptomatic individuals with 3.3% of cases being diagnosed as *E. histolytica*, 3.8% as *E. dispar* and 0.6% of mixed infections. *E. moshkovskii* has not been detected so far in these populations. These results make evident the over-diagnosis of amebiasis by conventional diagnostic methods and the lack of differentiation among the species of the genus, indicating the treatment in many more cases than really needed, which can lead to parasite resistance to common drugs to treat amebiasis.

322

CHANGES IN PROFILE OF EXPRESSED PROTEINS IN *TRYPANOSOMA BRUCEI BRUCEI* PRIOR TO BRAIN INVASION DURING EXPERIMENTAL AFRICAN TRYPANOSOMIASIS

Monica R. Namayanja

Makerere University, Kampala, Uganda

Trypanosoma brucei the causative agents of African Trypanosomiasis is known to invade the brain during the encephalitic stage of the disease. However mechanisms by which trypanosomes invade the brain are not well understood. During infections,the onset of the encephalitic stage is preceded by several waves of parasitemia, suggesting that some changes may occur in trypanosomes enabling them to traverse the blood brain barrier(BBB). We compared the traversal efficiency of trypanosomes harvested from experimentally infected mice during the early stage of the disease (early stage trypanosomes) and trypanosomes harvested during the late stage of the disease (late stage trypanosomes) *in vitro* using Mardin Darby Canine Kidney (MDCK) monolayer. Late stage trypanosomes were found to traverse the biological barrier more effectively than early stage trypanosomes. When analyzed by two dimensional gel electrophoresis,later stage trypanosomes were found to express proteins that were not expressed by early stage trypanosomes which points to possible differential transcriptional and translational events that enable an active crossing of the BBB. Identification of these proteins by mass spectrometry is underway.

323

SURVEILLANCE FOR CYCLOSPORIASIS IN THE UNITED STATES, 1997-2008

Rebecca L. Hall[1], Adriana Lopez[2], Michele C. Hlavsa[2], Jeffrey L. Jones[2], Barbara L. Herwaldt[2]

[1]Atlanta Research and Education Foundation, Decatur, GA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States

Cyclosporiasis is a food- and waterborne enteric disease caused by the parasite *Cyclospora cayetanensis*. Outbreaks have been investigated by CDC since the mid-1990s; however, U.S. data on sporadic cyclosporiasis cases have not been summarized previously. Health departments report cyclosporiasis cases via the National Notifiable Diseases Surveillance System. Of the 1,092 sporadic cases reported to CDC for the period 1997-2008, 338 (31%) had a history of international travel during the 14 days before illness onset, 382 (35%) did not (i.e., "domestic" cases), and the travel history was not reported for the other 372 (34%). The destination was known for 287 (85%) of the 338 travel-associated cases. The most frequent destinations were Mexico (59/338; 18%), Guatemala (40; 12%), and Peru (37; 11%). Domestic cases were reported largely from Florida (124; 32%) and the Northeastern United States (New York City [44; 12%], Massachusetts [31; 8%] and Connecticut [27; 7%]). Most

reported domestic cases (265; 69%) occurred or were diagnosed from April through August. Both travel-associated and domestic cases occurred equally among males and females (p=0.80). The median age of travel-associated and domestic case-patients was 41 years and 47 years. At least some food history was available for 52% of domestic cases, but varied widely due to state-specific differences in interview instruments, and case-patient response rates. While a large percentage of travel-associated cases visited known *Cyclospora*-endemic countries, our data suggest that *Cyclospora* could be transmitted more frequently than previously thought in some countries such as Mexico. Most domestic cases were reported by several eastern states during the spring and summer months. Vehicles of infection for sporadic cases are not usually identified because food histories are sparse or incomplete, a known limitation of sporadic case surveillance. Furthermore, because there is no *Cyclospora* molecular subtyping capability to link individual cases, some outbreak-associated cases were likely misclassified as sporadic.

324

FOLLOW-UP OF THE 1977 TOXOPLASMOSIS OUTBREAK FOR OCULAR DISEASE

Jeffrey L. Jones[1], Ricardo B. Akstein[2], Michele C. Hlavsa[1], Adriana S. Lopez[1], Marianna Wilson[1], Gary N. Holland[3]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Akstein Eye Center, Riverdale, GA, United States, [3]Jules Stein Eye Institute and Department of Ophthalmology, David Geffen School of Medicine at UCLA, University of California, Los Angeles, CA, United States

In 1977 an outbreak of *Toxoplasma gondii* infection occurred in Georgia associated with exposure in an indoor horse arena. Cat feces containing the organism were most likely stirred-up when horses ran on the dirt floor, and were inhaled or ingested by riders and observers. Thirty-seven persons were found to be infected with *T. gondii* (by clinical exam and/or serologically [IgM and IgG IFA]). Twenty-five persons received a follow-up eye examination 4 years later, and 1 person was identified with an ocular lesion diagnosed as toxoplasmosis. Starting in 2004, we again attempted to locate persons from the 1977 toxoplasmosis outbreak and offer them an ocular examination by a retinal specialist with ocular photographs if lesions were found. Of the 37 persons infected with *T. gondii* in the outbreak, 14 (38%) were located and agreed to an ocular examination. Of these 14, 13 (93%) were female, the median age was 16 years (range 10-47 years) in 1977, and the median age at time of examination was 42.5 years (range 35-72 years). Of the 23 persons not examined, 19 (83%) were female and the median age was 27 years (range 17-38 years) in 1977. Among the 14 persons examined, 3 (21%) were diagnosed with ocular lesions typical of toxoplasmosis. The person identified with an ocular lesion at four years had ocular symptoms at that time; the other 2 did not have ocular symptoms associated with their lesions. If these 3 persons were the only ones with ocular disease out of the 37 persons in the outbreak, the ocular disease rate would still be 8%. As a result of exposure to *T. gondii* during this outbreak, a relatively high percentage of persons developed ocular disease over time.

325

CRYPTOSPORIDIUM PARVUM PROTEINS RECOGNIZED BY PATIENTS WITH ACTIVE CRYPTOSPORIDIOSIS

Ynes R. Ortega, Roxana Sanchez

University of Georgia, Griffin, GA, United States

The protozoan parasite *Cryptosporidium* has been implicated in foodborne and waterborne outbreaks. The active infection is not necessarily detected with the current serological methods available using *Cryptosporidium* proteins. The present study was designed to determine *Cryptosporidium* proteins recognized by human sera from *Cryptosporidium*-infected individuals using one-dimensional and two-dimensional western blot analyses, and their identification by mass spectrometry in order to determine antigenic proteins for use in diagnosis of cryptosporidiosis during outbreak investigations. *C. parvum* antigens of 14, 16, 21 or 26 kDa detected in one-dimensional analysis, or 17.5 detected in two-dimensional analysis, reported previously in the literature, did not distinguish recent infections ($p < 0.05$). In one-dimensional analysis a *C. parvum* protein of 57 kDa reacted more strongly with acute human sera ($p < 0.05$) whereas the results of two-dimensional analysis suggest that *C. parvum* antigens of 43.4, 50.3, 50.3, 47.6, 64.7, and 50.3 kDa, with pIs of 5.4, 7.0, 7.2, 5.3, 6.6, and 6.7, respectively, may be used as markers of early *Cryptosporidium* infection. A *Cryptosporidium* serine/threonine phosphatase, actin protein, a dynein heavy chain, phosphoglycerate kinase, a chaperone-related protein, and three *Cryptosporidium* hypothetical proteins were identified by tandem mass spectrometry. Antigenic peptides were predicted from these proteins and *Cryptosporidium*-specific peptides are suggested. These proteins and peptides might be useful to detect early *Cryptosporidium* infections and to determine human immune response associated to a specific *Cryptosporidium* species.

326

INDUCTION OF INFLAMMATORY AND ANTI-APOPTOTIC RESPONSES BY DIFFERENT STRAINS OF *TOXOPLASMA GONDII* IN MACROPHAGES AND MICROGLIAL CELLS

Kathleen Glaser, Biniam Hagos, Edward Cedrone, **Robert E. Molestina**

American Type Culture Collection, Manassas, VA, United States

Toxoplasma gondii is a highly successful intracellular parasite capable of causing severe disease among immunocompromised and newborn populations. The majority of strains of *T. gondii* belong to three distinct clonal lines known as types I, II, and III. The outcome of the immune response to infection is influenced by the parasite strain type. This study examined the kinetics of gene expression in macrophages and microglial cells infected with types I, II, or III of *T. gondii*. Emphasis was placed in pro-inflammatory and anti-apoptotic factors as their expression have been reported to change significantly in parasite-infected cells. The kinetics of host gene expression was examined by RT-PCR in response to infection. In addition, the protective effects of parasite infection against apoptosis were assessed by inhibition of caspase-3. A type II strain of *T. gondii* (ME49) elicited a greater expression of pro-inflammatory cytokines in macrophages and microglial cells compared to types I (GT-1) and III (CTG). These differences were minimal early in infection (2-4 h), but became pronounced at middle (8-12 h) and late stages of infection (24 h). Contrary to this, the induction of anti-apoptotic genes was equivalent among the different type strains throughout infection. Of note, induction of the pro-inflammatory response occurred early in infection while the anti-apoptotic response exhibited a delayed profile regardless of the strain. Lastly, experiments with cells lacking the Toll-like receptor adaptor molecule Myd88 (Myd88-/-) showed a dependency on this factor for the pro-inflammatory response but not the anti-apoptotic response. The results suggest that the outcome of host gene expression in response to *T. gondii* is determined by the parasite type in a time-dependent manner and is selective to particular subsets of genes. The capability of *T. gondii* to induce an anti-apoptotic response in the absence of a critical immune regulator reflects a complex level of modulation of host functions that might extend to other intracellular parasites.

327

CPG-ODN, ARGININE OR ALANYL-GLUTMINE REDUCE CRYPTOSPORIDIAL INFECTION IN MALNOURISHED HUMAN INTESTINAL EPITHELIAL CELLS

Lourrany Borges Costa[1], Gregory Madden[2], Jesus E. Sevilleja[2], Cirle A. Warren[2], James K. Roche[2], Richard L. Guerrant[2]

[1]Federal University of Ceará, Fortaleza, Brazil, [2]University of Virginia, Center for Global Health, Division of Infectious Diseases and International Health, Charlottesville, VA, United States

Cryptosporidium parvum is a parasite that invades intestinal epithelial cells and is a leading cause of childhood diarrhea worldwide, and intensified by malnutrition, has substantial impact on immune, cognitive, and physical development. Oligodeoxynucleotides with unmethylated CpG motifs (CpG-ODN) act as immune adjuvant for vaccines by binding to Toll-Like receptor 9. Arginine (ARG) and alanyl-glutamine (AQ) are amino acids necessary for regulation of cell cycle, polyamines production, cell migration and proliferation. ARG also increases levels of nitric oxide. Previous studies showed that CpG-ODN, ARG and AQ reduce *C. parvum* infection in neonatal and malnourished mice. We now study the effect of these molecules on HCT8 cells (human intestinal epithelial cells) infected with the parasite. HCT8 cells were grown in culture plates (n=3-4 wells/group) and subjected to either 1% ("malnourished cells") or 10% ("nourished cells") Fetal Bovine Serum in glutamine-free media. Cells were then pre- treated with ARG (1mM), AQ (10mM), CpG-ODN (100µg/mL), or combinations of the two amino acids alone or together with CpG-ODN. 24 hours later, cells were infected with 105 *C. parvum* oocysts/well. At 6 hours after infection (time point=0 hours), cells were washed and received fresh media with the treatments. Cells were harvested for DNA extraction at 0 and 24 hours. A ratio of parasites per 106 cells was calculated using Real-Time qPCR for specific parasite and HCT8 gene. CpG-ODN (p=0.0109), ARG (p=0.0117) and AQ (p=0.0127) decreased the *C. parvum* infection mainly in the groups with 1%FBS but not in 10%FBS. These findings demonstrate the potential use of a combination of CpG-ODN, ARG and AQ as a novel approach to control *C. parvum* infection, especially during malnutrition, to enhance the host immune responses and repair the intestinal injury.

328

AN APICOMPLEXAN LINEAGE-SPECIFIC POLYTOPIC MEMBRANE PROTEIN AS A POTENTIAL DIAGNOSTIC AND DRUG TARGET FOR HUMAN CRYPTOSPORIDIOSIS

Thavamani Rajapandi[1], Najma Bhat[2], Honorine Ward[2], Timothy Stedman[3]

[1]BioServices, American Type Culture Collection, Manassas, VA, United States, [2]Division of Geographic Medicine and Infectious Diseases, Tufts Medical Center, Boston, MA, United States, [3]American Type Culture Collection, Manassas, VA, United States

Human cryptosporidiosis, a waterborne gastroenteritis, is an important cause of morbidity amongst infants in many tropical countries, and is a potentially life-threatening complication in HIV-infected and other immunocompromised individuals. Human cryptosporidiosis is caused by two major species of intestinal epithelial cell parasites, including *Cryptosporidium parvum* which infects both humans and farm animals and *C. hominis*, a primarily human parasite. We have recently identified a novel family of apicomplexan lineage-specific polytopic membrane proteins in *C. parvum* and *C. hominis*. One of the members of this unique family of proteins, CpAlp854, is abundantly expressed on the oocyst wall and in apical organelles of sporozoites. Immunofluorescence assays revealed that this protein is surface-accessible to antibodies. Interestingly, CpAlp854 is also abundantly expressed on merozoites of parasites cultured *in vitro* in HCT-8 cells. This is the first polytopic membrane protein that has been partially localized to the apical organelles in *Cryptosporidium.* Polyclonal anti-serum raised against an antigenic peptide of CpAlp854 blocks the excystation of the sporozoites from oocysts. We and others have recently shown that the orthologs of these groups of proteins in other apicomplexan parasites, including *Plasmodium falciparum* and *Toxoplasma gondii,* are associated with the cytoskeleton and may be involved in zoite invasion into host epithelial cells. The abundant expression and surface localization of CpAlp854 provide an attractive target as a diagnostic marker and potential target for the design of anti-cryptosporidial compounds or vaccine to combat human cryptosporidiosis.

329

MALNUTRITION IMPAIRS HOST DEFENSES AGAINST CRYPTOSPORIDIAL INFECTION AND VACCINE RESPONSES IN WEANED MICE

Lourrany Borges Costa[1], Jesus E. Sevilleja[2], James K. Roche[2], Patricio Manque[3], Gregory A. Buck[3], James Galen[4], Richard L. Guerrant[2]

[1]Federal University of Ceará, Fortaleza, Brazil, [2]University of Virginia, Center for Global Health, Division of Infectious Diseases and International Health, Charlottesville, VA, United States, [3]Center for the Study of Biological Complexity, Virginia Commonwealth University, Richmond, VA, United States, [4]University of Maryland, Baltimore, MD, United States

Cryptosporidium parvum is a protozoan that leads chronic diarrhea worldwide mainly in developing areas, where intensified by malnutrition, has devastating impacts on immune, cognitive, and physical development. The availability of the gene sequence for *C. hominis* surface antigen (CP15) opens opportunities to examine novel vaccine candidates. We developed a malnourished weaned mouse model with malnutrition induced by 2% versus 20% (malnourished and nourished respectively) protein diet for 5-7 days to study infection with *C. parvum* and immunization with CP15 vaccine. Malnourished and nourished groups included: vaccine alone, infection alone, vaccine plus infection, and uninfected. Vaccinated mice received the antigen as recombinant with cytolysinA in *Salmonella* serovar typhi CVD908-htrA given intranasally, followed by 2 intraperitoneal boosts (10 days between each) with synthetic CP15 and Freund's adjuvant. Infected mice received 5x107 oocysts/mouse via gavage. Mice were weighed daily and stools collected over 15 days post-infection. Parasite shedding determined by Real-Time qPCR in DNA extracted from stools. Mice were sacrificed 20-40 days after immunization; spleen, mesenteric nodes and serum were collected. Cytokines and antibodies were quantified by ELISA. qPCR results showed malnourished animals have heavier infection and more weight loss. In the group that was vaccinated and infected, the vaccine did not show significant protection against *C. parvum* infection. Immunologic results showed that among mice with vaccine alone, malnourished mice have increased levels of IFN-γ and IL-10, but reduced IL-6 and IL-2 and antigen-specific antibody responses compared with nourished mice. Among infection alone, malnourished mice have increased IFN-γ but decreased antigen-specific antibody responses compared with vaccine alone group. These findings demonstrate that malnutrition impairs immune responses to immunization and parasite challenge.

330

GENETIC CHARACTERIZATION, METRONIDAZOLE SUSCEPTIBILITY TESTING AND SECRETED PROTEASE ACTIVITY OF HISTORICAL AND CLINICAL *TRICHOMONAS VAGINALIS* ISOLATES

Denise C. Cornelius, William B. Lushbaugh, Leandro Mena, Christine Muzny, John C. Meade

University of Mississippi Medical Center, Jackson, MS, United States

Trichomonas vaginalis, a protozoan parasite that infects the human urogenital tract, causes, the most common non-viral, sexually transmitted disease in the world. Over 170 million cases of trichomoniasis occur worldwide annually. Trichomoniasis is associated with vaginitis, cervicitis,

chronic prostatitis, and non-gonococcal urethritis, and is a risk factor for HSV and HIV transmission. Trichomonas infection increases the incidence of premature rupture of placental membranes, low birth weight infants, and can be transmitted to neonates during passage through the birth canal. Frontline treatment is metronidazole, which is usually very effective and well tolerated. However, an estimated 2.5-10% of cases of trichomoniasis display some degree of resistance to initial treatment. We have characterized 23 historic and 170 clinical isolates of T. vaginalis based on genetic profiles and susceptibility to metronidazole. Restriction fragment length polymorphism (RFLP) analysis using a cytoplasmic heat-shock protein 70 (Hsp70) hybridization probe with digested genomic DNA was used in molecular typing of T. vaginalis isolates. RFLP results illustrate the substantial genomic diversity present in T. vaginalis and indicate that a large number of genetically distinct Trichomonas isolates of clonal lineage may be responsible for human trichomoniasis.

We have also developed a multi-locus sequencing typing (MLST) scheme for T. vaginalis for use with these strains. We expect that this method will provide a more rapid, easily reproducible technique for typing. Our initial scheme utilizes 12 house-keeping genes to determine the relatedness of individual T. vaginalis isolates. This should provide a sufficient level of discrimination power for typing this organism. We have successfully generated 400-500 bp PCR products for 8 T. vaginalis isolates. The number of single nucleotide polymorphisms (SNPs) observed at a single locus ranges from 1-7 SNPs. We compare the two typing techniques in terms of reproducibility, precision, and rapidity. The Alamar Blue ™ colorimetric assay was used to test isolate susceptibility to metronidazole. Susceptibility testing shows the prevalence of high drug resistance found in this study to be of 3-5%, similar to the national average. Secreted Cysteine protease activity was assessed via a fluorometric analysis. The variation in activity among T. vaginalis isolates is greater than threefold.

331

CRYPTOSPORIDIOSIS - AN INNER CITY HOSPITAL EXPERIENCE

Sina Helbig[1], Phyllis Andrews[2], Herbert B. Tanowitz[3], Christina M. Coyle[3]

[1]Department of Internal Medicine, Jacobi Medical Center, Bronx, NY, United States, [2]Albert Einstein College of Medicine, Division of Infectious Diseases, Bronx, NY, United States, [3]Department of Internal Medicine, Jacobi Medical Center; Albert Einstein College of Medicine, Division of Infectious Diseases, Bronx, NY, United States

Cryptosporidium spp. is a known cause of protracted diarrhea in immunocompromised patients. It is also reported with higher prevalence among children in underdeveloped countries. With HAART therapy the epidemiology of cryptosporidiosis may be changing and it is worth describing a recent experience from an inner city hospital in the Bronx with a large immigrant and HIV population. A retrospective chart review identified patients diagnosed with *Cryptosporidium* spp. from 2003 to 2009. Individuals that were HIV positive (HIV+) were compared to those that were HIV negative (HIV-). 47 patients with Cryptosporidiosis were identified. Of the 47, 29(62%) were HIV-, the mean age was 21±18 years and 16(33%) were female. Forty-one (87%) patients had diarrhea and 30(64%) were hospitalized at diagnosis. Twenty-three (49%) patients had traveled within one month to an underdeveloped country and all were visiting friends and relatives. Travel destinations in order of decreasing frequency were Latin America, Africa, Middle East, Eastern Europe and Asia. Fifteen of 47(32%) were treated with nitazoxanide. HIV+ patients had a median CD4=37/mm3 (Min 1/mm3 and Max 952/mm3) with 29% having a CD4>100/mm3. There was no difference with respect to sex or presence of diarrhea in those that were HIV+ and HIV-. HIV+ patients were more likely to be hospitalized, have a longer length of stay and be admitted to an ICU (p=<.001, p=.047 and p<.001, respectively). HIV- patients were younger than the HIV+ patients with a mean age of 10.9 ± 12 years (p<.001) and were more likely to have traveled (p=<.001). Three of 18 (17%) with CD4<10/mm3 died within 6 months and no HIV+ patients had biliary involvement. Ten patients with a mean CD4/mm3 of 54±1.2 had persistent diarrhea documented. In conclusion, *Cryptosporidium* spp. is an important cause of travelers' diarrhea in children undertaking high risk travel to endemic regions. Cryptosporidiosis continues to occur in HIV+ patients with CD4<100/mm3, causing persistent diarrhea, but is also seen in those with higher CD4 counts.

332

IMMUNOPHENOTYPIC LYMPHOCYTE ANALYSIS OF MEMORY CELL RESPONSES IN CRYPTOSPORIDIOSIS

Nina McNair, **Jan R. Mead**

Emory University and Atlanta Veterans Affairs Medical Center, Atlanta, GA, United States

Cryptosporidium parvum is a protozoan parasite that infects the epithelial cells of the small intestine causing diarrheal illness in humans. While T cells are known to be important in resistance and recovery from infection, little has been characterized as to the phenotypic expression of surface effector and memory markers after infection. Using a IL-12 KO model of acute infection, we depleted mice of CD4+ and CD8+ cell populations after either primary or secondary infection. Depletion of either CD4+ or CD8+ prior to primary infection significantly amplified infection in both groups. However, CD8+ depleted mice eventually recovered while CD4+ depleted mice were not able to recover from a primary infection. Mice depleted of either CD8+ or CD4+ cells after recovering from an acute infection and challenged demonstrated resistance to re-infection. We then used flow cytometry to characterize expression of different effector and memory cell markers 30 days after infection. Subpopulations with varying effector and memory cell potentials were defined based on the expression of specific cell surface molecules and activation markers. We found that infected mice had a higher percentage of activated CD4+ levels (CD69 and CD71) between days 3 and 7 post infection but that these markers rapidly declined over the course of infection. Increases in the percentage of effector markers (CD62Llow) on CD4+ and CD8+ cells were found in the spleen and mesenteric lymph node by day 7. Memory cell phenotype (CD62Lhi, CD44hi) increased and were the predominant cell population by day 30 post infection. Adoptive transfer of CD44hi cells of either CD4+ or CD8+ populations from immune mice into naïve mice provided protection from infection, suggesting that both subpopulations play a role in memory responses.

333

INFLUENZA SURVEILLANCE AMONG CHILDREN AND PREGNANT WOMEN PRESENTING TO HEALTH CARE FACILITIES IN BAMAKO, MALI

Mamadou B. Sylla[1], Doh Sanogo[1], Boubou Tamboura[1], Sandra Panchalingam[2], Adama M. Keita[1], Seydou Sissoko[1], Nana Kourouma[1], Mama N. Doumbia[1], Mahamadou M. Keita[1], Milagritos Tapia[2], Samba O. Sow[1], Karen Kotloff[2], Myron M. Levine[2]

[1]CVD-Mali, Bamako, Mali, [2]Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States

Virtually all epidemiologic data demonstrating an increased risk of complicated and fatal influenza illness among pregnant women and young infants derives from studies in industrialized countries in temperate or sub-tropical zones. To better define the burden of influenza in developing countries, we conducted surveillance among pregnant women and children < 36 months of age presenting to the Emergency Department (ED) of Hôpital Gabriel Touré and health centers in Banconi in Bamako, Mali. Women in the third trimester of pregnancy and children aged 0 to 35 months presenting with influenza-like illness (ILI) were identified. Per week of surveillance, up to 48 cases whose illness had lasted fewer than 4 days were sampled; this included up to 6 infants < 2 months of age, 24 children 2- 35 months of age and 18 pregnant women. After obtaining consent, nasal and throat swabs were obtained for analysis by real-time PCR for influenza virus. From November 2009 to February

2010, we recorded 1260 consultations for ILI including 1146 children and 114 pregnant women. Among these, samples were collected from 275 (24%) children and 40 (35%) women. Of the children, 24 (8.7%, 24/275) tested positive for 2009 H1N1 and 16 (5.8%, 16/275) had influenza B. Pregnant women tested positive for 2009 H1N1 in 10% (4/40) of cases and influenza B virus in 10% of cases (4/40). We found one case of co-infection (2009 H1N1 and B) in a pregnant woman. All cases were treated as outpatients and resolved without complications. In conclusion, ILI in Mali is associated with influenza A (2009 H1N1) and B infection. This preliminary surveillance suggests that broadening activities and conducting them throughout the year will provide invaluable information regarding the utility of vaccination in this setting.

334

HEPATITIS B IN IRAQ: CRISIS AND RESCUE OPTIONS

Husham Y. Ali

Dean, Technical and Health Institute, Zakho-Duhok-Kurdistan Region, Iraq

Hepatitis B is still of a global importance with more than 300 million people infected with hepatitis B virus (HBV) majority are resident in developing countries. In early eighties Iraq was considered as an area with intermediated endemicity of HBV infection and was pioneer among the Middle East countries in introducing HBV vaccination and incorporating it into Expanded Program of Immunization (EPI). The crisis escort the country from early eighties, inform of wars and sanctions, have created crisis which is continues smolder. The country suffered a humanitarian crisis from the massive degradation of the country's infrastructure and disability of health care systems and shortages of medicine and vaccines all over the country. Subsequences of the crisis, that many children who were born in eighties or early nineties either were non-vaccinated or have escaped one or two doses of HBV vaccine. This susceptible pool of children grows into adulthood and has been added to the preexisting reservoir of adult carriers of hepatitis B. Therefore, HBV carrier cases accumulate, especially HBeAg positive cases with increased tendency for transmission to others This paper will describe the breakdown of health care systems and it is impact on HBV infection rates during crisis and the challenges facing the country and the rescue options needed for control of this infection.

335

THE ROLE OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS NONSTRUCTURAL AND STRUCTURAL PROTEINS DURING INFECTION OF THE ENZOOTIC MOSQUITO VECTOR, *CULEX TAENIOPUS*

Joan L. Kenney, Rodion Gorchakov, A. Paige Adams, Scott C. Weaver

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

Venezuelan equine encephalitis generally exists in two ecological cycles: enzootic and epizootic. Viral strains that circulate in the enzootic cycle (ID and IE) are maintained in rodents and mosquito vectors (*Culex taeniopus*) and are typically unable to cause disease in equids. Epizootic viruses (IAB and IC) are able to infect equids and persist in a replication cycle between equids and epizootic mosquito vectors (*Aedes taeniorhynchus*). Previous studies indicate that the genotypic determinate for the epizootic phenotype is primarily found in the E2 glycoprotein of the structural regions of the virus. Interestingly, recent studies comparing the efficiency of epizootic, enzootic, and intermediate phenotypic strains suggest that there might be regions outside of the structural regions that play a role in determining the viral phenotype. We hypothesize that while the structural regions clearly play a role in establishing the epizootic phenotype, that regions outside of the structural regions may be important for fitness in the enzootic mosquito, *Cx. taeniopus*. To evaluate our hypothesis, we generated various chimeric VEEV viruses between epizootic (IAB Trinidad Donkey) and enzootic (IE 68U201) viruses to evaluate infection and dissemination within the enzootic mosquito vector. As expected, the IAB wild type was unable to infect or disseminate in Cx. taeniopus. The IE wild type virus infected 81% and disseminated in 44% of exposed mosquitoes. Both chimeras IAB/IE/IAB and IE/AB/IE infected 53% of exposed mosquitoes, but only the chimera with IE derived structural proteins (IAB/IE/IAB) was able to disseminate in 16% of exposed mosquitoes. The fact that the IE/IAB/IE chimera was able to infect Cx. taeniopus, while the wild type IAB was not, indicates that the genetic determinates for the enzootic phenotype include regions outside of the structural proteins.

336

INNATE IMMUNE PATTERN RECOGNITION RECEPTOR UTILIZATION BY RIFT VALLEY FEVER VIRUS

Megan Ermler[1], Ekaterina Yerukhim[1], Katherine Fitzgerald[2], Charles H. King[1], Amy G. Hise[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]University of Massachusetts, Worcester, MA, United States

Rift Valley fever virus (RVFV) is a zoonotic pathogen endemic to regions of Africa and the Arabian Peninsula. In the most severe cases, RVFV infection can cause retinitis, encephalitis, or hemorrhagic fever. The innate immune response to RVFV is suspected to be important in viral clearance but is still poorly defined. In animal models of RVFV infection, a strong protective role has been identified for type I interferon responses. In human infection, a delayed onset of interferon production is associated with the more severe forms of RVFV-induced clinical disease. Members of the Toll-like receptor (TLR) and RNA helicase families recognize viral patterns and stimulate type I interferon responses. In this study, human embryonic kidney (HEK) cells were used as a model for defining key innate recognition receptors during RVFV infection. HEK cells intrinsically express helicase RIGI and some basal levels of TLR3. HEK cells that overexpressed individual TLRs of interest were transiently transfected with luciferase reporter plasmids for NFκB and IFNβ signaling pathways. In addition, exogenous RIGI was transfected into cells to drive increased induction of the helicase pathway. Dominant negative constructs were utilized to block activity of adaptor molecules and subsequent signaling pathways. Results from *in vitro* studies were verified using primary bone marrow-derived dendritic cells and macrophages from wild-type and knockout mice. Recognition by endosomal TLRs drove NFkB mediated cytokine responses, whereas activation through helicases was the primary source for interferon production. The degree of dependence upon these receptors varied among cell types.

337

HOST GENETIC FACTORS AND SUSCEPTIBILITY TO HTLV-1-ASSOCIATED MYELOPATHY/TROPICAL SPASTIC PARAPARESIS IN PERUVIAN HTLV-1 INFECTED

Michael J. Talledo[1], Giovanni Lopez[1], Jeroen R. Huyghe[2], Kristien Verdonck[1], Elsa Gonzales[1], Daniel Clark[3], Guido Vanham[4], Eduardo Gotuzzo[1], Guy Van Camp[2], Lut Van Laer[2]

[1]Institute of Tropical Medicine Alexander von Humboldt Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Department of Medical Genetics, University of Antwerp, Antwerp, Belgium, [3]Facultad de Ciencias y Filosofía, Laboratorios de Investigación y Desarrollo (LID), Universidad Peruana Cayetano Heredia, Lima, Peru, [4]Virology Unit, Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium

HTLV-1 is a retrovirus associated to HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), a chronic neurodegenerative disease characterized by a paraparesis of the lower limbs. The causative factors that predispose to HAM/TSP development are not well known; high proviral load (PVL) has been associated to HAM/TSP in several populations. However PVL and viral factors *per se* do not explain fully HAM/TSP development. A multifactorial background is proposed for HAM/TSP, with host genetic, viral and environmental factors as contributors to disease susceptibility. A two stage study was performed to ascertain whether human genetic factors are associated to HAM/TSP disease in Peruvian HTLV-1-infected. Allelic distribution of 6 HLA, and 94 SNPs belonging to

45 genes were evaluated in 55 HAM/TSP patients and 114 asymptomatics (AC) HTLV-1 infected. SNPs with a trend of association ($P<0.1$) were evaluated in a second stage of 85 HAM/TSP and 146 AC. 36 Ancestry informative markers (AIMs) were analyzed to correct for population stratification. Logistic regression analysis was done in both stages to test for association between disease status and candidate genes. Age, gender, PVL and the first three principal components based on the AIMs were used as covariates. 12 SNPs from 9 genes showed a $P<0.1$ in the first stage: *IFN-γ -874*, *MMP2*-1306, *NFKB1A*, *NKG2D*, *NKG7*, *PD1*-1.9, *RANTES*-403, *TGF-β* -509, *TLR2*. In the second stage three of the twelve SNPs evaluated showed a $P\leq0.1$, SNPs belongs to *NFKB1A* and *NKG2D* genes. *P*-values for the full data set were calculated to determine if the trend of association was in the same direction, $P<0.05$ were observed for SNPs from *NFKB1A* (2 SNPs) and *NKG2D* (3 SNPs) genes. In conclusion, although no correction for multiple testing was performed due to the exploratory nature of the study, the P-values observed suggest that *NFKB1A* and *NKG2D* genes might influence susceptibility to HAM/TSP. These findings need to be confirmed in a larger population and/or in a different population of HTLV-1 infected to asses the implication of these genes over HAM/TSP disease.

338

LOW RATES OF HBV AND HIV CO-INFECTION IN TANZANIA

Jose Debes, William Stauffer, David Boulware

University of Minnesota, Saint Paul, MN, United States

Hepatitis B is a leading cause of liver disease in the developing world. Hepatitis B virus (HBV) shares infection routes with the human immunodeficiency virus (HIV).Therefore, areas with high endemicity of HIV, such as sub-Saharan Africa, tend to present high rates of infection with HBV. In the setting of HIV co-infection, both morbidity and mortality from HBV is increased compared to non co-infected populations. Moreover, with the use of antiretroviral therapy and its potential liver toxicity this relation becomes critically important. We conducted a prospective study to address the role of HBV co-infection in HIV-positive patients that were starting antiretroviral therapy in northern Tanzania. Appropriate approval was obtained from the research committee at Selian Lutheran Hospital in Arusha and the University of Minnesota in Minneapolis. Consent was obtained from participating subjects. Patients who started antiretroviral therapy were tested for hepatitis B s-antigen (HBsAg) and ALT in addition to routine laboratory tests, with the purpose of identifying those with chronic hepatitis B. Follow up with ALT and clinical symptom was intended to happen at 6 and 12 weeks when subjects returned for routine controls. Approximately 38% of our subjects were males and 62% females. Average age was 33 years. Surprisingly of 120 recruited patients, in two different periods of 3 months each, within 2 years, only 3 (2.5%) were positive for HBsAg. This rate of con-infection is much lower than previously reported in Tanzania for both general population and HIV-positive patients. The study was repeated in a smaller population using different reagents and laboratory settings and results were similar. Although occult hepatitis B (active hepatitis B virus with negative HBsAg) has been reported in HIV-positive patients, this is unlikely to account for such low numbers of HBV-infected individuals in an HIV-positive population. Further research to understand these results is warranted.

339

ESTABLISHMENT OF A NATIONAL VIRAL HEMORRHAGIC FEVER SURVEILLANCE PROGRAM IN UGANDA

Trevor Shoemaker[1], Stephen Balinandi[2], Jon Towner[1], Adam MacNeil[1], Pierre Rollin[1], Stuart Nichol[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention, Entebbe, Uganda

Uganda has experienced many past outbreaks of viral hemorrhagic fevers (VHFs), including recent outbreaks due to Ebola (Bundibugyo) and Marburg virus, in 2007. It is also an endemic region for many other viral zoonotic diseases, including Rift Valley Fever (RVF) and Crimean Congo Hemorrhagic Fever (CCHF), which have the potential to cause large outbreaks in animal and/or human populations. Because of this, the Special Pathogens Branch (SPB), Centers for Disease Control and Prevention, has collaborated with the Uganda Virus Research Institute (UVRI) to initiate a national VHF surveillance and laboratory network in Uganda. The overall goals of the VHF surveillance system are to enhance Uganda's capability to detect, diagnose, and respond to endemic VHFs in a timely manner. Through implementation, we will improve the collection and analysis of surveillance data, improve laboratory capacity for detecting viral pathogens and strengthen its role in epidemiologic surveillance and outbreak response. SPB has developed and implemented a standardized three tiered (suspect, probable, and confirmed) case definition for VHFs and a standardized case reporting form. The laboratory at UVRI has the capacity to perform diagnostic antigen-detection, IgM, and IgG enzyme-linked immunosorbent assays, as well as reverse-transcriptase polymerase reaction. These assays will be employed to confirm suspect VHF cases including Ebola hemorrhagic fever, Marburg hemorrhagic fever, RVF, CCHF, as well as arenaviruses and hantaviruses which may be endemic in Uganda. A comprehensive program, including training on collection of epidemiological data and clinical samples, has been initiated at multiple sites throughout Uganda. The sites were chosen based on proximity to areas with past VHF activity in human populations, or proximity to suspect natural zoonotic reservoirs. This network will help provide a baseline for VHF activity in Uganda and improve the early detection, diagnosis, and response to VHF outbreaks in Uganda.

340

RESPIRATORY SYNCYTIAL VIRUS IN CENTRAL AND SOUTH AMERICA: GENETIC VARIABILITY IN STRAINS CIRCULATING FROM 2007 TO 2009

Merly Sovero[1], Josefina Garcia[1], Victoria Espejo[1], Victor A. Laguna-Torres[1], Jorge Gomez[2], Melvin Barrantes[3], Felix Sanchez[4], Guillermo Comach[5], Roberto Agudo[6], Ana E. Arango[7], Nicolas Aguayo[8], Tadeusz J. Kochel[1], Eric S. Halsey[1]

[1]Naval Medical Research Center Detachment, Lima, Peru, [2]Directorate of Epidemiology, Lima, Peru, [3]Hospital F. Solano, Buenos Aires, Argentina, [4]Hospital Infantil Manuel de Jesus Rivera, Managua, Nicaragua, [5]LARDIDEV-Biomed-UC, Maracay, Bolivarian Republic of Venezuela, [6]Servicio Departamental de Salud, Ministerio de Salud, Cochabamba, Plurinational State of Bolivia, [7]Universidad Nacional de Antioquia, Medellin, Colombia, [8]NGO Asociacion Rayos de Sol, Asuncion, Paraguay

Respiratory syncytial virus (RSV) is a major cause of viral lower respiratory tract infections among infants and young children. It has a negative-sense, nonsegmented, single-stranded RNA genome. The significance of subgroup differentiation has been suggested by some studies. Subgroup A RSV may be been more virulent than subgroup B, and infection may result in greater disease severity among hospitalized infants. This virus expresses three transmembrane glycoproteins: the attachment glycoprotein (G), the fusion protein (F), and the small hydrophobic protein. The F and G proteins are important antigenically because they stimulate the production of protective immune responses. The G protein is of particular interest because variability in this protein is greater than that in the other proteins, both between and within the major antigenic groups of RSV. In this study, we evaluated the genetic diversity of both group A and B RSV strains by sequencing a variable region of the G protein gene of isolates collected during a three year period (2007-2009) in Central and South America. Nasopharyngeal throat swab specimens were collected at hospitals throughout Central and South America from patients who presented with a febrile, respiratory syndrome. Virus identification was accomplished using RT-PCR and this was followed by characterization and sequencing. From 7,198 samples collected, 185 (2.5%) were positive for RSV. Participants under 12 years of age accounted for 97% of the samples, clearly showing this virus is predominantly seen in children. We randomly selected 50 samples to be sequenced for genetic variability. Our results revealed the existence of two major antigenic groups of RSV, groups A and B, as two established strains circulating in Central and South

America during the last year. Two main branches were identified in group A: genotypes GA2 and GA5, with GA2 being predominant. The subgroup B that we observed was genetically similar to the virus isolated in Buenos Aires in 1999 (BA virus). These data provide a better understanding of the patterns of RSV strain circulation and the possible importance of strain differences to the consistency of yearly community outbreaks of RSV disease.

341

EVIDENCE OF ARENAVIRUS INFECTION AMONG FEBRILE PATIENTS IN PERU

Alfredo Huaman[1], Roger Castillo[1], Patricia V. Aguilar[1], Carolina Guevara[1], Manuel Cespedes[2], Victor Suarez[2], Luis Suarez[3], Tadeusz J. Kochel[1], Eric S. Halsey[1]

[1]Naval Medical Research Center Detachment, Lima, Peru, [2]National Institute of Health, Lima, Peru, [3]Directorate of Epidemiology, Lima, Peru

Viruses from the family *Arenaviridae* are usually associated with hemorrhagic diseases that are transmitted to humans by distinct species of rodents. Arenaviruses are grouped into Old World and New World complexes based on their geographic distribution and serological cross-reactivity. Among the New World arenaviruses, Machupo, Junin, Guanarito, Chapare, and Sabia are known to cause severe hemorrhagic disease in humans, whereas Tacaribe virus has resulted in a single example of febrile disease with mild central nervous symptoms. In Peru, Allpahuayo virus (New World complex) has been the only arenavirus isolated from arboreal rice rats (*Oecomys bicolor* and *Oecomys paricola*) in Iquitos. However, human infections associated with Allpahuayo and other arenaviruses remain unknown. In 2000, the U.S. Naval Medical Research Center Detachment (Naval Medical Research Center Detachment), Lima, in collaboration with the Ministry of Health of Peru, initiated a passive surveillance study to investigate etiology of febrile illnesses. Patients presenting at health posts or clinics with fever ≥ 38°C of no more than seven days duration and headache, myalgia, or other nonspecific symptoms were enrolled in the study. Two paired-blood samples were collected, one during the acute phase of illness and the second sample 2-4 weeks after onset of symptoms. Samples were processed for virus isolation and serological evidence of fourfold or greater increase in antibody titer to a variety of vector-borne viruses. Serological testing for arenaviruses was done for evidence of anti-Allpahuayo and anti-Tacaribe IgM by enzyme immunoassay (EIA). A four-fold or greater increase in antibody titer (indicating seroconversion) to Tacaribe and Allpahuayo was found in six and three patients, respectively. A presumptive case was defined as having an acute sample with titers ≥ 1:400. We found eight Tacaribe and one Allpahuayo case fulfilling this criteria. Virus isolation attempts were unsuccessful in all cases. The most common symptoms among Tacaribe and Allpahuayo patients with seroconversion included headache, chills, malaise, anorexia, myalgia, and arthralgia. Three of the Tacaribe cases had petechiae and one Allpahuayo case had bleeding gums. Additional studies are needed to confirm whether the patients were infected with Tacaribe and Allpahuayo virus or a related arenavirus. Results of this study suggest that arenavirus infection may be the cause of undifferentiated febrile illness in Peru.

342

FIRST SEROLOGIC EVIDENCE OF HANTAVIRUS IN PERU

Roger Castillo[1], Alfredo Huaman[1], Brett M. Forshey[2], Tadeusz J. Kochel[1], Carolina Guevara[1], Eric S. Halsey[1]

[1]Naval Medical Research Center Detachment, Lima, Peru, [2]Naval Medical Research Center Detachment, Iquitos, Peru

Hantaviruses are rodent-borne virus of the family *Bunyaviridae* and have been identified as etiological agents of two human diseases: hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome (HPS). HPS resulting from infection by hantavirus species such as Andes virus or Laguna Negra virus (LNV) has been reported in numerous countries of South America including Chile, Bolivia, Paraguay, and Brazil. Surprisingly, there have been no reports of human hantavirus infection in nearby Peru, although a Rio Mamore-like virus (RMV) has been isolated from a rodent (*Oligoryzomys microtis*) in the Amazon basin city of Iquitos, Peru. The objective of this study was to provide serological evidence of human hantavirus infection in Peru. A cross-sectional serosurvey was conducted with serum samples obtained from 1,316 healthy volunteers residing in urban areas of Iquitos. Serum samples were tested for IgG reactive to LNV, Sin Nombre Virus (SNV), and RMV antigens using an enzyme immunoassay (EIA). From 1,316 serum samples, a total of 30 (2.3%) contained IgG reactive to one or more hantaviruses. Two (0.2%) were positive for anti-LNV-RMV-SNV IgG by EIA, nineteen (1.4%) were positive only for anti-SNV IgG, seven (0.5%) were positive only for anti-RMV IgG and two (0.2%) were positive only for anti-LNV IgG. There were no significant age or gender differences between the hantavirus-exposed and unexposed populations. In conclusion, the finding of this study indicates human exposure to one or more hantaviruses in Peru.

343

PILOTING COMMUNITY INTERVENTION TO PREVENT NIPAH VIRUS TRANSMISSION USING BAMBOO SKIRTS

Rebeca Sultana, Utpal Kumar Mondal, Dawlat Khan, M. Jahangir Hossain, Shamim Azad, M. Abu Sharker, Nazmun Nahar, Stephen P. Luby

ICDDR,B, Dhaka, Bangladesh

Human Nipah virus (NiV) outbreaks have occurred regularly in central and north-western Bangladesh. Fruit bats are the natural reservoir of NiV. When people drink raw date palm sap, apparently contaminated with infected bats' saliva and urine, they occasionally contract NiV infection. In prior studies, gachhis (date palm sap collectors) expressed interest in placing a bamboo skirt over the sap flow and pot to prevent bat access to date palm sap. The aim of this study was to assess the acceptability and take-up of a promotion campaign to encourage the use of bamboo skirts by the date palm harvesting community. We conducted an intervention trial from December 2009- February 2010, in Boalmari sub-district in Faridpur district of Bangladesh. We facilitated 15 community meetings to introduce NiV infection, possible way of NiV transmission through date palm sap and how bamboo skirt interrupts bat access to sap. We identified gachhis and tree owners through a baseline survey. We randomly sampled 79 tree owners out of 1303 and took an equal sample of 79 gachhis out of 168. After one month, we assessed the early impact of the intervention by interviewing gachhis and tree owners, and observing the date palm trees. At baseline, no bamboo skirts were used in the community. One month after the intervention, 34% of gachhis (20/59, p-value <0.001) and 14% of tree owners (7/49, p-value <0.001), who drink raw sap, used bamboo skirts. In addition, 5% (3/59) of gachhis and 2% (1/49) of tree owners used jute skirts. Gachhis reported that jute stalk is locally available and required less time to make into a skirt compared to bamboo. Thirteen gachhis and five tree owners also reported covering their trees with cloth, mosquito nets and thorns. Observations confirmed the reported practices. In conclusion, many respondents adopted bamboo skirts implying the community's receptiveness to the intervention. Inclusion of locally available materials and increased duration of the intervention might further improve uptake.

344

ASSESSMENT OF PLAQUE ASSAY METHODS FOR ALPHAVIRUSES

Diana Juarez[1], Kacey Long[2], Patricia V. Aguilar[1], Tadeusz J. Kochel[1], Eric S. Halsey[1]

[1]Naval Medical Research Center Detachment, Lima, Peru, [2]University of Texas, Medical Branch, Galveston, TX, United States

Alphaviruses have been responsible for outbreaks involving thousands of human and equine cases of severe disease in the Americas. Confirmation

of alphavirus cases is based on viral isolation or a four-fold or greater increase in antibody titers between acute and convalescent samples. Specificity of antibodies to an alphavirus is usually confirmed by plaque reduction neutralization assays. However, the performance of two standard methods of PRNT (semisolid and solid) have not been compared for this group of viruses. In an attempt to identify the best method for alphavirus and neutralizing antibody recognition, we evaluated: 1) a semisolid method using a 0.6% carboxymethyl cellulose overlay, and 2) a solid method using a 0.4% agarose overlay. Initially, Mayaro virus (MAYV), UNA virus (UNAV) and Venezuelan equine encephalitis virus (VEEV) were titrated using both methods. Next, positive controls were evaluated to determine neutralizing antibody titers. To confirm results, we tested acute and convalescent sera from 19 patients who had MAYV isolated from their acute sample. The solid method consistently showed greater sensitivity than the semisolid method. The alphaviruses provided ~5-9 fold higher viral titers using the solid method and also gave a higher neutralization titer on positive controls. Specific MAYV neutralizing antibodies (40 or higher) were detected in 17 of 19 of the convalescent sera (and none of the acute) using the solid method while all samples were negative with the semisolid method. Neutralizing antibodies against VEEV and UNAV were not detected in these samples further confirming the specificity of the solid assay. In conclusion, our results provide evidence that the solid method is superior in detecting alphaviruses and alphavirus neutralizing antibodies and should be the method of choice when using PRNT as a confirmatory test.

345

AVIAN FLU: PILOTING AN INTERVENTION TO REDUCE THE RISK OF TRANSMISSION TO BACKYARD POULTRY-RAISING FAMILIES IN RURAL BANGLADESH

Nadia A. Rimi, Rebeca Sultana, Kazi I. Ahmed, Marufa Hasin, Md. Z. Rahman, Afroza K. Roza, Nazmun Nahar, Salah U. Khan, Andrea B. Mikolon, M. S. Islam, Emily S. Gurley, Stephen P. Luby

International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Available evidence suggests that most backyard poultry raisers in Bangladesh are not implementing recommended practices to reduce the risk of H5N1 transmission to humans. We developed and piloted preventive messages focused on slaughtering sick poultry, one of the riskier behaviors. We explored the acceptability and feasibility of the messages in the community. We implemented the intervention in two villages during June-August, 2009. We held five one-hour meetings to introduce bird flu, its clinical signs, transmission, and preventive messages through a pictorial flipchart and posters. Each participating villager attended one session. The messages encouraged covering one's nose and mouth with clothing while slaughtering, burying offal, hand washing, and cleaning slaughtering tools and site. We also suggested isolating sick poultry, burying carcasses, and avoiding buying, selling or consuming sick poultry. We documented community response using observation, in-depth interview, group discussion, and informal conversation. We observed both before and after intervention that villagers occasionally separated sick poultry, buried offal of sick and healthy poultry, and cleaned the slaughtering site. During interviews after the intervention, residents expressed willingess to slaughter their poultry that were sick with bird flu using the safer methods, but were unwilling to avoid slaughtering or selling sick poultry as they would lose household income. They reported they had never observed this disease in their area. Later, when poultry die-offs of unidentified cause occurred, their slaughtering practices were unchanged. In conclusion, the villagers already practiced some preventive behaviors, however, their normal practices risk transmission of influenza from poultry to people. More intensive communication and follow-up may increase these poultry raisers' risk perception, but financial loss is an important impediment to safer behavior. Encouraging safer slaughtering for all sick poultry or all poultry may be an achievable objective for behavior change.

345A

MOLECULAR EPIDEMIOLOGY AND GENETIC DIVERSITY OF RABIES VIRUS ASSOCIATED WITH BATS IN PERU

Rene Edgar Condori[1], Andres Velasco-Villa[2], Sergio Recuenco[2], Charles Rupprecht[2], Cesar Cabezas-Sanchez[1]

[1]Instituto Nacional de Salud, Lima, Peru, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States

During the last decade in South America, rabies perpetuated by the common vampire (*Desmodus rotundus*) increased considerably, especially in remote areas of the Amazon. Typically, domestic animals and humans are exposed by contact with infected wildlife. To determine current epidemiological links, viral diversity and disease spread, complete and partial sequences of the nucleoprotein (N) gene of viral isolates were studied from 2002 - 2007 in different mammalian species and distinct geographical areas. Phylogenetic analysis revealed that Peruvian rabies viruses could be grouped in at least seven distinct lineages. *D. rotundus* was incriminated as a principal reservoir and transmitter throughout Peru. Viruses isolated from vampire bats and livestock suggest long-term transmission pathways Overlap of some lineages was found, as was evidence of spillover to other species, and a suggestion of geographic translocations. Two viral variants not previously identified in the Americas were found in a kinkajou (*Potus flavus)* and the insectivorous bat *Histiotus montanus*, from the south eastern part of the Amazon. One RV lineage was widely distributed from Colombia to Madre de Dios. This is the first comparative study on genetic diversity of rabies virus in Peru and its relationship with other variants in the Americas. Our results provide a better understanding of the molecular epidemiology of rabies associated with bats in Peru.

346

DEVELOPMENT AND FIELD USE OF A SIMPLE, PORTABLE TEST TO QUANTIFY H_2S-PRODUCING FECAL BACTERIA IN DRINKING WATER AS PREDICTORS OF DIARRHEAL DISEASE RISK

Ku L. McMahan, Mark D. Sobsey

University of North Carolina at Chapel Hill School of Public Health, Chapel Hill, NC, United States

Lack of access to safe water, improper sanitation and poor hygiene contribute to an ongoing global health and development crisis resulting in millions of deaths and infectious disease morbidity burdens affecting billions of persons annually. Inadequate water, sanitation and hygiene account for roughly 94 percent of the 4 billion cases of diarrhea that WHO estimates occur globally each year. In order to know if water is safe to drink and if WHO-recommended Water Safety Plans for hygienic water management are achieving microbially safe water, drinking water and its sources must be tested regularly. Given the lack of access to microbial testing of water in resource-limited settings and especially in most water supply settings in developing countries, there is a need for simple, low cost tests for fecal indicator microbes that can be performed by the water consumer at the point of use or by others on their behalf (water suppliers, community health workers, government agencies, etc.). This research focused on the development of a compartmentalized plastic bag for water quality testing for hydrogen sulphide (H_2S)-producing fecal microbes in water to estimate their concentration as Most Probable Number (MPN). Analysis of lab spiked-sewage samples, natural water samples from North Carolina, and household drinking water samples from community water supplies in central Vietnam showed that there are significant relationships between H_2S producing bacteria and E. coli (97% of samples were either both positive or both negative for E. coli and H_2S producing bacteria in lab studies, as were 69% of samples from Vietnam). Furthermore, molecular analyses of the bacterial community structure of select Vietnamese water samples show that there is a strong relationship between positive H_2S tests and water containing enteric pathogens and fecal indicator bacteria

of concern. Moreover, a household water and sanitation study in central Vietnam communities showed that there was a significant relationship between increasing levels of H_2S -producing bacteria and diarrheal disease{Odds Ratio 1.28 (95% CI 1.051328-1.388585), p=0.008}. We conclude that the low-cost MPN compartment bag test for H2S-producing bacteria is simple and easy-to-use in field settings (as little or less time than both the IDEXX Colilert system and standard membrane filtration techniques), is low cost and is amenable to widespread commercial production and distribution.

347

A QUALITATIVE EXPLORATION OF BARRIERS TO PROMOTE WATER TREATMENT TECHNOLOGY IN RURAL BANGLADESH

Shaila Arman, Nazmun Nahar, Leanne Unicomb, Mohammad Abdul Kadir, Fazlul Kader Chowdhury, Md. Al Mamun, Subas Chandra Biswas, Smriti Roy, Stephen P. Luby

International Center for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Drinking contaminated water is major cause of morbidity and mortality among children <5 in Bangladesh. Point of use water treatment technologies could provide safe drinking water at the household level. To date, such interventions have failed to achieve high rates of regular use. This study was conducted to understand the barriers affecting the uptake of water treatment technology at the household level. We conducted this study in two villages where a local non government organization had promoted subsidized arsenic removal filters. We enrolled both users and non users who had a child < 5 years and a monthly income of < TK 5000 (US$ 72). In-depth interviews identified practices of household water management, experience with water treatment technology, and perceptions of water quality and water born diseases. Water from tube-wells was the main source of drinking water. It was considered safest because it originated underground, despite being contaminated with iron and arsenic. Surface water was not preferred because it was turbid, and used for bathing and washing utensils. Safe drinking water was only judged by its appearance, not linked to germs or pollutants. Diarrheal diseases were not considered serious and were believed to be related to food, not water. Since arsenic poisoning is not immediately visible, informants did not recognize it as a serious health hazard. Barriers to use of water filters included collecting water directly from tube-wells for immediate consumption without storage. Users disliked the taste and smell of filtered water. The water flow was slow, maintenance was difficult and spare parts were not available. In conclusion, the idea of pathogen free water was not particularly meaningful to these village residents; rather they focused on its appearance, taste, and smell. Stressing the positive aspects of water treatment technology to the non-users, such as visible filtering of iron and improvement in color, along with ensuring access to affordable filters, and the necessary spare parts, could increase uptake.

348

AN ASSESSMENT OF USE OF SIPHON FILTERS IN LOW INCOME COMMUNITIES OF URBAN DHAKA, BANGLADESH

Nusrat Najnin[1], Leanne Unicomb[1], Minhaj Mahmud[1], David Levine[2], Jill Luoto[2], Jeff Albert[3], Jaynal Abedin[1], Stephen Luby[1]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]University of California, Berkeley, California, CA, United States, [3]The Aquaya Institute, San Francisco, CA, United States

Siphon water filters are a low cost point of use water treatment technology providing safe drinking water at household level in low income populations. There is little evidence of why this group continues to use these filters or not. This study aimed to identify both motivators and barriers to sustained use. We conducted a 3 month follow up survey among a low income community in urban Dhaka, Bangladesh who received siphon filters at the end of a randomized control trial as part of an assessment process of willingness to pay, and who had recent experience using the filters as part of this study. Some participants received this hardware free of cost while others had to pay up to 5 US$. We interviewed household caregivers and tested water quality using the H_2S method, a 24 hour color-coded system to confirm contamination. Among the 178 study participants, 82% (n=145) reported ever using the filters. Of these, 49% (n=71) reported using it within seven days of our interview. From household spot checks we observed that 37% (n=54) of households filters were used recently. There was no difference in usage between people who did or did not purchase the filters (28% vs. 30%, p=0.84). A higher proportion of observed recent users had better quality stored water compared to non users (30% vs. 18%, p=0.07), determined by bacterial growth from the H2S test. The most frequent reasons for not using the filter were using other treatment methods (11%, n=19), filter being broken or clogged (10%, n=18) and too troublesome (10%, n=17). People who perceived they are 'treating water like a modern person' were more likely regular filter users on multivariate regression analysis (OR: 7.4, 95% CI: 1.4, 38.4). Regular use was not strongly associated with perception of 'improving health' (OR: 2.4, 95% CI: 0.6, 10.2). In conclusion, although filter users had better water quality compared to nonusers, only one third of the people continued to use it after 3 months. Non health benefits were the primary determinant of regular filter usage.

349

PERCEPTIONS, PRACTICES AND BARRIERS OF HANDWASHING IN RURAL BANGLADESH

Aasma Afroz[1], Sharifa Nasreen[1], Leanne Unicomb[1], Emily S. Gurley[3], Shaila Arman[1], Mohammad Abdul Kadir[1], Dorothy Southern[1], Peter J. Winch[2], Eduardo Azziz Baumgartner[3], Stephen P. Luby[3]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

Handwashing with soap can substantially reduce the risk of diarrhea and respiratory disease, yet, observational studies in Bangladesh show that practice of hand washing with soap, soil, or ash is infrequent. We conducted a qualitative study in three rural villages of Bangladesh to explore perceptions, practices, and barriers to hand washing with different cleansing agents at different times. From the findings of structured observations from a previous study we knew that these villagers were using an agent for handwashing. To understand the perceptions and barriers of using an agent for handwashing a convenience sample of adult males and females was selected for in-depth interview (25). We used pocket voting (30), an interactive exercise with school children to assess the actual hand washing practices with different cleansing agents. Habitually, people rinse their hands with only water. They said, soap helps to get rid of stickiness, bad odor after defecation or to remove poison after spraying pesticides. Using soil after defecation was embedded in the context of religion, which is mainly practiced by the elderly. To avoid direct contact with stool, the anus is cleaned with three pieces of hard soil and then hands are rubbed with soil or ash before rinsing with only water. From pocket voting, we found girls practice better handwashing than boys. Most of the children indicated that they used a hand washing agent after defecation and the most common agent was soap followed by ash. Ash is only used after defecation, when soap is not available. After defecation, the absence of soap inside latrines was an important barrier to handwashing with soap. Respondents were reluctant to use the same soap for handwashing at other times which they used for washing hands after defecation. None of the informants washed their hands before cooking. According to most, hands should be washed when visibly dirty, while only a few perceived that hands should be cleansed to remove germs or to prevent diarrheal disease. In conclusion, these residents of rural Bangladesh wash their hands to remove visible dirt and not to reduce the risk of disease transmission. Messages to promote hand washing with soap may be more effective if they capitalize on people's desire for cleanliness.

350

BARRIERS TO CHILDREN ADOPTING A HANDWASHING HABIT IN URBAN BANGLADESH

Md. Fosiul Alam Nizame[1], Leanne Unicomb[1], Stephen P. Luby[1], Shuburna Chodhuary[1], Md. Nuruzzaman[1], Hoshne Nur Rob[1], Jaynal Abedin[1], Pavani Kalluri Ram[2]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]University at Buffalo, State University of New York, Buffalo, NY, United States

Handwashing with soap can reduce diarrhea, a major cause of mortality in Bangladesh. Increasingly, hygiene promotion programs seek to inculcate handwashing habits among young children. We sought to understand physical and behavioral barriers to establishing handwashing habits in the home early in a child's life in low-income community in Dhaka. We conducted a household survey by visiting the 5th household in each compound of houses in the Dhaka study community. Through observations and interview, we recorded physical and behavioral barriers to use of soap for handwashing by children aged 2-7 years. Of 146 households enrolled, 143 (98%) had a handwashing station, defined as the place where hands are usually washed after using the toilet. The station was outside both toilet and cooking place in 82% of households and the mean distance from the latrine was 6 steps (SD=7.6). Water was available at 96% of the 143 handwashing stations on the interview day, although only 76% of respondents stated that water is always available. Soap was observed at the handwashing station in only 40 (28%) and was located at a mean height of 101 cm (40 inches). Among households that did not keep soap at the handwashing station, 75 % reported bringing soap from inside the house to wash hands. Respondents indicated that bar soap cannot be kept at the station because others might use it (42%), soap could be stolen (31%), and there is no convenient place to keep the soap at the station (13%). Of 54 respondents who had children 2-7 years old, 81% reported that the child knows where soap is kept at home. In 22 households that had children 2-7 years old and that had bar soap present at the handwashing station, 10 (45%) allowed those young children to access that bar soap. In conclusion, soap at the handwashing station is accessible to children aged nearly 4 years, according to international child growth standards. While many young children could reach soap at this height, soap is not actually kept at the handwashing station in a majority of households. Even when soap is kept at a handwashing station, a minority of children are allowed to access it, preventing many young children from independently forming a habit of handwashing after using the toilet. Community- and school-based handwashing programs seeking improvement of children's handwashing behavior in Bangladesh must aim for parental behavior change with respect to children's access to soap at home.

351

PILOT STUDY OF SERIAL SOAP WEIGHTS AS A NEW METHOD OF MEASURING HANDWASHING; DHAKA, BANGLADESH

Meghana A. Gadgil[1], M. Abu Yushuf Sharker[1], Pavani Ram[2], Leanne Unicomb[1], Stephen Luby[1]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]SUNY Buffalo School of Public Health and Health Professions, Buffalo, NY, United States

Handwashing with soap reduces diarrhea and respiratory mortality for children < 5 in low-income countries. Accurate, inexpensive measures of handwashing are needed to evaluate handwashing interventions. Current measures are invalid or prohibitively resource-intensive. We tested the feasibility of serial soap weights to measure handwashing. Fieldworkers conducted 8 bi-weekly visits to 180 households with at least 1 child < 5 in an urban slum of Dhaka. At each visit, we interviewed participants and weighed soap products. Soap weights were included in analysis if there was no soap replacement in the interval. Soap weight differences were compared to several measures of handwashing: visual inspections of respondent palms for cleanliness, presence of soap and water at the handwashing station, and handwashing with soap at critical times such as after defecation, as observed during 5hr structured observations (SO). Main handwashing products were bar soap (87%) and laundry soap (13%); all households also used their main handwashing product for either bathing or laundry. Soap weight differences were stable over the course of 8 visits (by regression on visit number, bar soap p-value 0.42, laundry soap p-value 0.86). Most (63%) respondents reported increased soap consumption on Fridays (a weekend day in Muslim Bangladesh). Mean bar soap use was 1.5g/day/person (95% CI 1.56- 1.62) & mean laundry soap use was 3.2g/day/person (95% CI 3.17 - 3.31). Compared to 3, 4, and 5-day intervals, the 2-day interval had the most soap weight data included in analysis (63%) due to fewer replacements between visits. Bar soap and laundry soap weight changes were not correlated with the SO measure of handwashing (Pearson's r = -.04 and -.09 respectively). Similarly, soap weight changes were not correlated with palm inspections or with presence of soap and water at the handwashing station. In conclusion, accurate soap consumption measurements may be possible with a few household visits 2 or 3 days apart, provided weekly spikes in soap use are accounted for. Soap weights had poor correlation with three different measures of handwashing, each with its own limitations. However, given the reliability of soap weight differences, the serial soap weight method merits further validation as a measure of handwashing behavior by testing its correlation with health outcomes.

352

ORAL REHYDRATION USE BY CAREGIVERS OF YOUNG CHILDREN WITH DIARRHEA IN A POOR PERI-URBAN DISTRICT IN THE DOMINICAN REPUBLIC

John D. McLennan

University of Calgary, Calgary, AB, Canada

Oral rehydration is an effective strategy for decreasing morbidity and mortality from diarrhea. However, gaps have been reported in its use at the household level. This study examined the extent of use of oral rehydration and predictors of its use by caregivers of young children with diarrhea. Three samples of caregivers of young children from peri-urban Dominican Republic participated in a structured interview on child health which contained closed-ended questions about responses to childhood diarrhea. Of the 541 participating caregivers, 156 (29%) reported that the index child had had an episode of diarrhea within the last four weeks. Bivariate analysis was used to explore the relationship between hypothesized predictors and oral rehydration use. The following pattern of responses were reported for those whose child had had a recent diarrheal episode: went to the doctor (59%), used tea (49%), used package oral rehydration solution (40%), used coconut oil (33%), used an antibiotic (20%), used a homemade sugar/salt solution (4%). Those reporting having used oral rehydration were more likely to perceive their child to have been dehydrated, to have taken the child to a doctor, and to have also used an antibiotic. Children receiving oral rehydration were more likely to be younger and to have had more days of diarrhea. In conclusion, there are still gaps in the extent to which caregivers employ oral rehydration as a response to childhood diarrhea. Variables associated with oral rehydration use may provide a preliminary understanding of factors influencing its use.

353

TRENDS IN DIARRHEAL DISEASE MORTALITY IN CHILDREN <5 YEARS OLD, ACCESS TO IMPROVED WATER SOURCES AND USE OF HOUSEHOLD WATER TREATMENT, CDC/ KEMRI DEMOGRAPHIC SURVEILLANCE SYSTEM, NYANZA PROVINCE, KENYA, 2003-2008

Kashmira A. Date[1], Robert Quick[1], Daniel Feikin[2], Kayla Laserson[2], Sheila Ogwang[3], Mary Hamel[2]

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

Diarrheal diseases are a leading cause of death in children <5 years old in Kenya. In 2003, a nationwide social marketing campaign was initiated in Kenya to reduce diarrhea risk through sale of chlorine solution for household water treatment; from 2003-2008, >7.1 million bottles were sold. To measure the impact of this program on diarrheal disease mortality, we examined data from the CDC/Kenya Medical Research Institute (KEMRI) Demographic Surveillance System (DSS), a longitudinal, population-based health and vital event registration system, designed to monitor health and demographic dynamics in Nyanza Province of rural western Kenya. We conducted an exploratory ecological analysis of DSS demographic and verbal autopsy data from 2003-2008 in two DSS sites. We calculated the diarrhea mortality rate per year in children <5 years old, and determined the proportion of households per year reporting access to improved water sources, and use of household water treatment. From 2003-2008, 999 (6%) of 17,232 deaths were determined by verbal autopsy to be diarrheal deaths; 628 (63%) were in children <5 years old. During this period, the diarrhea mortality rate decreased by 51%, from 73 to 36 per 10,000 children <5 years old, with the greatest decrease in 2005. During the same period, the proportion of households reporting access to improved water sources increased from 38% to 48%, with almost all of the increase occurring after 2006. The reported use of chlorine water treatment products steadily increased from 4% of households in 2003 to 32% of households in 2008, with 66% of the increase occurring after 2005.Although reported household chlorine water treatment and access to improved water sources increased during this period, most of these changes appeared after the largest decline in diarrheal mortality. Further study is warranted to determine other possible explanations for the decline in diarrheal mortality including trends in diarrhea treatment, association with other illnesses, such as malaria, HIV infection and malnutrition, and other environmental factors.

354

EXAMINING THE SOCIAL AND ENVIRONMENTAL FACTORS THROUGH WHICH NEW ROAD DEVELOPMENT IMPACTS DIARRHEAL DISEASE INCIDENCE

Darlene Bhavnani[1], Bhramar Mukherjee[1], William Cevallos[2], Gabriel Trueba[2], Joseph N. Eisenberg[1]

[1]University of Michigan, Ann Arbor, MI, United States, [2]Universidad San Francisco de Quito, Quito, Ecuador

Road development has been linked to an increase in infectious disease incidence. A previous study found higher rates of diarrhea in villages that were less remote due to new road construction in northern coastal Ecuador. While remoteness is a distal factor that is associated with diarrhea, what remains to be examined are the proximal social and environmental factors through which decreasing remoteness impacts diarrhea incidence. In this study, we investigated the causal pathway between remoteness and diarrhea. We estimated specific indirect effects of remoteness through various proximal factors hypothesized to be associated with diarrhea, the direction of which were sometimes opposing. For example, decreasing remoteness was correlated with shorter residence time in the community, a proxy for increased movement of people and pathogens, increasing risk of diarrhea. However, development also brought with it improved sanitation, decreasing risk for diarrhea. We used case-control data collected between July 2003 and February 2008 from 21 communities of varying remoteness in northern coastal Ecuador. Each community was visited seven times for a period of 15 days, during which all cases of diarrhea were identified through daily household visits. Both household and community controls were randomly selected upon case identification. Cases were defined as individuals with three or more loose or watery stools passed in a 24-hour period. Stool samples collected from cases and controls were used to estimate pathogen-specific diarrhea (*E.coli*, rotavirus and *Giardia*). For analysis, we employed a structural equations modeling approach, bootstrapping our 95% confidence intervals to determine relative effect sizes of the hypothesized specific indirect effects of remoteness on diarrhea. This study provides insight on the social and environmental changes that accompany development in rural areas as well as their impact on diarrheal disease burden.

355

PERSPECTIVES ON DIARRHEA AND HOUSEHOLD WATER CHARACTERISTICS IN TRINIDAD, BOLIVIA

Emily R. Smith, Vaughn Barry, Christine L. Moe

Emory University, Rollins School of Public Health, Atlanta, GA, United States

Diarrhea is a primary cause of morbidity and mortality around the world. Improved water, sanitation, and hygiene are primary pathways of diarrhea prevention. The objective of this research was to identify household knowledge, attitudes, and practices surrounding household water use and diarrhea. We conducted 374 household interviews in 40 neighborhoods in peri-urban Trinidad, Bolivia during summer 2009. Median self-reported household income was $2300 Bolivianos and an average of 5.9 people were reported living in each household. The majority of respondents considered the community to be healthy or very healthy (72%), and 94% considered diarrhea to be dangerous or very dangerous. At least half of respondents reported water and rotten food to be causes of diarrhea, while fewer than 15% identified poor hygiene behavior, such as lack of hand-washing at key moments, to be a cause of illness. Only half of households reported that they always or usually treating drinking water, and more than one third of households reported never treating water. Among households that reported always or usually treating water, most reported boiling (31%) or the use of a fabric, ceramic or sand water filter (23%). Notably, 22% of respondents report owning a ceramic filter, while only 7% report this type of filter as a method they use to treat water. The majority of households with ceramic filters obtained the devices from NGOs during widespread flooding (2007-2008). Approximately half of the households indicated that they would pay up to 200 Bolivianos ($28 USD) for a ceramic water filter. These results indicate that improved hygiene education is needed in peri-urban Trinidad. Furthermore, improved education should accompany distribution of filters during natural disasters to improve uptake and consistent use of these devices for water treatment. Ultimately, these results will be utilized to design water, sanitation, and hygiene interventions for residents in Trinidad, Bolivia.

356

THE IMPACT OF A WATER TREATMENT PROGRAM ON GASTROINTESTINAL PARASITE FREQUENCY

Jeffery L. Deal[1], **James T. McElligott**[2], George C. Greene[1], Andrea P. Summer[2]

[1]Water Missions International, Charleston, SC, United States, [2]Medical University of South Carolina, Charleston, SC, United States

Previously published results revealed a reduction in the proportion of individuals with parasites present in stool in Honduran villages with potable water and sanitation interventions. The communities received no intervention (Control), a water treatment intervention (Water) or water treatment and sanitary pit latrine with pour flush toilet (Toilet).

Any intervention was associated with a lower proportion of positive (+) tests, though the proportion + was higher in the Toilet group than with Water only. The purpose of this analysis is to generate a hypothesis for this discrepancy. The primary outcome is the magnitude of difference in proportion of stool + (*Giardia, Cryptosporidium, Entamoeba*) between groups with subgroup analysis for age and gender. There were 4 Control communities (N = 86), 4 Water (N=112) and 4 Toilet (N=53). Magnitude is measured with relative risk (RR) with 95% confidence intervals (CI) and logistic regression. The relative risk of a + test for the Control group versus any treatment was 1.98(CI 1.28-3.08). The Toilet group had less + than Control (RR1.32,CI.77-2.25), but more than Water group alone (RR 1.97,CI 1.03-3.78). By gender a trend towards increased + in the female group is seen in the Toilet group only (RR 1.49,CI.58-3.9). By multivariate analysis controlling for age and gender, the odds (OR) of having a + test were 0.42 (CI.23-.79) for any treatment compared to no treatment. For the Toilet group alone there was a trend towards increased odds for female gender (OR1.6,CI.43-5.9) not present when all patients were analyzed (OR.98,CI.52-1.8). In conclusion, though underpowered for subgroup analysis, these results indicate that the reduced improvement associated with communities who received toilet and water interventions may be due to the greater proportion of females + for parasites. The fact that women clean the toilets may account for their suspected increased parasite burden. Future investigations will include adjustments to the Toilet intervention to further test this hypothesis.

357

SEASONAL DIARRHEA INCIDENCE IN NICARAGUA, 2001-2002: IMPLICATIONS FOR THE EFFECTIVENESS OF UNIVERSAL ROTAVIRUS IMMUNIZATION

Rodolfo Pena[1], Magdaly Torres[1], **Sylvia I. Becker-Dreps**[2], Wilton Perez[1], Douglas R. Morgan[2], Peter Allebeck[3], Carina Kallestal[4]

[1]Centro de Investigacion en Demgrafic y Salud (CIDS), Leon, Nicaragua, [2]University of North Carolina, Chapel Hill, NC, United States, [3]Karolinska Institutet, Stockholm, Sweden, [4]Women's and Children's Health, IMCH, University Hospital, Uppsala, Sweden

Approximately 90% of diarrhea episodes are treated at home and are not captured by commonly-used hospital-based surveillance systems. In order to estimate the potential benefit of rotavirus immunization in Nicaragua, we measured the diarrhea incidence among a community-based sample of children during the dry and rainy seasons. Rotavirus, the most common cause of childhood diarrhea, is transmitted most intensely during the dry season in Central America, while bacterial pathogens are typically more common during the rainy season. The study was conducted using the Health and Demographic Surveillance System in León, Nicaragua. We randomly selected 414 households from the sampling frame of the Surveillance System. Field interviewers enrolled 726 children under age 5 from these households and returned every 2 weeks to record any diarrhea episodes. The children were followed for 13 weeks during the dry season and 20 weeks during the rainy season in 2001-2002. The diarrhea incidence rate was calculated by season and compared using Poisson regression analysis. Among a total of 726 enrolled children, 216 children experienced diarrhea; 108 occurred during the dry season and 194 occurred during the rainy season. Diarrhea incidence in all age groups increased during the rainy season. Overall, incidence was 0.072 (95% CI 0.058, 0.085) episodes per person-month during the dry season and 0.090 (95% CI 0.077, 0.102) episodes per person-month during the rainy season. In conclusion, we found a higher incidence of diarrhea during the rainy season, using a community-based sample of children. These findings have implications for the recent introduction of the rotavirus vaccine, which will likely reduce the burden of diarrhea during the dry season, but have less of an impact during the rainy season.

358

AN EXAMINATION OF THE RELATIONSHIP BETWEEN LEVELS OF DRINKING WATER QUALITY INDICATORS AND THE OCCURRENCE OF SELF-REPORTED DIARRHEAL DISEASE: A PROSPECTIVE COHORT STUDY IN THE DOMINICAN REPUBLIC, 2005-2006

Shannon M. Kraft[1], Christine E. Stauber[1], Gloria M. Ortiz[2], Mark D. Sobsey[2]

[1]Georgia State University, Atlanta, GA, United States, [2]University of North Carolina-Chapel Hill, Chapel Hill, NC, United States

Based on the 2010 report of the Joint Monitoring Program, 884 million people do not have access to clean water. Lack of access to clean water is a known contributor to the occurrence of diarrheal disease (JMP, 2010). The purpose of this study was to examine the potential associations between the occurrence of diarrheal disease and the levels of three water quality indicators, turbidity, total coliforms, and Escherichia coli (E. coli), in 185 households in Bonao, Dominican Republic in a four-month observational study of diarrheal disease in 2005-2006. Datasets included a biweekly water quality dataset and a weekly diarrheal disease occurrence dataset. These two datasets were merged using three different methods, which impacted the number of observations. T-tests and odds ratios were calculated for all three different datasets. Multivariate logistic regression was also conducted. P-values of <0.05 and 95% confidence intervals were used to determine statistical significance of water quality indicators in predicting diarrheal disease. There were 430 cases of diarrhea out of 14,245 observations. In the age-adjusted multivariate logistic regression, turbidity (OR = 1.36; p-value =.012) was the only water quality indicator found to be positively associated with the occurrence of diarrhea disease. In conclusion, this study strengthens the evidence supporting a positive association between turbidity and the occurrence of diarrhea as has been shown in two recent studies examining drinking water quality and diarrheal disease in the United States. Future studies are needed to further clarify which water quality variables are predictive of diarrheal disease.

359

EXAMINING THE INFLUENCE OF ECONOMIC AND POLITICAL FACTORS UPON ACCESS TO IMPROVED WATER AND SANITATION IN SELECT AFRICAN NATIONS, 2005-2008

Dlorah Jenkins, Christine Stauber, Monica Swahn

Institute of Public Health, Georgia State University, Atlanta, GA, United States

Today, 884 million people worldwide lack access to safe drinking water and 2.6 billion are without access to improved sanitation facilities. While many nations are on track towards meeting the Millennium Development Goals of decreasing the proportion of those without improved water and sanitation, progress in many developing nations is lacking**.** The purpose of this study was to determine what influence political and economic factors have on the availability of improved water and sanitation in developing nations, focusing on sub-Saharan Africa. This study addressed the following research questions: 1) Do political factors, specifically political stability (PS) and government effectiveness (GE), have an impact upon the availability of improved water and sanitation resources in sub-Saharan Africa? 2) Is gross national income (GNI) associated with the availability of improved water and sanitation resources? Data from the Demographic and Health Surveys of 11 sub-Saharan African nations conducted from 2005-2008 and from the World Bank indicators on PS, GE and GNI were analyzed using logistic regression models to examine the association between political and financial indicators and access to water and sanitation. A total of 109,606 observations were included in this study. The majority had access to improved drinking water sources (65.9%) and travel times < 30 minutes (83.3%). Most used no form of household water treatment (81.1%) and did not have an improved sanitation facility (64.1%). Overall, the strength and direction of the

association between economic/political factors and access to water and sanitation varied. GE and GNI had the strongest positive associations with access to improved water source and household water treatment. GNI was positively associated with access to improved sanitation; political stability was inversely associated with travel time to water source. The results of this study indicate that GNI, PS, and GE are associated with water and sanitation access in sub-Saharan Africa. With this information, context-specific interventions to improve and expand water and sanitation services in the region can be developed, focusing on building stable, effective governments, and alleviating the burden of poverty.

360

SCHISTOSOMA MANSONI CERCARIAE DETECTION IN WATER SAMPLES USING DEAD-END ULTRAFILTRATION AND PCR

Bonnie Mull[1], Evan Secor[2], Karen Foo[3], Vincent Hill[2]

[1]Centers for Disease Control and Prevention/AREF, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Centers for Disease Control and Prevention/APHL Emerging Infectious Disease Fellowship, Atlanta, GA, United States

An estimated 207 million people worldwide are infected with *Schistosoma spp.,* a water-borne parasitic helminth, with an estimated 779 million people at risk in 76 endemic countries. Transmission of schistosomiasis is spatially and temporally restricted to water bodies inhabited by competent intermediate host snails. Because humans become infected through contact with cercariae-infested water, identification of active transmission sites can help maximize the effectiveness of control interventions. The standard cercariometric filtration method relies upon suction and requires technically skilled staff to perform the collection. Moreover, water turbidity can drastically limit the volume sampled. We applied a previously described dead-end ultrafilration (DEUF) method (Smith, 2009) using commercially available hollow-fiber dialysis ultrafilters for concentrating *Schistosoma* cercariae from 100-liter surface water samples followed by detection using real-time PCR (Gomes, 2006). Seeded water samples were pumped through the ultrafilter; the filter was then backflushed with a surfactant solution. The backflush solution was further concentrated through two 150 µm screens using a 60cc syringe. Lysis buffer (1.5 mL) was then slowly pushed through the filter screens and cercarial DNA was extracted and analyzed by PCR. The DEUF method consistently detected 5 cercariae in 100 liters of surface water with turbidities of up to 90 NTU. The limit of detection with other cercariometric methods is 1 cercariae in 5-10 liters of 120 NTU water. DEUF is a simple and cost effective method that can be utilized by untrained field personnel for rapid sample collection (~1 hr to collect 100 L). PCR detection of cercariae in DEUF samples offers a reliable technique for detection of cercariae in natural waters, which could be of great value in mapping areas of schistosomiasis infection risk, estimating the force of transmission and assessing whether transmission has been reduced after an intervention.

361

USE OF THE BRISTOL STOOL CHART TO COMPLEMENT SELF-REPORTED DIARRHEA AS AN OUTCOME MEASURE FOR WASH RESEARCH

Clair Null[1], Michael Kremer[2], Edward Miguel[3], Alix Zwane[4]

[1]Emory University, Rollins School of Public Health, Atlanta, GA, United States, [2]Harvard University, Cambridge, MA, United States, [3]University of California, Berkeley, CA, United States, [4]The Bill and Melinda Gates Foundation, Seattle, WA, United States

We discuss the use of the Bristol Stool Chart as a potential outcome measure for public health research on water, sanitation, and hygiene interventions. The literature on WASH interventions has relied on mothers' reports of whether or not their children suffered from diarrhea, since more objective measures such as stool samples are difficult to obtain and expensive to analyze. As an alternative health indicator, mothers could be asked to rank their child's most recent stool according to the Bristol Stool Chart, a 7-point pictorial scale that ranges from (1) "separate hard lumps" to (4) "like a sausage or snake, smooth and soft" to (7) "watery, no solid pieces". The Bristol Stool Chart has been used for clinical purposes among adults in upper-income countries, but no previous studies have used the scale as an indicator of gastroenteritis among children in developing countries. We asked over 300 mothers in rural western Kenya to rank their child's most recent stool on repeat survey visits, with an average of 7 observations per child under age 5 for over 1100 unique children. We would not expect a perfect correlation between this measure and the clinical definition of diarrhea used in the same survey ("3 or more looser than normal stools in a 24 hour period over the past 7 days"), since the stool chart measure is only the previous stool compared to an absolute scale whereas the clinical definition is compared to the child's normal stool and references a 7 day period. Nonetheless, the prevalence rate of loose stools (ranking of "7") according to the chart (21%) is almost twice as high as for the clinical definition of diarrhea (11%), p-value<0.001. Use of the chart is limited in that mothers were unable to rank stools of children over age 3, as they did not know what older, more mobile children's stools looked like, and the chart did not adequately represent the stools of infants under 6 months, as evidenced by the fact that in over a third of such cases mothers selected the option "other" rather than one of the 7 categories on the scale.

362

AN EXAMINATION OF HOUSEHOLD DRINKING WATER STORAGE AND MANAGEMENT PRACTICES IN BONAO, DOMINICAN REPUBLIC FROM SEPTEMBER 2005-JANUARY 2006

Kathryn Holt, Christine Stauber, Gloria Ortiz, Mark Sobsey

Georgia State University, Atlanta, GA, United States

More than 2.2 million people die each year from diarrheal disease. Most cases of diarrheal disease can be linked to a lack of access to clean water and sanitation. The proper usage of sanitation, hygiene and safe drinking water are all mechanisms by which to prevent or limit fecal contamination, and in turn, will reduce the risk of diarrheal disease. In an attempt to better understand the roles of drinking water storage, we analyzed data collected during a prospective cohort study performed in the Dominican Republic from September 2005 to January 2006. The purpose of this study was to determine if characteristics of household drinking water storage containers influenced the concentration of E. coli in the stored household drinking water in communities of Bonao, Dominican Republic. Drinking water samples were taken at approximately two week intervals and tested for turbidity, total coliforms and E. coli. In addition, information was collected about the storage container, drinking water source and use of dippersor other utensils for serving the water. After testing independent risk factors for E. coli contamination using t-tests and chi-squared tests, it was established that household storage practices have a significant impact on drinking water quality. Specifically, water samples collected from narrow-mouthed containers had significantly less E. coli (geometric mean of 10.5 MPN/100 ml) than those collected from wide-mouthed containers (geometric mean of 25.1 MPN/100mL water). In addition, household drinking water samples that were reported to be treated via boiling or chlorination had significantly lower concentrations of E. coli compared to samples that were reported to be untreated. The geometric average E. coli for all untreated samples was 19.5 E. coli MPN/100mL and for all treated samples was 7 E. coli MPN/100mL. The results of this study suggest an association between household storage practices and concentrations of E. coli in household drinking water. This highlights the importance of understanding the role of household water storage practices and the need to encourage hygienic practices that might prevent or reduce contamination of drinking water during storage in the home.

363

RELATIONSHIPS BETWEEN TURBIDITY, WATER QUALITY AND CHLORINATION IN RURAL COASTAL ECUADOR

Karen Levy[1], Shannon Oliver[1], Katharine Robb[1], Larissa Anderson[2], Marla Luckey[2], Emily Smith[1], Marina Fernandez[1], Joseph N. Eisenberg[2]

[1]Emory University, Atlanta, GA, United States, [2]University of Michigan, Ann Arbor, MI, United States

Chlorination can provide an effective and low-cost method of treating drinking waters, but the specific efficacy and effectiveness of chlorination of turbid waters under real world conditions remains under-studied. We present the results of a study of the impacts of turbidity on water quality and chlorination of water in rural coastal Ecuador. This study follows up two previously published studies from the same region. In the first, we observed on average a more than half-log reduction of indicator organisms between the source of drinking water to its point-of-use. In the second, no significant differences were observed in log reductions between drinking water of households that reported chlorination of their water. This follow-up study explores the role of source water turbidity in explaining these previously observed results. We report on E.coli contamination levels from samples collected in four villages from source waters, under household storage conditions (with and without water chlorination), and under controlled conditions. We report on water sampled from households both before and after agitation of the container, to address whether our previously observed reductions in indicator organisms during storage in the household are due to settling of turbid source waters or die-off of organisms in the storage container. In addition we report water quality results from trials examining the efficacy of chlorine dosage regimes currently recommended by the CDC for waters of varying turbidity. The results address the following questions: (1) Is turbidity related to microbial contamination in source waters and/or stored water? (2) Are our previously observed reductions in microbial contamination during storage due to settling or die-off of organisms? (3) What is the efficacy of recommended chlorination dosage of turbid waters? (4) What is the effectiveness of chlorination of turbid waters in the household context? This research provides important new insight about the relationship between turbidity, water quality, and chlorination under village conditions.

364

CHARACTERIZATION OF AN *IN VITRO* MALARIA LUMINESCENCE-BASED LIVER STAGE DRUG SENSITIVITY SCREEN

Jacob D. Johnson[1], Erin H. Harris[1], Karen M. Kopydlowski[2], Thomas H. Hudson[1], Michael P. Kozar[1], Mark R. Hickman[1], Michael T. O'Neil[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]United States Army Medical Materiel Development Activity, Fort Detrick, MD, United States

Plasmodium liver stages represent an ideal target for antimalarial prophylaxis since elimination of liver stages prevents development of the disease-causing erythrocytic stages. Although rodent and non-human primate models are available for evaluating causal prophylactic activity, the amount of compound needed for *in vivo* testing is substantial, and thus, requires costly and time-consuming scale-up synthesis. Since *in vivo* models are not a viable option for high-throughput drug screening or developing a proper structure-activity relationship around lead molecules, the need exists for an *in vitro* liver stage screen to complement the existing antimalarial blood-stage assays. In this study, we report the development of a 96-well luciferase expressing *P. berghei*-HepG2 *in vitro* liver stage screen to identify potential antimalarials. Our assay protocol does not require post-drug washes, media changes, or cell lyses, resulting in high inter-experimental reproducibility, throughput, and automation amenability. Furthermore, our approach utilizes soluble, non-toxic d-luciferin, which allows measurement of parasite growth over time. A panel of antimalarial drugs with known blood- and liver-stage activities were benchmarked in the system by determining their respective 50% inhibitory concentrations (IC50s). The results compare to other previously published *in vitro* liver-stage drug IC50 values. Counterscreening using MTT whole-cell toxicity assays was performed and selectivity indices calculated. Additionally, drug susceptibility time courses on select drugs were explored. Finally, activity profiles of novel compounds displaying liver-stage inhibition will be briefly highlighted. The results obtained indicate that the assay significantly increases our screening capabilities to discover potential antimalarials affecting liver-stage infection.

365

HEPATOCYTE-BASED ASSAY TO ASSESS THE EFFECTS OF DRUGS ON THE PRE-ERYTHROCYTE STAGES OF *PLASMODIUM VIVAX*

Soundarapandian Velmurugan[1], Rana Chattopadhyay[1], Chinnamma Chakiath[1], Alan Magill[2], Michael O'Neil[2], Wilbur Milhous[2], John Barnwell[3], William E. Collins[3], Stephen L. Hoffman[1]

[1]Sanaria Inc., Rockville, MD, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Centers for Disease Control and Prevention/NCID/Malaria Branch, Chamblee, GA, United States

Recent data indicate that the impact of *Plasmodium vivax* (Pv) malaria on the health and economies of the developing world has been dramatically underestimated. Pv has a unique dormant stage in its hepatic cycle, the hypnozoite, which allows the blood stage infection to relapse in the absence of reinfection. Hypnozoites are characterized as persistent parasites of around 4 mm diameter. There have been no reports in the literature of assessment of drugs against the liver stages of Pv *in vitro*. We are developing a medium to high throughput assay to identify new drugs against Pv liver stages, including hypnozoites. As a first step we established a system for infecting human hepatoma (HepG2) cells with cryopreserved Pv sporozoites (PvSPZ). The cryopreserved PvSPZ invade HepG2 cells and develop into late liver stage schizonts or remain as dormant structures resembling hypnozoites. We next assessed the effects of primaquine (PQ), atovaquone (AQ) and chloroquine (CQ) on liver stage parasite development in a 3 day assay in a 96 well format. PQ showed 22%, 56%, 77% inhibition of liver stage parasite numbers at 1, 10, 100 µg/mL concentrations, AQ showed 20%, 66%, 86% inhibition at 1, 10, 100 ng/mL concentrations respectively and CQ did not show any significant inhibition. The high concentration of PQ needed to achieve inhibition similar to AQ could be due to an inability of the HepG2 cells to metabolize PQ. Dose dependent effects of PQ and AQ, but not by CQ indicate that our assay has the capacity to reproducibly evaluate drugs against the hepatic stages initiated from cyropreserved PvSPZ. These data provide the foundation for finalizing a medium to high throughput assay to identify new drugs for the elimination of Pv liver stages including hypnozoites.

366

EFFICACY OF ARTEMETHER-LUMEFANTRINE (AL) IN THE TREATMENT OF BLOOD STAGES OF *PLASMODIUM VIVAX*

Quique Bassat[1], Aurel Allabi[2], Marc Cousin[3], Srivicha Krudsood[4]

[1]Barcelona Centre for International Health Research (CRESIB), Barcelona, Spain, [2]Unité de Pharmacologie, Faculté des Sciences de la Santé-Université d'Abomey-Calavi, Cotonou, Benin, [3]Novartis Pharma AG, Basel, Switzerland, [4]WHO CC for Clinical Management of Malaria, Mahidol University, Bangkok, Thailand

The significant burden of *Plasmodium vivax,* traditionally underappreciated, is currently in the spotlight, specially in southeast Asia, where chloroquine (CQ) resistant strains to this species led to the use of artemisinin-based combination therapies (ACTs) for its treatment. The effect of the currently recommended dosing regimen of AL on *P. vivax* was reviewed in the 4 Novartis sponsored studies which included patients

(pts) with mixed infection (*P. vivax* / *falciparum*) at baseline (n=13, 5, 16, 2). All pts were cleared from *P vivax* by 48, 24, 42 and 24 hours, for each study, respectively. Reappearance of *P. vivax* was observed between day 18-49 in 6/13, 2/5, 3/16 and in 0/2 of pts, respectively (primaquine (PQ) was administered after day 7 in 1 pt). Literature review suggests that AL achieves a mean PCT of 1.2 days [0.9, 1.6] with 80% of pts having cleared parasites at D1, as reported previously. However, if not administered with PQ, the incidence of recrudescence, being re-infection or relapse, is around 50%, observed mainly after D28 (1;2). This is higher than observed with dihydroartemisinin-piperaquine (DP), probably due to the relatively shorter half life of lumefantrine compared to piperaquine. Moreover, AL combined with PQ (in pts not G6PD-deficient) (n= 38) is safe and provides faster PCT than the combination of CQ/PQ (mean 41.6 h vs 55.8 h, p<0.001), with a cure rate at D28 of 97.4%, as reported previously. AL is effective against blood stages of *P vivax* and allows fast parasite clearance. If AL is administered alone, the risk of recrudescence, re-infection or relapse, remains however high and needs to be monitored. The combination of AL and PQ provides high rates of radical cure.

367

EFFECTIVENESS AND SAFETY OF ARTEMETHER-LUMEFANTRINE IN THE USA: SURVEILLANCE IN PEDIATRIC AND ADULT PATIENTS WITH *PLASMODIUM FALCIPARUM* MALARIA

Marc Cousin, Raymond Schlienger, Verena Walter, Jaquie Mills, Anne Claire Marrast

Novartis Pharma AG, Basel, Switzerland

Most studies of the safety and efficacy of artemether-lumefantrine (AL) have been conducted in malaria endemic regions, mainly in Southeast Asia and sub-Saharan Africa. However, fewer data are available in non-immune patients, as the largest clinical trial so far was performed in 165 non-immune patients from Europe and non-malarious areas of Colombia. This study showed that AL was effective (PCR corrected 28-day parasitological cure rate of 96.0%) and well tolerated, including in patients >70 kg of body weight. In April 2009, AL received marketing approval from the US Food and Drug Administration (FDA), who requested that a "descriptive study of the use of AL in non-immune travelers" be conducted as a post-marketing requirement. Therefore, the current evaluation, designed by Novartis in partnership with the US Centers for Disease Control and Prevention (CDC), aims to assess for a period of 5 years, pediatric and adult patients (US and foreign residents) diagnosed with malaria and treated with AL with regard to demographics, malaria immune status, treatment effectiveness, and adverse events (AEs). As malaria is a reportable disease in the US, the evaluation was designed to assess public health surveillance data reported to CDC using the 'Malaria Case Surveillance Report' form. This form was recently adapted to capture additional information including AEs and serious AEs. The evaluation will provide a unique opportunity to gather data in elderly patients as well as in patients with a BMI ≥25 kg/m^2. Data collection and reporting methods will be described.

368

PATHWAYS TO DISCOVERY OF NON-HEMOLYTIC 8-AMINOQUINOLINE ANTIMALARIALS

Larry A. Walker[1], Babu L. Tekwani[1], N.P. Dhammika Nanayakkara[1], Aruna Sampath[2], Alan Magill[2], Colin Ohrt[2]

[1]University of Mississippi, University, MS, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States

8-Aminoquinolines (8AQ) are the only drug class that kills the key parasite stages necessary for the survival of malaria. For *Plasmodium falciparum* (Pf) malaria, this is the mature (stage 5) gametocytes (Pfg) which transmit the infection. For *P. vivax* (Pv) and *P. ovale* (Po), this is the sleeping liver stage or hypnozoite, which emerges weeks to months after the initial infection and causes relapse. Primaquine is the only approved drug that can do this but it is not widely deployed because of toxicity concerns, especially hemolytic toxicity in population with glucose 6-phosphate dehydrogenase (G6PD) deficiency. G6PD deficiency is the most common enzymopathy and it is prevalent in malaria endemic regions. A Non-hemolytic 8AQ Consortium was formed to identify 8AQ or 8AQ combinations with an improved therapeutic index. The first objective of the consortium is to develop predictive models for hemolytic toxicity in G6PD-deficiency. Clear progress being made with one *in vitro*, two mouse and one Rhesus model. Once the models are fully qualified, they will be used to identify drug combinations with or isomers of existing drugs to mitigate the risk of existing drugs as high priority. Unique approaches are also being used to select new 8AQ from the 1800+ in the Walter Reed Army Institute of Research chemical information system and literature for assessment in the G6PD models, with priority to those with documented efficacy in humans or the Rhesus relapsing model. Investigations on mechanism of hemolytic toxicity are also being pursued. The current overall status of this project will be presented.

369

INESS-INDEPTH EFFECTIVENESS AND SAFETY STUDIES FOR ANTIMALARIALS IN AFRICA: A PHASE IV STUDY PLATFORM

Aziza J. Mwisongo

INDEPTH-NETWORK, Dar es salaam, United Republic of Tanzania

Researcher-driven Phase I, II and III randomized, controlled, clinical trials have been well supported and establish the initial safety and efficacy of such products when delivered under ideal conditions. However, large scale Phase IV studies to determine the effectiveness and frequency of severe adverse events when the intervention is delivered in real-life systems is a missing piece of the drug development pipeline. In Africa, new drugs are offered for national policy decisions with as few as 6,000 patient exposures and no long term follow-up. Phase IV safety and effectiveness data, generated outside trial conditions, will be valuable for national malaria control programmes in Africa before the widespread use of such new treatments. INESS undertakes Phase IV effectiveness and safety studies of new combination therapies (and other drugs and vaccines) for malaria in at least 8 INDEPTH Demographic Surveillance System (DSS) sites in 4 countries of Africa over a four year period. The main product of the platform is a longitudinal evidence base to allow assessment of efficacious drugs in real life settings. The study employs several modules under two main modules of system effectiveness and safety. In the past one year, data has been collected from five DSS sites in Ghana and Tanzania on the current antimalarials. This year the study extends to include Burkina Faso and Mozambique and will begin data collection on newly registered ACTs. The experience of setting and carrying out a phase IV platform for investigation of not only antimalarials as well other health commodities in future is a rare experience. Experinces derived from the implementation of this platform are unique and tedious, particularly the set up of electronic data capture and biometric system for identification of patients and linking of health facility and DSS data. All these experiences are documented within INESS and is worth sharing with other researchers, academicians as well as policymakers.

370

AN ATYPICAL PROTEIN PHOSPHATASE IS CRITICAL FOR THE INTRAERYTHROCYTIC DEVELOPMENT OF *PLASMODIUM FALCIPARUM*

Bharath Balu[1], Christopher O. Campbell[1], Steven P. Maher[1], Anatolie Naumov[1], Jennifer Sedillo[1], Julian C. Rayner[2], John H. Adams[1]

[1]University of South Florida, Tampa, FL, United States, [2]The Wellcome Trust Sanger Institute, Cambridge, United Kingdom

An insertion mutagenesis screen of *Plasmodium falciparum* identified PF13_0027, annotated as a hypothetical protein, to be crucial for intraerythrocytic development. This protein with unknown function

consists of two distinct conserved eukaryotic domains: 1) an inactive rhodanese domain and 2) an atypical protein tyrosine phosphatase (PTP) domain. This tandem domain structure is similar to many eukaryotic dual specificity phosphatases, including the MAP kinase phosphatases. The atypical PTP putative catalytic domain of PF13_0027 lacks the signature arginine residue and has an insertion adjacent its catalytic site. Most merozoites produced from the PF13_0027 KO clone fail to initiate or complete invasion of new host erythrocytes resulting in a severely attenuated blood-stage growth phenotype. Although early asexual development appears normal, a defect in cell cycle regulation becomes evident in late trophozoites due to a significantly longer pre-S phase (52 hrs) versus wild-type parent (46 hrs). Another significant defect becomes noticeable in late schizont development as premature degradation of the parasitophorous vacuole membrane leaves merozoites free in the erythrocyte cytoplasm and the explosive rupture of egress is not usually observed. As a result of this defective development unusually large numbers of merozoites accumulate in the culture supernatants of this phosphatase null mutant, as merozoites fail to invade host erythrocytes. Microarray analysis revealed significant alterations of specific metabolic pathways suggesting a regulatory function for this phosphatase in asexual development and a key role in regulating progression into sexual stage development, consistent with its role in crucial signaling cascades.

371

EVIDENCE FOR THE EFFLUX OF A RANGE OF ANTIMALARIAL DRUGS AND 'CHLOROQUINE RESISTANCE REVERSERS' FROM THE DIGESTIVE VACUOLE IN MALARIA PARASITES WITH MUTANT FORMS OF THE CHLOROQUINE RESISTANCE TRANSPORTER

Adele M. Lehane[1], David A. Fidock[1], Kiaran Kirk[2]

[1]Columbia University, New York, NY, United States, [2]The Australian National University, Canberra, Australia

Chloroquine resistance in the malaria parasite *Plasmodium falciparum* is conferred by mutations in the *P. falciparum* Chloroquine Resistance Transporter (PfCRT). PfCRT localises to the membrane surrounding the digestive vacuole, an acidic organelle in which chloroquine accumulates to high concentrations and exerts its toxic effect. Previously, we have shown that chloroquine-resistant malaria parasites show an increased leak of H+ ions from their digestive vacuole in the presence of chloroquine. This observation was attributed to the transport of chloroquine, together with H+, out of the digestive vacuole via mutant PfCRT. Here, we show that chloroquine:H+ efflux from the digestive vacuole is present in transfectant parasites expressing mutant PfCRT in the context of different genetic backgrounds, including in parasites that are not rendered highly chloroquine resistant by the introduction of the mutant protein. Further, we show that a range of other antimalarial drugs, as well as various 'chloroquine resistance reversers' induce an increased leak of H+ ions from the digestive vacuole in parasites expressing mutant forms of PfCRT, consistent with these compounds being substrates for mutant forms, but not the wild-type form, of PfCRT. The finding that chloroquine resistance reversers are substrates for mutant PfCRT has implications for the mechanism of action of this class of compound.

372

THE MECHANISMS OF LATENCY OF MALARIA PARASITES IN THE MOSQUITO SALIVARY GLANDS

Min Zhang[1], Clare Fennell[2], Lisa Ranford-Cartwright[2], Ramanavelan Sakthivel[3], Pascale Gueirard[4], Stephan Meister[5], Anat Caspi[6], Christian Doerig[2], Ruth S. Nussenzweig[1], Renu Tuteja[7], William J. Sullivan, Jr.[8], David S. Roos[6], Robert Ménard[4], Beatriz M. Fontoura[3], Elizabeth A. Winzeler[5], Victor Nussenzweig[1]

[1]New York University Medical Center, New York, NY, United States, [2]University of Glasgow, Glasgow, United Kingdom, [3]University of Texas Southwestern Medical Center, Dallas, TX, United States, [4]Institut Pasteur, Paris, France, [5]The Scripps Research Institute, La Jolla, CA, United States, [6]University of Pennsylvania, Philadelphia, PA, United States, [7]International Centre for Genetic Engineering and Biotechnology, New Delhi, India, [8]Indiana University School of Medicine, Indianapolis, IN, United States

Sporozoites, the invasive form of malaria parasites transmitted by mosquitoes, are quiescent while in the insect salivary glands. Sporozoites only differentiate inside of the hepatocytes of the mammalian host. We show that sporozoite latency is an active process controlled by a eukaryotic initiation factor-2α (eIF2α) kinase (IK2) and a phosphatase. IK2 activity is dominant in salivary gland sporozoites leading to an inhibition of translation and accumulation of stalled mRNAs into granules. When sporozoites are injected into the mammalian host, an eIF2α phosphatase removes the PO4 from eIF2α-P, and the repression of translation is alleviated to permit their transformation into liver stages. In IK2 knockout sporozoites eIF2α is not phosphorylated and the parasites transform prematurely into liver stages and lose their infectivity. Thus, to complete their life cycle, *Plasmodium* sporozoites exploit the mechanism that regulates stress responses in eukaryotic cells.

373

TARGETING *PLASMODIUM* SPOROZOITE-KUPFFER CELL INTERACTIONS WITH A PHAGE DISPLAY LIBRARY

Sung-Jae Cha, Monique Stins, Marcelo Jacobs-Lorena

Johns Hopkins University, Baltimore, MD, United States

After inoculation by the bite of an infected mosquito, the *Plasmodium* sporozoite enters the blood stream and infects the liver with unique specificity. After capture by protruding glycosaminoglycans, sporozoites migrate along the sinusoidal wall until they find and invade a Kupffer cell, thus gaining access to the underlying hepatocytes. Previous evidence suggests that specific sporozoite-Kupffer cell interactions are required for invasion to occur. A screen of a phage display library for peptides that bind to rat Kupffer cells yielded three peptides. Notably, peptide binding to Kupffer cells strongly inhibited *Plasmodium berghei* sporozoite invasion. These observations support the hypothesis that the peptides bind to Kupffer cell receptor(s) for sporozoite invasion. In a separate set of experiments we found that antibodies against the candidate peptides recognize sporozoite protein(s) and importantly, inhibit invasion, suggesting that the candidate peptides structurally mimic sporozoite ligands that are required for invasion. These findings could provide the basis for the identification of novel protective antigens for use in pre-erythrocytic vaccines.

374

LIMITED ABILITY OF *PLASMODIUM FALCIPARUM PFCRT, PFMDR1*, AND *PFNHE-1* POLYMORPHISMS TO PREDICT QUININE *IN VITRO* SENSITIVITY OR CLINICAL EFFICACY IN UGANDA

Frederick N. Baliraine[1], Samuel L. Nsobya[2], Jane Achan[2], James K. Tibenderana[3], Ambrose O. Talisuna[4], Bryan Greenhouse[1], Philip J. Rosenthal[1]

[1]University of California, San Francisco, San Francisco, CA, United States, [2]Makerere University, Kampala, Uganda, [3]Malaria Consortium, Kampala, Uganda, [4]Medicines for Malaria Venture, Kampala, Uganda

Quinine (QN) remains a standard drug for treating severe malaria in Africa, and it is increasingly used to treat uncomplicated malaria. However, failures of QN therapy are common. A recent effectiveness study in Uganda found that 23% of children treated with QN experienced recrudescence over 28 days of follow-up. Mechanisms of resistance to QN are uncertain, and it is unknown if QN treatment failures in Africa are due to drug resistance. Recent studies have identified associations between *in vitro* QN sensitivity and polymorphisms in the *pfcrt, pfmdr1*, and *pfnhe1* genes, all of which encode putative transporters. In particular, for *pfnhe1*, which encodes a putative Na+/H+ exchanger, varied numbers of DNNND or DDNHNDNHNND repeats in ms4760 have been associated with different levels of *in vitro* QN sensitivity. To better characterize mediators of QN sensitivity and treatment failure, we characterized associations between genetic polymorphisms, *in vitro* QN sensitivity, and QN treatment responses in Uganda. Among 172 fresh clinical isolates tested (IC50 range 15.4 - 760.9 nM, based on HRP-2 ELISA assays), a trend of decreasing sensitivity to QN was observed with accumulation of *pfmdr1* mutations at codons 86, 184 and 1246. All parasites had the *pfcrt* 76T mutation and wild type sequences at *pfmdr1* 1034 and 1042. Considering *pfnhe1*, sequence analysis showed that ms4760 is highly polymorphic, with 29 novel genotypes identified in addition to 19 previously reported. Two copies of either the DNNND or DDNHNDNHNND repeat, compared to 1 or >=3 repeats, were weakly associated with decreased QN sensitivity, but differences were not significant. Considering samples from 66 subjects treated in the clinical trial, none of the polymorphisms noted above predicted QN treatment failure. Our data suggest that known polymorphisms in *pfcrt*, *pfmdr1*, and *pfnhe1*, while associated with QN sensitivity in some studies, are not robust markers for QN resistance, and each likely will be of limited value as a tool for the surveillance of QN resistance in Africa.

375

NOVEL CHIMERIC VACCINES FOR CHIKUNGUNYA: IMMUNOGENICITY AND EFFICACY STUDIES IN A129 MICE

Charalambos Partidos[1], Kenneth Plante[2], Eryu Wang[2], James Weger[1], Joe Brewoo[1], Dal Young Kim[2], Rodion Gorchakov[2], Ilya Frolov[3], Ann C. Powers[4], Dan T. Stinchcomb[5], Scott C. Weaver[2], Jorge E. Osorio[6]

[1]Inviragen, Madison, WI, United States, [2]Center for Biodefense and Emerging Infectious Diseases and Department of Pathology, University of Texas Medical Branch, Galveston, TX, United States, [3]Department of Microbiology, University of Alabama, Birmingham, AL, United States, [4]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [5]Inviragen, Fort Collins, CO, United States, [6]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI, United States

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that causes explosive outbreaks of febrile illness associated with rash, painful arthralgia and sometimes arthritis. There is currently no commercial vaccine for CHIKV. Therefore, the development of a new, safer vaccine is needed. We constructed a candidate CHIKV vaccine based on introducing RNA sequence elements to the wild-type CHIKV strain that prevent efficient expression of the structural proteins in insect cells. To this end, we used the Encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES), which is nonfunctional in insect cells (LR CHIKV/mutSG/IRES). Infection with this new virus construct was assessed in A129 homozygote mice, which are defective in interferon α/β signaling, and compared it to the 181/25 live attenuated vaccine. Groups of adult A129 mice were injected intradermally with each of the CHIKV candidate vaccines. All mice injected with the 181/25 vaccine survived with no apparent signs of disease except a temporary ruffled appearance and body weight loss. The severity of this transient morbidity correlated inversely with the infecting dose of virus. In contrast, mice receiving the LR-CHIKV/mutSG/IRES construct remained healthy with no apparent signs of morbidity, weight loss or foot pad swelling. Mice immunized with either vaccine construct seroconverted with detectable neutralizing antibody responses measured on day 21 and 35 post immunization. When challenged intradermally with the 10^2 PFU wild-type CHIKV, all mice were protected with no clinical signs of disease (weight loss, fever or foot pad swelling). In contrast, control mice immunized with PBS succumbed to infection by day 3 post challenge. Immune sera collected from mice vaccinated with either candidate vaccine conferred full protection against lethal CHIKV challenge in passively immunized A129 mice. This result indicates the protective role of antibodies against CHIKV infection. Overall, these findings highlight that our new vaccine candidate is safe and efficacious and offers a promising strategy to prevent CHIKV epidemics..

376

LONG-LASTING OVERMORBIDITY AND IMPAIRED QUALITY OF LIFE 30 MONTHS AFTER CHIKUNGUNYA INFECTION: COMPARATIVE COHORT OF FRENCH GENDARMES EXPOSED TO CHIKUNGUNYA IN 2006 IN REUNION ISLAND

Catherine Marimoutou[1], Elodie Vivier[2], Fabrice Simon[2]

[1]DESP, Institut de Médecine Tropicale du Service de Santé des Armées, Marseille, France, [2]Service de Pathologie infectieuse et tropicale, Hôpital militaire Laveran, Marseille, France

In June 2006, a survey studying the prevalence of Chikungunya (CHIKV) was settled among the French gendarmes exposed to the large 2005-2006 outbreak in Reunion Island (southwest Indian Ocean). The chikungunya prevalence in this cohort was 18.8% (126/671 participants). Our objective was to observe the long-lasting morbidity due to CHIKV infection within the same cohort by comparison of clinical symptoms and quality of life (QOL) between gendarmes infected with CHIKV (CHIK+) and non infected (CHIK-). Self-questionnaires collecting clinical symptoms, health care service consumption and QOL (SF-36) were sent by mail to all gendarmes who participate to the 2006 survey. Based on the two questions: "Do you think that you got chikungunya infection during your stay in Reunion Island?" and "Do you consider that you are healed?", patients were ordered in three groups: healed CHIK+, non healed CHIK+ and CHIK-. Among the 398 responders (92% male, median aged of 42.8 years), 101 (25.4%) were CHIK+. Between July 2006 and June 2008, CHIK+ subjects remained at least 5 times more symptomatic for rheumatic symptoms than CHIK- and their healthcare consumption was 1.7 time higher (9.5 consultations versus 5.5 consultations). In June 2008 (in median 30 months after infection), CHIK+ subjects still significantly more frequently complained for joints pain, swelling and stiffness than CHIK- with a gradient of severity between healed and not healed CHIK+ subjects. Moreover, at the same time, CHIK+ subjects considered that their pain moderately (52%) or highly (18%) reduced their activity while 81% of CHIK- declared no pain limitation. As well, all dimensions of SF36 and both physical (PCS) and mental component (MCS) summaries were impaired in CHIK+ subjects with a decreasing gradient from not healed (Mean PCS: 43.6; Mean MCS: 41.6) to healed CHIK+ (Mean PCS: 52.0; Mean MCS: 47.5), then to CHIK- (Mean PCS: 54.8; Mean MCS: 50.8, $p<0.001$). In conclusion, this comparative study among a young active male population concludes to a persistent high overmorbidity and impaired QOL due to CHIKV at 30 months of infection, even in patients considering themselves healed.

377

THE EFFECTS OF IMMUNE SUPPRESSION ON CHIKUNGUNYA VIRUS PATHOGENESIS IN MICE

Sarah A. Ziegler, Hilda Guzman, Amelia P. Travassos da Rosa, Robert B. Tesh

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

Chikungunya virus (CHIKV) is a mosquito-transmitted *Alphavirus* that causes an illness, characterized by fever, rash and incapacitating joint and muscle pain. CHIKV-induced arthralgia can be recurrent and persistent in some people for months and up to 5 years after infection. CHIKV is endemic and sporadically epidemic in Asia and Africa; the recent epidemic in Asia involved 2 million people. Despite its frequency, the pathogenesis of CHIKV in people is poorly understood. To help elucidate CHIKV pathogenesis, a mouse model of CHIKV infection was developed in our laboratory. Previous work has demonstrated that needle inoculation of CHIKV in young mice causes a self-limiting infection characterized by 3-4 days of viremia with persistence of virus in spleen and skeletal muscle for several more days as well as severe focal necrosis in the skeletal muscles. Recent published work has shown that CHIKV pathogenesis may be dependent on cells of the immune system. Suppression of the immune response of mice prior to infection with CHIKV is helping us to understand how immune-suppression changes CHIKV pathogenesis. The use of cyclophosphamide and glucocorticoid steroids decrease the inflammation associated with CHIKV infection in mice with no increase in mortality or sickness. Viremia levels are similar in treated and untreated mice. Results to be presented include histopathologic analysis of skeletal with and without immune suppression as well as the effect of CHIKV infection on blood chemistry and liver enzyme values. These results help us to better understand CHIKV infection in an at risk population as well as aiding in the development of therapeutic options in people.

378

INNATE IMMUNE RESPONSE TO RIFT VALLEY FEVER VIRUS INFECTION

Kimberly K. Gray[1], Alexander N. Freiberg[1], Michael R. Holbrook[2]

[1]University of Texas - Medical Branch, Galveston, TX, United States, [2]National Institutes of Health Integrated Research Facility, Frederick, MD, United States

Rift Valley fever virus (RVFV), a member of the Buyanviridae family, is an important pathogen in Africa and on the Arabian Peninsula. RVFV can cause severe disease; hemorrhagic fever or encephalitis, in humans and animals, particularly ruminants. Due to the rapid progression from symptoms to death and the high containment facilities needed to work on RVFV, there is little data on the effect of infection on the host innate immune response and how this correlates with pathogenesis. In an effort to characterize the host response to a vaccine strain (MP-12) and a wild-type strain (ZH501) in the mouse model, cytokine levels in infected C57BL/6 mice were measured in serum, liver, brain and spleen. These data have shown that ZH501 infection significantly increases chemokines (KC, MIP-1α, MIP-1β, and MCP) while it decreases Th1 [IFNγ and IL-12(p70)] associated cytokines when compared to MP12 infections in the organs examined. Interleukin-1α and IL-1-responsive cytokines increase in ZH501 infection with peak concentration levels at 72 hpi. There is no change in type II interferon response between the three treatments except in the brain of ZH501 infected animals, where there appears to be an IFNγ response late in infection. The most dramatic differences in cytokine concentration are seen between 48 and 84 hpi with 60 and 72 hpi appearing to be critical time points in mouse infection as these points represent the largest differences in cytokine response in this model. Blood chemistry analysis demonstrated an increase in liver enzymes and a decrease in liver and kidney function markers (glucose, bilirubin, and BUN) at 60-72 hpi in ZH501 infected animals while MP-12 and mock infected animals were largely unchanged. Given the recognized hepatotropism of RVFV, loss of liver function is not surprising. Data shown here demonstrate that, unlike the vaccine strain, RVFV wild-type virus targets the brain and visceral organs and causes a significant inflammatory response. These significant differences in viral pathogenicity indicate that, despite inducing a productive infection in mice, the host is able to limit the effect of viral infection on the innate immune response.

379

PROTEOMIC CHARACTERIZATION OF THE RIFT VALLEY FEVER VIRUS NSS PROTEIN

Rebecca Easley[1], Alan Baer[1], Lee Dean[1], Dana Grindel[1], Aarthi Narayanan[1], Weidong Zhou[2], **Kylene Kehn-Hall**[1]

[1]National Center for Biodefense and Infectious Disease, Department of Molecular and Microbiology, George Mason University, Manassas, VA, United States, [2]The Center for Applied Proteomics and Molecular Medicine, George Mason University, Manassas, VA, United States

Rift Valley Fever Virus (RVFV), a member of the Bunyaviridae family, is a priority pathogen for both the CDC and USDA. While RVFV has a significant effect on livestock, it can also cause hemorrhagic fever in humans. Our research is aimed at determining critical RVFV host interactions to identify therapeutics targeted against the host. RVFV is a negative stranded RNA virus that encodes three segments: large (L), medium (M) and small (S). The S segment codes for the NSs protein which is nonstructural and is not required for viral replication, although it does play a major role in viral pathogenesis. NSs protein exists in both the nucleus and cytoplasm and forms distinct filamentous structures in the nucleus. It is known to suppress transcription of host mRNA through interactions with TFIIH subunit p44. In this study we used proteomic methods to identify novel host proteins that interact with NSs. Using mass spectrometry we identified multiple novel NSs interacting proteins, including Protein Arginine Methyltransfrase 5 (PRMT5) and Heat Shock Protein 70 (HSP70). The association of NSs with PRMT5 led us to investigate methylation of NSs. Interestingly, Methylation Modification Prediction Server (MeMo) predicted multiple arginine methylation sites, but no lysine methyation sites. Treatment of MP-12 infected Vero cells with the general methyltransferase inhibitor, AdOx, resulted in decreased viral replication. Current studies are focused on identifying the site(s) of NSs methylation and effects of methylation on NSs function.

380

PATHOGENESIS OF MONKEYPOX VIRUS IN CYNOMOLGUS MACAQUES INFECTED BY THE INTRAVENOUS OR INTRABRONCHIAL ROUTE

Joseph Blaney[1], Reed Johnson[1], Julie Dyall[2], Louis Huzzella[2], Dan Ragland[2], Dar-Yeong Chen[2], Daniel Mollura[2], Marisa St. Claire[2], Russ Byrum[2], Catherine Jett[2], Jason Paragas[2], Peter Jahrling[2]

[1]Emerging Viral Pathogens Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [2]Integrated Research Facility, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, MD, United States

Monkeypox virus (MPXV), an orthopoxvirus, is a zoonotic disease of primarily Central Africa which causes outbreaks of disease associated with case fatality rates of up to 10%. In addition to variola virus, the causative agent of smallpox, MPXV is also a potential agent of bioterrorism. Infection of nonhuman primates (NHPs) with MPXV, primarily by the intravenous (IV) route, serves as a model of orthopoxvirus disease in humans although disease course is considerably accelerated. The objective of this study was to further characterize the pathogenesis of the IV model of MPXV infection and to determine the utility of intrabronchial (IB) inoculation of NHPs as a novel model that might more closely resemble the progression of MPXV disease in humans. Three NHPs inoculated IB with $5x10^6$ PFU of MPXV (66% moribundity) were compared to NHPs inoculated IV with the standard $5x10^7$ PFU dose (83% moribundity). Mean time to fever onset was 2.8 and 4.2 days for the IV and IB groups,

respectively. Lesions and infectious virus in oral and nasal swabs were detected 4 days post IV inoculation and 7 days post IB inoculation. In addition, mean day of moribundity for the IV route was 9.8 days and 20 days for the IB route. Virus distribution across 19 tissues was unaffected by route, although virus load was typically 10-fold higher after IV inoculation. Disease course in a subset of NHPs was also evaluated using PET/CT imaging. NHPs were imaged at several time points using ^{18}F-FDG as a nonspecific indicator of inflammation. Inflammation and consolidation of lungs in NHPs infected by the IB route was visualized during the progression of disease and resolution was observed in one animal. Lymphadenopathy and suspected immune activation was also detected by PET/CT imaging in the axillary lymph nodes of NHPs infected by both routes. Taken together, these results indicate that the IB route results in severe lesional disease with a delay in the onset of disease symptoms and moribundity, but the severe viral pneumonia associated with this route may limit the application of this model.

381

PATHOPHYSIOLOGIC ASSESSMENT OF EBOLA VIRUS INFECTION WITH AND WITHOUT INTRAVENOUS FLUID TREATMENT IN A NONHUMAN PRIMATE MODEL USING A MULTI-SENSOR TELEMETRY SYSTEM

Mark G. Kortepeter[1], Anna Honko[1], Joshua C. Johnson[1], Gene G. Olinger[1], Bret K. Purcell[1], Robert Rivard[1], Matthew J. Hepburn[1], Lisa E. Hensley[1], James V. Lawler[2]

[1]United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, United States, [2]National Institute of Allergy and Infectious Diseases, Fort Detrick, MD, United States

The pathophysiology of Ebola virus infection in humans and NHPs is not well characterized. We sought to characterize the pathophysiology of Ebola virus infection and impact of IV fluids in an NHP model. Nine adult rhesus macaques (6M/3F) were implanted with multi-sensor telemetry devices and internal jugular catheters and subsequently challenged with ~100 pfu IM *Zaire ebolavirus* (Kikwit, 1995). Five animals were controls and four animals received IV normal saline using a treatment algorithm. Physiologic signals were recorded continuously. Daily labs were collected for viremia, chemistries, hematology, and cytokines. All animals became ill. Pre- and post-challenge physiologic parameters were compared. 2/5 untreated and 1/4 treated animals survived beyond D10: 1 untreated animal was euthanized on D18 for an eye infection and two animals survived without sequelae beyond 5 months until euthanized. Viremia levels were lower in surviving animals. Pre-illness diurnal patterns for BP, pulse, temp, RR, and contractility index disappear after fever onset. Late-stage infection was associated with a progressive and steady decline in mean arterial blood pressure and systolic blood pressure that began 36 to 48 hours before ultimate obtundation and euthanasia. Lactic acidosis and renal failure developed in the final 24-48 hours in non-survivors. A decline in the cardiac contractility index was observed in animals that succumbed to infection. IV fluid boluses appeared to result in transient improvement in hemodynamic parameters. Early aggressive IV fluids in one animal appeared to stabilize BP and improve contractility index. A rise in respiratory rate and end-diastolic pressure was also observed in this animal. Graphs will be used to illustrate physiologic trends in individual animals and across animals. In conclusion, this data provides insight into physiologic changes in Ebola virus infection. Late-stage infection manifests as progressive hemodynamic compromise and may be responsive to fluids.

382

A MATRIGEL PLUG MODEL TO STUDY FILARIAL PATHOLOGY IN RATS

Tiffany S. Weinkopff[1], Rob Eversole[2], Charles Mackenzie[3], Patrick Lammie[4]

[1]University of Georgia, Athens, GA, United States, [2]Western Michigan University, Kalamazoo, MI, United States, [3]Michigan State Univeristy, East Lansing, MI, United States, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States

Lymphatic filariasis is caused by the parasitic nematodes *Wuchereria bancrofti* and *Brugia malayi* which infect over 120 million people worldwide. Most microfilaria-positive individuals are asymptomatic, but with subclinical lymphangiectasia. We hypothesized that the excretory-secretory products (ES) of the worms activate the lymphatic endothelium directly or indirectly through an accessory cell. Initial experiments suggested that the ES did not directly activate lymphatic endothelial cells (LEC). Monocytes have been shown to play a role in lymphangiogenesis by secreting soluble factors; so we analyzed the production of IL-8, IL-6 and VEGF-A by peripheral blood mononuclear cells (PBMC) from naïve donors following stimulation with filarial ES as these factors can support endothelial proliferation and function. ES-stimulated PBMCs produced significantly increased levels of IL-8, IL-6 and VEGF-A compared to cells cultured in media alone and CD14+ monocytes were the primary producers of IL-8 and VEGF-A. Furthermore, IL-8, IL-6 and VEGF-A induced *in vitro* tubule formation by LEC in Matrigel cultures. Therefore, we tested the ability of IL-8, IL-6 and VEGF-A to stimulate *in vivo* vessel formation in August rats using a Matrigel plug model to measure lymphangiogenesis. Haematoxylin and eosin staining as well as immunohistochemical analysis using antibodies specific for LEC markers such as LYVE-1, podoplanin and VEGFR-3 revealed that stimulation with rat recombinant IL-8, IL-6 and VEGF-A induced cellular infiltration into the Matrigel plugs and *in vivo* LEC tubule formation. In addition, supernatants from human naïve PBMCs stimulated with filarial ES products were also incorporated into the Matrigel plugs and induced *in vivo* vessel formation. Collectively, these data suggest that monocytes may support lymphatic function through the secretion of soluble factors to encourage vessel growth and contribute to the pathogenesis of filarial disease. Our Matrigel experiments establish a useful model to study the molecular mechanisms associated with filarial pathology *in vivo*.

383

BASOPHILS ARE NOT NECESSARY FOR PROTECTION AGAINST CHALLENGE INFECTION IN A FILARIA VACCINE MODEL

Marina N. Torrero, Marc Hübner, David Larson, Ellen Mueller, Edward Mitre

USUHS, Bethesda, MD, United States

Live, irradiated L3 larvae induce substantial protection against challenge infection with *Litomosoides sigmodontis*, the only murine model of filariasis in which the parasites develop to mature, microfilaria-releasing adult worms. Recently, other investigators have demonstrated that basophils play an important role in protective immunity against intestinal helminth infections. To establish the role of basophils during vaccine-induced protection against filariae, Balb/c mice were immunized with three weekly s.c. injections of 25 irradiated L3 larvae, depleted of basophils with weekly i.p. injections of anti-CD200R3 antibody, and subsequently challenged s.c. with 40 infective L3 larvae. Mice were sacrificed 4 weeks p.i. Vaccine-induced responses in mice were characterized by high levels of parasite-specific IgE and IgG1, eosinophilia, and elevated concentrations of circulating IL-4. In addition, splenic CD4+T cells of vaccinated mice produced IL-4 and proliferated in response to parasite-antigen. Vaccinated mice that were depleted of basophils exhibited an attenuated type 2 response characterized by reduced levels of parasite-specific IgE and IgG1, less eosinophilia, and lower concentrations of IL-4 in blood. Even

though type 2 immune responses induced by vaccination were lower in basophil-depleted mice, basophil depletion did not alter protective immunity induced by the vaccination regimen. Specifically, vaccinated and vaccinated+ basophil-depleted mice had average worm recoveries of only 3.5 and 3.2 worms per mouse, respectively, whereas infected mice had an average of 20 adult worms per mouse. These findings suggest that basophils amplify type 2 immune responses, but are not necessary for vaccine-mediated protection against this tissue-invasive nematode.

384

EARLY EXPOSURE TO HELMINTH ANTIGENS INDUCES SPECIFIC TH1 AND TH2 CYTOKINES IN INFANTS WITH NO EFFECT ON SUBSEQUENT RESPONSE TO BCG

Yenny Djuardi[1], Heri Wibowo[1], Taniawati Supali[1], Erliyani Sartono[2], Maria Yazdanbakhsh[2]

[1]Faculty of Medicine University of Indonesia, Jakarta, Indonesia, [2]Leiden University Medical Center, Leiden, The Netherlands

Helminth infection during pregnancy have been reported to affect immune responses of the fetus and therefore may impact on how newborns respond to new antigens encountered early in life. Children and adults infected with helminths generally develop Th2-skewed immune responses as well as regulatory T cells which can compromise the immune response to Th1-producing vaccines such as BCG. We asked whether this is also the case in infants with developing immune maturity. Heparinized venous blood was collected from a total of 74 pairs of pregnant mothers and their infants at the age of 2 and 5 months, 1 year, 2 and 4 years of age. Whole blood assay was performed and samples were stimulated with crude *Brugia malayi* or *Ascaris lumbricoides* antigens. Measurement of Th1 (IFN-γ) and Th2 (IL-5) cytokines was done by luminex. Filarial specific IgG4 from plasma was analyzed by ELISA. Maternal filarial status was determined by filarial antigen detection and intestinal helminths by direct examination of stool. While maternal cytokine responses to helminths showed more Th2 dominance, children showed much lower helminth-specific Th2 responses but these responses increased with increasing age. Th1 responses to helminth antigens were more pronounced in children at early age. Starting from 2 years of age, Th2 response to BmA was higher in children born to helminth positive mothers compared to those born to helminth-free mothers. When we examined responses to PPD after BCG vaccination, both children born to helminth-infected or helminth-free mothers showed no significant differences in the Th1 and Th2 productions against PPD for up to 4 years of age. The results indicate the priming of child immune responses to helminth antigen resulted in both Th1 and Th2 responses, with no effect on subsequent cytokine responses to PPD.

385

CHRONIC HUMAN FILARIAL INFECTION LEADS TO ALTERED T CELL MEMORY AND A DEFECT IN EFFECTOR CELL TRANSITION

Cathy Steel, Thomas B. Nutman

National Institutes of Health, Bethesda, MD, United States

Chronic filarial infection has been associated with defects in parasite-specific T cell responses. Whether this defect reflects altered T cell memory, as occurs in chronic viral infections, has not been examined. Using multiparameter flow cytometry to identify T memory cell subpopulations in well-characterized patient groups from the filarial-endemic Cook Islands, we examined the role of persistent infection in the maintenance of T cell memory. Compared to filarial-uninfected endemic normals (EN, n=6), phenotyping of CD45RA- cells demonstrated a much smaller CD4 central memory (CD27+CCR7+IL7Rα+) compartment (Tcm) in mf+ infected patients (INF, n=5; GM% of CD45RA- cells=4.6 vs 8.4 in EN) as well as a smaller CD8+CD27-CCR7-IL7Rα- effector compartment (Teff; GM%=32 vs 42). These contracted Tcm and Teff populations were still evident in patients previously mf+ who had cleared their infection (CL-Inf; n=6; CD4+Tcm=3.8%, CD8+Teff=22%). In contrast, the CD8+CD45RA+ effector memory compartment (Temra+), containing both Ag-experienced effector as well as anergic cells, was expanded in both the INF and CL-INF compared to the EN (GM% of CD45RA+ cells=14 and 12 vs 9). Moreover, the density of IL7Rα, necessary for T memory cell maintenance (but decreased in T effector cells), was significantly higher in the INF as well as the CL-INF for CD4+T effector memory cells (Tem; EN vs INF and CL-INF, p=0.030 and 0.009), CD8+Tem cells (EN vs CL-INF, p=0.026) and CD8+Temra+ cells (EN vs INF, p=0.017). The increased expression of IL7Rα on these memory cell populations may indicate a defect in the ability of these cells to transition from memory to effector status. The fact that the Tem:Teff ratio was higher in both the INF and CL-INF compared to the EN group (CD4: EN vs INF and CL-INF, p=0.017 and 0.065; CD8: EN vs CL-INF, p=0.041) further supports this notion. Taken together, these data indicate that filarial-infected patients have contracted memory compartments and a defect in effector cell development compared to EN, which persists even following clearance of infection.

386

CONCOMITANT INFECTIONS: REDUCED INFECTIVITY OF *PLASMODIUM GALLINACEUM* TO THE MOSQUITO, *ARMIGERES SUBALBATUS*, IS MEDIATED BY *BRUGIA* MICROFILARIAE

Matthew T. Aliota, Jeremy F. Fuchs, Bruce M. Christensen

University of Wisconsin, Madison, WI, United States

Co-occurrence of multiple species of malaria parasites and co-occurrence of malaria and filarial worm parasites in a single human have been reported frequently in the tropics. But, little is known about the occurrence and prevalence of lymphatic filariasis and malaria transmitted by the same *Anopheles* vector and how these two parasites interact within this vector; therefore, it is important to gain an understanding of the interactions among parasites in a concomitantly infected vector to better design effective disease management programs. Herein, we present data evaluating the hypothesis that immune activation and/or development by filarial worms might negatively impact *Plasmodium* development in co-infected mosquitoes. To test this hypothesis in the laboratory, we conducted studies using the mosquito Armigeres subalbatus and the parasites *Brugia malayi*, *Brugia pahangi*, *Dirofilaria immitis*, and *Plasmodium gallinaceum*. Although they are a laboratory strain, *Ar. subalbatus* used in this study are natural vectors of *P. gallinaceum* and *B. pahangi* and they are naturally refractory to *B. malayi* (melanization-based refractoriness); therefore, using *Ar. subalbatus* as a model may provide a better depiction of the competition between filarial worm and malaria parasites within the same vector. Mosquitoes were dissected and oocyst mean intensities were analyzed six days after blood feeding on either *P. gallinaceum* alone or after taking a bloodmeal containing both *P. gallinaceum* and *B. malayi* or a bloodmeal containing both *P. gallinaceum* and *B. pahangi*. There was a significant reduction in oocyst mean intensity for all three biological replicates in mosquitoes that had a dual infection, regardless of *Brugia* species, as compared to those mosquitoes that were infected with *Plasmodium* alone; and this could have a significant impact on the measurement of vector infection and transmission dynamics, i.e., if filarial worm infection reduces the intensity of *Plasmodium* transmission, the elimination of filarial worms in a co-endemic locale could enhance malaria transmission.

387

SECRETED FILARIAL PRODUCTS INDUCE HUMAN MONOCYTES TO HAVE THE FUNCTIONAL AND PHENOTYPIC CHARACTERISTICS OF ALTERNATIVE ACTIVATION

Roshanak T. Semnani, Lily Mahapatra, Vivornpun Sanprasert, Thomas B. Nutman

National Institutes of Health, Bethesda, MD, United States

A defect in antigen presenting cell (APC) function is among the many mechanisms proposed to mediate the profound filarial-specific T cell hyporesponsiveness seen in lymphatic filariasis. This concept is based on findings that monocytes from patients with patent filarial infections are studded with internalized filarial antigens and express markers associated with alternative activation of macrophages. To explore the role of the filarial-derived parasite antigens in the differentiation process of human monocytes, monocytes were exposed to microfilariae (mf) of *Brugia malayi* and their phenotypic and functional characteristics were compared to monocytes exposed to factors known to generate either alternatively activated (IL-4) or classically activated (MCSF) macrophages. Like IL-4, exposure to mf did not alter the mRNA expression of ARG-1 or iNOS, but did induce significant upregulation of CCL17, CCL18, CCL22 and down-regulation of monocyte HLA-DR surface expression as compared to mf-unexposed monocytes. Secreted products from mf also significantly downregulated the monocyte surface expression of PDL2 and CD86 and mRNA expression of TLR3, TLR5, TLR7 and TLR8 resulting in decreased production of IL-10 and TNF-α following TLR ligand stimulation. In contrast to MCSF-cultured monocytes, exposure of monocytes to mf resulted in significant inhibition of the phagocytic capacity of these cells similar to IL-4 driven monocytes. Despite a phenotype reminiscent of alternatively activated macrophages (AAMΦ), mf failed to alter the ability to monocytes to mediate CD4+ and CD8+ T cell proliferation in response to anti-CD3. In summary, our data suggest that although, on balance, secreted filarial products skew monocytes toward an immunoregulatory phenotype suggestive of alternative activation, they also induce monocyte differentiation signals that involve innate immune pathways not typically found in AAMΦ. The role of these mf-altered monocytes in functioning to medicate the filarial-specific T cell hyporesponsiveness in human filarial infections is currently under study.

388

HELMINTH-MEDIATED PROTECTION AGAINST AUTOIMMUNE DIABETES IN NOD MICE IS NOT DEPENDENT ON FOXP3$^+$ REGULATORY T-CELLS

Marc P. Hübner[1], Yinghui Shi[1], Marina N. Torrero[1], Ellen Mueller[1], David Larson[1], Kateryna Soloviova[1], J. Thomas Stocker[1], Kristin V. Tarbell[2], Edward Mitre[1]

[1]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, United States

Helminth infections exert beneficial effects on autoimmune diseases. Previously we showed that infection with the filarial nematode *Litomosoides sigmodontis* prevents the onset of diabetes in nonobese diabetic (NOD) mice and that the protective effect is independent of a Th2 immune response. In this study we tested the hypothesis that *L. sigmodontis*-induced regulatory immune responses prevent the onset of diabetes in NOD mice. Infection of NOD mice with the filarial nematode *L. sigmodontis* prevented the onset of diabetes and was associated with significantly increased frequencies of splenic and pancreatic lymph node CD4+CD25+FoxP3+ cells. Splenic FoxP3+ cells from infected NOD mice showed significantly increased proliferation as measured by Ki67 positivity and increased expression of CTLA-4. Transfer of spleen cells from 12-week old NOD mice into NOD.scid mice demonstrated that spleen cells from uninfected, but not *L. sigmodontis*-infected NOD mice, induced diabetes in NOD.scid mice. Lack of diabetes induction by splenocytes of *L. sigmodontis*-infected mice did not appear due to active regulation of effector lymphocytes by FoxP3+ T-regulatory cells as splenocytes of *L. sigmodontis*-infected eGPF FoxP3 NOD mice depleted of FoxP3+ cells also did not induce diabetes in recipient mice. These results suggest that *L. sigmodontis* alters the potency of autoimmune-inducing cells in infected mice through a mechanism independent of active regulation by FoxP3+ cells. Similarly, continuous depletion of CD25+ regulatory T-cells with PC61 did not reduce the protective effect *L. sigmodontis* has on NOD mice. As *L. sigmodontis* infection in NOD mice increases splenic IL-10 and TGFβ production, we are currently treating helminth-infected and uninfected NOD mice with anti-IL-10R and anti-TGFβ to test whether helminth-induced protection is dependent on these immunoregulatory cytokines. These studies demonstrate that filarial worms protect against the onset of Type 1 diabetes in NOD mice by reducing the effector capability of autoimmune cells through a mechanism that is independent of Th2 responses or FoxP3+ T-regulatory cells.

389

DENGUE OUTBREAK -- KEY WEST, FLORIDA, 2009

Christopher Gregory[1], Elizabeth Radke[2], Kristina Weis[2], Erin Sauber-Schatz[3], Glen Gallagher[1], Elizabeth Hunsperger[1], Kay Tomashek[1], Brad Biggerstaff[4], Lillian Stark[5], Jorge Munoz[1], Carina Blackmore[2]

[1]Centers for Disease Control and Prevention, San Juan, Puerto Rico, [2]Florida Department of Health, Gainesville, FL, United States, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [5]Florida Department of Health, Tampa, FL, United States

In September 2009, three cases of autochthonous dengue were identified in Key West, Florida after a 70-year absence. An outbreak investigation to determine the incidence of and risk factors for recent dengue infection in Key West was conducted by the Florida Department of Health and the CDC's Dengue Branch. A stratified random sample of households within 1 kilometer radius of the index cases was selected. At each household, residents were asked to provide blood samples and medical and travel histories. Blood was tested for anti-dengue IgM and IgG antibodies. Antibody-positive samples were tested with a plaque-reduction neutralizing test (PRNT) to determine the infecting flavivirus serotype. For participants with fever in the past 7 days, samples were tested for dengue virus (DENV) by RT-PCR and non-structural protein-1 (NS-1) assay. Mosquitoes were collected from the area and tested for DENV by RT-PCR. Blood was collected from 240 persons in 175 households. Eight (3.3%) participants who had not recently traveled had evidence of recent dengue infection by IgM, RT-PCR, or NS1; 5 were identified as having DENV-1. Ninety-one (37.9%) participants were IgG positive, indicating possible past flavivirus infection. Of these, 5 (2.1%) persons with a dengue-like illness in the past 3 months were classified as having probable recent dengue infection by PRNT. Genotyping showed that mosquito and human isolates were closely related to each other and to a strain of dengue 1 isolated in Mexico. In multivariate analysis having a bird bath or wading pool in the yard or having ever lived outside the US were risk factors for dengue. Emptying water-filled containers or using prevention measures such as repellent were protective factors. Approximately 5% of Key West residents tested positive for recent dengue infection, making this the largest dengue outbreak in the continental United States outside the Texas-Mexico border region in over 60 years. With increasing international travel and ample *Aedes aegypti* mosquito populations, Key West and southern Florida may be at risk of future dengue outbreaks.

390

QUANTIFYING THE SPATIAL DIMENSION OF DENGUE VIRUS EPIDEMIC SPREAD WITHIN A TROPICAL URBAN ENVIRONMENT

Gonzalo M. Vazquez-Prokopec[1], Uriel Kitron[1], Brian Montgomery[2], Peter Horne[2], Scott A. Ritchie[3]

[1]Emory University, Atlanta, GA, United States, [2]Queensland Health, Cairns, Australia, [3]James Cook University, Cairns, Australia

Dengue infection spread in quasi-naive populations occurs in an explosive and widespread fashion. Knowledge on the contribution different factors such as human movement, vector dispersal and the built environment to the propagation of dengue virus infection has been limited. We analyzed the spatio-temporal pattern a large dengue virus-2 (DENV-2) outbreak that affected the Australian city of Cairns (north Queensland) in 2003, quantified the relationship between dengue transmission and distance to the epidemic's index case (IC), evaluated the effects of indoor residual spraying (IRS) on the odds of dengue infection, and generated recommendations for city-wide dengue surveillance and control. We retrospectively analyzed data on the exact position of the most likely place of infection for 383 DENV-2 confirmed cases and on the location and timing of 1,163 IRS applications. Spatial and space-time analyses determined the intensity and directionality of clustering of dengue cases, whereas a Bayesian space-time regression assessed the impact of IRS in the odds of weekly dengue infection. About 63% of the cases clustered up to 800 m around the IC's house. Most cases were distributed in the NW-SE axis as a consequence of the arrangement of the built environment and, possibly, the prevailing winds. Infection spread rapidly, generating 18 clusters (comprising 65% of all cases); clusters varied in severity and extent as a function of their distance to the IC's house. Human movement contributed to ~40% of the observed transmission. IRS applications had a significant protective effect in the occurrence of dengue cases only when reached coverage of 60% or more of a house's neighboring premises. By applying sound statistical analysis, we described the spread of dengue virus with high detail and quantified the spatio-temporal dimension of dengue virus transmission within a complex urban environment. We foresee that some of the results and recommendations derived from our study may also be applicable to other areas currently affected or potentially subject to dengue epidemics.

391

CONTACT CLUSTER INVESTIGATIONS REVEAL A KEY ROLE OF HUMAN MOVEMENT PATTERNS IN THE TRANSMISSION OF DENGUE VIRUS

Steven T. Stoddard[1], Amy C. Morrison[1], Brett M. Forshey[2], Valerie Paz-Soldan[3], Gonzalo Vazquez-Prokopec[4], Helvio Astete[5], Wilder Carrasco[5], Shirley M. Guedez[5], Wendy L. Quiroz[5], Esther J. Rios[5], Eric Halsey[2], John Elder[6], Uriel Kitron[4], Tadeusz J. Kochel[2], Thomas W. Scott[1]

[1]University Of California, Davis, Davis, CA, United States, [2]Naval Medical Research Center Detachment Lima, Lima, Peru, [3]Tulane University, New Orleans, LA, United States, [4]Emory University, Atlanta, GA, United States, [5]Naval Medical Research Center Detachment, Iquitos, Peru, [6]San Diego State University, San Diego, CA, United States

Aedes aegypti, the principal vector of dengue virus (DENV), feeds during the day when people are active. Consequently, exposure is not limited to the individual's home, and the daily movement of individual humans will play a key role in defining the pattern and spatial extent of DENV transmission. This hypothesis predicts that there should be evidence of dengue infection in the locations visited recently by dengue infected individuals. To test this, we collected blood samples on day 0 and day 15 from febrile individuals (index) and contacts living in locations the index recently visited. Between August of 2008 and April of 2010, we conducted 66 contact cluster investigations initiated by DENV-positive (30) and negative (36) febrile participants in Iquitos, Peru. We identified a total of 166 DENV infections by RT-PCR, IFA, and/or IgM ELISA (titer ≥ 1:100): 124 infections among participants within positive clusters and 42 among participants in negative clusters, for attack rates of 0.25 and 0.07 respectively ($P<<0.001$). Overall, 56% of all infections occurred in sites outside the home. Within negative clusters, 74% of infections occurred outside of the home and attack rates were 0.06 for the home and 0.08 for other sites. Within positive clusters, 51% of infections occurred outside the home with attack rates of 0.32 for the home and 0.21 for sites outside the home ($P=0.02$). Patterns of increased dengue transmission in sites recently visited by dengue positive participants were especially marked in 2008 when DENV-4 first invaded Iquitos, but were maintained over the study period despite rapidly rising herd immunity. Our data indicate that 1) movement patterns of individuals play an important role in defining the pattern and spatial extent of transmission and 2) consideration of human behavior and movement patterns will increase the effectiveness of surveillance and control programs.

392

THE ROLE OF IMPORTED CASES AT DIFFERENT STAGES OF DENGUE EPIDEMICS

Chuin-Shee Shang[1], Chi-Tai Fang[1], Chung-Ming Liu[2], Tzai-Hung Wen[3], Kun-Hsien Tsai[1], Chwan-Chuen King[1]

[1]Graduate Institute of Epidemiology, National Taiwan University, Taipei City, Taiwan, [2]Global Change Research Center, National Taiwan University; Department of Atmospheric Sciences, National Taiwan University, Taipei City, Taiwan, [3]Department of Geography, National Taiwan University, Taipei City, Taiwan

Dengue/dengue hemorrhagic fever is the world's most widely spread mosquito-borne arboviral disease and threatens more than two thirds of the world's population. Cases are mainly distributed in tropical and subtropical areas in accordance with vector habitats for *Aedes aegypti* and A. albopictus. Rapid and frequent international travel also contributes to the geographical expansion of dengue epidemics. However, the role of imported cases in those countries/areas where dengue has not become endemic yet remains unclear. The specific aims of this study are to investigate the interplays between imported and indigenous dengue cases and climate conditions at different stages of epidemics. We analyzed bi-weekly, laboratory-confirmed dengue cases at their onset dates of illness from 1998 to 2007 to identify correlations between indigenous dengue and imported dengue cases in the context of local meteorological factors across different time lags. Our results revealed that imported cases have a role in igniting indigenous outbreaks in southern Taiwan (a non-endemic area) when favorable weather conditions are present and where *Aedes aegypti* mosquitoes are mainly distributed. In addition, the imported and indigenous dengue cases had a significant quantitative relationship only at the onset of local epidemics. However, this relationship became less significant once indigenous epidemics progressed past the initial stage. These findings imply that imported dengue cases are able to initiate indigenous epidemics when appropriate weather conditions are present. An early-warning surveillance system, that is able to integrate local meteorological data, is crucial to successful prevention and control of dengue, particularly in countries where dengue is non-endemic. The deployment of such an integrated system will be an invaluable tool for averting the global risk of dengue/dengue hemorrhagic fever epidemics in an era of climate change.

393

A STOCHASTIC SIMULATION TOOL FOR SPATIALLY PRIORITIZING RISK IN THE TRANSMISSION OF DENGUE VIRUSES

Karen M. Campbell[1], C.D. Lin[1], Thomas W. Scott[2]

[1]San Diego State University, San Diego, CA, United States, [2]University of California, Davis, CA, United States

One of the unanswered challenges in providing cost effective allocation of integrated resources to prevent outbreaks of dengue viruses is that space-time virus transmission is reported to be erratic and unpredictable from place to place and year to year. Tools to provide improved understanding of transmission dynamics of a region in order to more effectively anticipate the location and timing of epidemics would be important contributions to prevention programs. We developed a Reversible Jump Markov Chain Monte Carlo Bayesian simulation tool to examine the space-time pattern of incidence in a large region over many years of transmission. The tool is used to identify geographic patterns in transmission that are innate to a region as well as spatial migrations in incidence behavior over time. It is also used to partition a large region into innate geographical structures with highly correlated incidence behavior over time. We applied this tool in evaluating the space-time transmission dynamics of dengue viruses throughout Thailand over 2+ decades (1983-2004). Our analysis revealed a definitive geographic pattern that subdivides the region into zones of synchronized risk and predicts geographically varying spatial extent in the spread of seasonal epidemics. We developed graphical representations of spatially correlated risk across the region at a range of spatial scales that are intrinsically defined by the data. We analyzed the spatial structure of risk in the context of space-time influences from the environment (geography and weather) and from human population dynamics and observed clear evidence of the interaction between both sets of forces (human and entomological) in regulating the spread of dengue viruses. This tool is also being applied to regions in other parts of the world. The simulation requires no initial assumptions about the spatial dynamics of the region in order to produce a space-time assessment of dynamics of risk. We will present an overview of the tool, the statistical model that drives the simulation, and the results of our analyses.

394

A MULTI-HOST, MULTI-VECTOR SIR MODEL OF DENGUE 2 VIRUS IN SENEGAL

Benjamin Althouse[1], Amadou Sall[2], Kathy Hanley[3], Mawlouth Diallo[2], Douglas Watts[4], Scott Weaver[5], Derek Cummings[6]

[1]Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Institut Pasteur de Dakar, Dakar, Senegal, [3]Department of Biology, New Mexico State University, Las Cruces, NM, United States, [4]Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, United States, [5]Center for Biodefense and Emerging Infectious Diseases and Department of Pathology, University of Texas Medical Branch, Galveston, TX, United States, [6]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Epidemics of Dengue 2 virus (DENV) have been observed in Senegal with interepidemic periods between 7 and 8 years. This cycle may be the result of persistence of DENV in one primate reservoir species with periodic spillover transmission to other primates including humans, or ongoing sustained transmission in multiple primate species. DENV infections have been detected in several primate species in Senegal, each with distinct demographic dynamics and potential differences in DENV transmission efficiencies. Here we report the results of a time series analysis of DENV incidence in multiple vector species in Senegal, and investigate the dynamics of models that incorporate multiple hosts and vectors, and determine which exhibit long interepidemic periods. We present a multi-host, multi-vector deterministic seasonally forced SIR model to investigate the impact of multiple host species on the incidence of DENV in Senegal. We consider human, patas monkey, baboon, and green monkey as host species and *Aedes aegypti*, and *Ae. furcifer* as vector species. We first examine each primate-mosquito system as uncoupled, and then as coupled to the other primate-mosquito systems through non-zero cross-species biting rates. We find long-period multiannual cycles predominantly when transmission probabilities (primate to mosquito and mosquito to primate) are low. As we increase the coupling between systems we find that for low values of coupling (1/1000th of normal biting rates) we find large epidemics occur in all species at once with long interepidemic periods. We consider multiple formulations of scaling of biting rates of the two vectors by primate body size and determine the impact on long-term dynamics. In conclusion, little work has been done to model the impact of multiple host species on the incidence of DENV in sylvatic settings. The long interepidemic periods in Senegal have yet to be explained. Our work demonstrates that the inclusion of multiple host species has little impact on either length of period or the size of epidemic outbreaks in humans. We identify critical ranges of both coupled and uncoupled models that exhibit the long periods observed in the data.

395

FIRST YEAR OF ENHANCED DENGUE SURVEILLANCE AT AN ACUTE CARE SETTING IN PUERTO RICO

Rosa L. Rodríguez-Acosta, D. Fermin Argüello, Gladys González-Zeno, Kay M. Tomashek

Centers for Disease Control and Prevention - Dengue Branch, San Juan, PR, United States

Dengue is an acute febrile mosquito borne illness caused by four dengue virus serotypes. In Puerto Rico, dengue is endemic and reportable by law. The Centers for Disease Control and Prevention (CDC) and the Puerto Rico Department of Health (PRDH) has operated a passive dengue surveillance system (PDSS) for over 30 years. In June 2009 CDC implemented an enhanced dengue surveillance system (EDSS) at a 161 bed, acute care hospital in Guayama, Puerto Rico. Upon implementation physicians and nurses were trained in dengue diagnosis, case management, and reporting. Case reporting was initiated by clinicians, and CDC staff oversaw day-to-day case reporting, reviewed case forms for accuracy and completeness, and prepared serum samples for delivery to the CDC laboratory. Clinical and epidemiological data and serum samples were collected from patients fitting dengue clinical criteria as defined by the World Health Organization. From June 1, 2009 to April 30, 2010, 837suspected dengue cases from 12 municipalities across the Southeastern region were reported, 474 (57%) and 15 (2%) fit criteria for dengue fever (DF) and dengue hemorrhagic fever (DHF), respectively. Laboratory-positives (408, 49%) were identified in 7 municipalities; 351 (86%) were RT-PCR positive and 57 (14%) were dengue IgM positive. DENV-1, -2, and -4were detected during in these municipalities, but DENV-1 made up the majority (94%) of infections. There were 155 (18%) laboratory-negative and 255 (30%) indeterminate cases. Laboratory-positives were equally distributed by gender, with a mean age of 23 years (median =17, sd=17). Most cases were between the ages of 10-14 (92, 23%) and 15-19 (65, 16%). Many laboratory-confirmed cases (46%) were hospitalized and no deaths occurred. Two hundred and fifty nine (64%) met the case definition for DF while 12 (3%) met the criteria for DHF. In its first year EDSS was instrumental in providing timely identification and characterization of dengue cases leading to a better description of dengue occurrence and severity in the Southeastern region of Puerto Rico.

396

EXPRESSION AND KINETIC CHARACTERISATION OF NON DISCRIMINATING *PLASMODIUM FALCIPARUM* APICOPLAST GLUTAMYL-TRNA SYNTHETASE (PFGLURS)

Boniface M. Mailu[1], Megan Peterson[1], Jen Arthur[1], Gowthaman Ramasamy[1], Devaraja Mudeppa[2], Pradipsinh K. Rathod[2], Malcolm J. Gardner[1]

[1]Seattle Biomedical Research Institute, Seattle, WA, United States, [2]University of Washington, Department of Chemistry, Seattle, WA, United States

The genome sequences of malaria parasites have revealed many novel insights into parasite biochemistry and the identification of potential drug targets. We identified *Plasmodium falciparum* genes encoding glutamyl-tRNA synthetase (GluRS) and glutamyl-tRNA amidotransferase (Glu-AdT), enzymes that comprise an indirect aminoacylation pathway for the production of Gln-tRNAGln, a key substrate for protein biosynthesis. In archaea, most bacteria, and (probably) plastids, this pathway is the sole route for the production of Gln-tRNAGln, but it is not found in the eukaryotic cytosol. Bioinformatic analyses suggest that the *Plasmodium* GluRS and Glu-AdT orthologs are targeted to the apicoplast. Aminoacyl tRNA synthetases are potential drug targets because their catalytic activities determine the genetic code and therefore they are essential for protein synthesis and cell viability. We expressed the full-length as well as truncated forms (minus the apicoplast targeting sequence) of PfGluRS. Recombinant PfGluRS glutamylated the cognate substrate apicoplast tRNAGlu as well as the non-cognate substrate apicoplast tRNAGln. The latter activity is diagnostic of GluRSs that participate in indirect aminoacylation, demonstrating that this pathway is functional in *Plasmodium*. Kinetic characterization was performed using conditions established to produce linear kinetics within a 3-minute reaction. Both forms of the enzyme exhibited a higher affinity towards the cognate tRNAGlu substrate as compared to the non-cognate tRNAGln substrate. The truncated enzyme exhibited a marked lower preference towards tRNAGlu than the full-length enzyme while at the same time exhibiting a higher velocity (Kcat) and catalytic efficiency (Kcat/Km), suggesting that the removal of the apicoplast targeting sequence affects enzyme activity. Like many tRNA synthetases, PfGluRS is subject to product inhibition by PPi but upon addition of inorganic pyrophosphatase a 5-fold stimulation of the reaction rate was observed. We will describe the biochemical characterization of the apicoplast GluRS as well as progress towards expression and reconstitution of the PfGlu-AdT, the second enzyme in the pathway.

397

TRANSCRIPTIONAL ANALYSIS OF FATTY ACID STARVATION IN *PLASMODIUM FALCIPARUM*

Claire E. Brown, Johanna P. Daily

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

Plasmodium falciparum causes millions of infections worldwide and a high burden of mortality. As half the earth's population remains at risk, and drug-resistance remains a challenge, investigating parasite biology is essential. We previously described a novel *in vivo* transcriptional profile in *P. falciparum* obtained from blood samples of infected patients. The novel state appears to represent a starvation response, with induction of metabolic pathways and genes, including those for fatty acid biosynthesis. Parasites require exogenous fatty acids, and thus we test the hypothesis that this novel state may occur in response to limited availability of host fatty acids. We cultivated the 3D7 strain of *P. falciparum* in lipid-depleted media reconstituted with incremental concentrations of fatty acids known to support parasite growth *in vitro*. Samples were analyzed by gas chromatography coupled to a flame ionization detector. When cultures were subjected to the lowest concentration of fatty acids, we detected the induction of multiple fatty acid species, stearic acid being most abundant. We also performed metabolic labeling using 14C-acetate, and confirmed increasing synthesis of lipids as a result of decreasing fatty acid content in the culture media. Using parasites also cultivated under lipid-depleted conditions, we extracted RNA to analyze the gene transcripts for fatty acid synthesis. Using real-time PCR, we demonstrated a step-wise induction of FAS II gene transcripts as the fatty acid content of the culture media was decreased. We will present the correlating whole genome transcriptional profile in order to identify alterations in other GO functions associated with the fatty acid starvation state. Through the *in vitro* recapitulation of the starvation state we can characterize *P. falciparum's* unique biology and its impact on the human host.

398

CORRELATION OF αII-TUBULIN AND PFG377 ORTHOLOG GENE EXPRESSIONS IN *PLASMODIUM VIVAX* GAMETOCYTES AND MOSQUITO INFECTION

Nakit Chansamut[1], Sureemas Buates[1], Rachanee Udomsangpetch[1], Somporn Krasaesub[2], Nattawan Rachaphaew[2], Ratree Takhampunya[2], Jetsumon Sattabongkot[2]

[1]Mahidol University, Bangkok, Thailand, [2]United States Army Medical Component Armed Forces Research Institute of Medical Science, Bangkok, Thailand

Plasmodium vivax transmission from humans to mosquitoes requires the presence of infectious gametocytes in human peripheral blood. An index of infectiousness based solely on an analysis of gametocyte counts from blood films is unreliable. There is no correlation between gametocyte density, which is generally low, and mosquito infection. This may due to the gametocyte examination by microscope is not sensitive and cannot identify the infective or mature gametocyte from the dead or immature ones. Gametocyte stages display a distinct pattern of gene expression than asexual stages. In *P. falciparum*, αII-tubulin and Pfg377 are gametocyte-specific genes essential for microgametocyte formation and the emergence of macrogametes from erythrocytes during the gametogenesis, respectively. The expression of these genes may be useful for predicting infectiousness of the gametocytes to the mosquito vectors. In this study, the transcripts of αII-tubulin and Pfg377 ortholog genes in *P. vivax* were determined by quantitative real-time PCR using 73 clinical blood samples. *Anopheles dirus* mosquitoes were fed on these blood samples to determine the infectiousness of the gametocytes. The parasites from those samples infective to mosquitoes expressed significantly higher levels of αII-tubulin and Pfg377 ortholog than those of in the non-infective group. However, there were the weak correlations between the expression of αII-tubulin and Pfg377 ortholog genes and the mean oocyst number in mosquitoes' midgut. The levels of expression of these genes may be useful to predict the *P. vivax* gametocyte infectiousness for malaria surveillance.

399

PLASMODIUM FALCIPARUM QUANTITATIVE TRAIT LOCI DETERMINING INFECTIVITY TO *ANOPHELES GAMBIAE* MOSQUITOES

Jonathan M. Mwangi, Lisa Ranford-Cartwright

Division of Infection and Immunity, Faculty of Biomedical and Life Sciences, University of Glasgow, Glasgow, United Kingdom

Malaria parasites differ in the prevalence (proportion infected) and intensity (number of oocysts per mosquito) of mosquito infections. Our main aim is to determine the number of parasite genetic loci that contribute to infectivity differences and locate them on a genetic map. We have previously identified two genetically distinct clones of *Plasmodium falciparum* (denoted 3D7 and HB3) that differ significantly ($P < 0.0001$) in their ability to establish mature oocyst infections in *Anopheles gambiae* mosquitoes, natural vectors of human malaria. We have used a quantitative trait locus (QTL) approach to map the parasite loci contributing to differences in prevalence and intensity of infection. The prevalence and intensity of infection in *A. gambiae* mosquitoes were measured for 20 progeny clones from the 3D7 X HB3 genetic cross. The

progeny clones were genotyped using a custom-built Affymetrix molecular inversion probe 10K malaria panel array with a coverage of ~1 SNP per 3 kb to generate a genetic map. Here we present data for a major locus on chromosome 12 of the parasite which contributes both to prevalence and to infection intensity. This locus is responsible for 94% of the observed phenotype for infection intensity and 44% of the observed prevalence phenotype. The locus contains ~27 open reading frames. Additional loci on other chromosomes make minor contributions to the traits. This study demonstrates for the first time a single parasite QTL having a major effect on mosquito infection.

400

PERUVIAN *PLASMODIUM FALCIPARUM*: HISTORICAL BOTTLENECKS OR RECENT INTRODUCTIONS?

Sean M. Griffing[1], Tonya Mixson-Hayden[2], Sankar Sridaran[3], Md T. Alam[4], Andrea M. McCollum[2], César Cabezas[5], Wilmer Marquiño[6], John W. Barnwell[7], Alexandre Macedo De Oliveira[7], Carmen Lucas[8], Nancy Arrospide[5], Ananias A. Escalante[9], David Bacon[8], Venkatachalam Udhayakumar[7]

[1]Emory University, Centers for Disease Control and Prevention, Atlanta Research and Education Foundation, Chamblee, GA, United States, [2]Malaria Branch, Centers for Disease Control and Prevention, Atlanta Research and Education Foundation, Chamblee, GA, United States, [3]Malaria Branch, Centers for Disease Control and Prevention, Association of Public Health Laboratories, Chamblee, GA, United States, [4]Malaria Branch, Centers for Disease Control and Prevention, Chamblee, GA, United States, [5]Instituto Nacional de Salud, Lima, Peru, [6]Pan American Health Organization, Washington, DC, United States, [7]Centers for Disease Control and Prevention, Chamblee, GA, United States, [8]Naval Medical Research Center Detachment, Lima, Peru, [9]Arizona State University, Phoenix, AZ, United States

Population genetics principles have previously been used to explain the impact of natural selection on the spread, maintenance, and decline of drug-resistant alleles and global population structure of *Plasmodium falciparum*. In South America, this population structure was demonstrated to be the least diverse in the world yet highly differentiated. In Peru, malaria control efforts reduced the incidence of malaria after the 1950s, which could have induced bottlenecks. During the 1990s, multiple epidemics of malaria occurred in Peru. We tested the hypothesis that Peruvian *P. falciparum* populations rapidly expanded from locally bottlenecked populations or founder migrants from neighboring areas. We investigated the genetic relatedness of *P. falciparum* parasites (n=220) using samples from the Peruvian Pacific Coast (Bellavista, La Arena, Zarumilla) and the western (Pampa Hermosa, Ullpayacu), central (Padre Cocha), and eastern (Caballococha) Peruvian Amazon collected in 1999 and 2000. We sequenced *dhfr*, *dhps*, *pfcrt*, and *pfmdr1* (genes linked to sulfadoxine-pyrimethamine and chloroquine resistance), 54 proximal microsatellite markers, and 12 neutral markers. We tested our data with pairwise Fst, AMOVA, median joining network diagrams, pairwise linkage disequilibrium, and the Bottleneck application. Our findings include the first description of genotypes collected in coastal Peru. Across all sites, parasite lineages demonstrated limited genetic diversity and multilocus linkage disequilibrium (LD) across all 4 resistance genes and proximal microsatellites, and neutral markers. Our results indicate there were 5 clonal lineages that rapidly expanded, with some representing bottlenecked local populations and others recent introductions. In addition, population structure exhibited admixture rather than isolation by distance. *P. falciparum* population structure should be carefully considered when planning and interpreting molecular epidemiology-based surveillance data.

401

GENOME-WIDE ASSOCIATION STUDIES FOR ANTIMALARIAL RESISTANCE UNCOVERS NOVEL TARGETS IN *PLASMODIUM FALCIPARUM*

Daniel J. Park[1], Daria Van Tyne[2], Stephen F. Schaffner[1], Daniel E. Neafsey[1], Elaine Angelino[3], Joseph F. Cortese[1], Kayla G. Barnes[2], David M. Rosen[2], Amanda K. Lukens[2], Rachel F. Daniels[1], Danny A. Milner[2], Charles A. Johnson[1], Ilya Shlyakhter[3], Sharon R. Grossman[3], Justin S. Becker[2], Daniel Yamins[3], Elinor K. Karlsson[3], Daouda Ndiaye[4], Ousmane Sarr[4], Souleymane Mboup[4], Christian Happi[5], Nicholas A. Furlotte[6], Eleazar Eskin[6], Hyun Min Kang[7], Daniel L. Hartl[3],Bruce W. Birren[1], Roger C. Wiegand[1], Eric S. Lander[1], Dyann F. Wirth[2], Sarah K. Volkman[2], Pardis C. Sabeti[3]

[1]Broad Institute, Cambridge, MA, United States, [2]Harvard School of Public Health, Boston, MA, United States, [3]Harvard University, Cambridge, MA, United States, [4]Cheikh Anta Diop University, Dakar, Senegal, [5]University of Ibadan, Ibadan, Nigeria, [6]University of California, Los Angeles, CA, United States, [7]University of Michigan, Ann Arbor, MI, United States

Plasmodium falciparum malaria's rapid adaptation to new drugs allows it to remain one of the most devastating infectious diseases of humans. Understanding the genetic basis of these adaptations is critical to successful intervention. Genome-wide association studies (GWAS) are a promising approach to directly identifying genetic variation that may be contributing to both established and emerging drug resistance. We developed a high-density genotyping array and applied it to 57 culture-adapted parasites, characterizing population structure and applying long-haplotype tests to identify signatures of selection. Coupled with drug-sensitivity phenotyping, we performed association studies with 13 antimalarials. Using recently developed GWAS tools such as the EMMA and HLR tests, we were able to control for population structure and detect known and novel resistance loci at genome-wide significance. Functional analysis of one of the novel halofantrine hits revealed that PF10_0355 overexpression decreases sensitivity to halofantrine, mefloquine and lumefantrine but not to structurally unrelated antimalarials, and that resistance is mediated by increased gene copy number. This demonstrates the effective application of these GWAS methods, and shows the usefulness of GWAS more generally for understanding the genetic basis for antimalarial drug resistance in the wild, potentially identifying important biomarkers for surveillance as elimination and eradication efforts are pursued.

402

NEXT-GENERATION SEQUENCING FOR BASIC BIOLOGICAL AND TRANSLATIONAL STUDIES OF THE MALARIA PARASITE

Daniel E. Neafsey[1], Alex Melnikov[1], Andreas Gnirke[1], Kevin Galinsky[1], Rachel Daniels[1], Danny A. Milner[2], Carsten Russ[1], Tharanga Niroshini Senaratne[2], Jonathan Hermann[2], Kayla Barnes[2], Daoudo Ndiaye[3], Ousmane Sarr[3], Souleymane Mboup[3], Bruce W. Birren[1], Roger F. Wiegand[1], Dyann F. Wirth[1], Pardis C. Sabeti[3], Sarah K. Volkman[1]

[1]Broad Institute, Cambridge, MA, United States, [2]Harvard School of Public Health, Boston, MA, United States, [3]Cheikh Anta Diop University, Dakar, Senegal

We report on advances in adapting next-generation Illumina sequencing into a 'post-genomic' research tool useful for investigating pertinent biological questions associated with malaria parasites. We have devised a hybrid selection protocol to significantly enrich parasite DNA in samples containing large amounts of host DNA, and demonstrated the viability of this enrichment approach on whole-genome-amplified samples. These strategies will not only allow for far more efficient sequencing of non culture adapted parasite samples, but will enable sequencing of samples previously considered unsuitable, such as DNA derived from blood spots on filter papers. We have also developed a method to estimate the multiplicity of infection (MOI) with unprecedented accuracy in clinical

samples that leverages the enormous power of Illumina sequencing in an economical way using a multiplexed approach to sequence PCR amplicons of highly polymorphic loci. We report on the multiplicity as well as the proportional representation of parasite strains within a collection of complex infections from Senegal, and will discuss the biological relevance of accurate MOI estimation for future drug, vaccine, and epidemiological studies. Finally, we will report on methodologies developed for field assessment of key genetic variants in epidemiological and surveillance studies using high resolution melting technologies. Collectively, we will discuss methodological and technical advances to identify genetic loci of biological interest and survey for these loci directly from patient-derived materials as well to assess parasite numbers and types as intervention strategies are applied.

403

RANDOMIZED STUDY COMPARING ARTESUNATE PLUS AMODIAQUINE TO ARTHEMETER PLUS LUMEFANTRINE FOR THE REPEATED TREATMENT OF RECURRENT *PLASMODIUM FALCIPARUM* UNCOMPLICATED MALARIA OCCURRING IN COHORT FOLLOWED DURING TWO YEARS IN SENEGAL

Jean Louis A. Ndiaye[1], Babacar Faye[1], Ali Gueye[1], Corinne Tchania[1], Ibrahima Ndiaye[1], Brigitte Charron[2], Valerie Lameyre[2], Oumar Gaye[1]

[1]Department of Parasitology, Faculty of Medicine, Cheikh Anta Diop University, Dakar, Senegal, [2]Impact Malaria, Sanofi Aventis Group, Paris, France

The use of artemisinin combination therapy (ACT) is currently recommended for treating uncomplicated malaria. Our objective was to assess the efficacy and safety of repeated administrations of two fixed-dose combination ACT: artesunate + amodiaquine (ASAQ) and artemether-lumefantrine (AL) in consecutive episodes of *Plasmodium falciparum* malaria. A randomized, investigator-blinded, comparative study was conducted in a rural community of central Senegal from August 2007 to January 2009. Children and adult patients with uncomplicated *P. falciparum* malaria were randomized to receive ASAQ once daily, or AL twice daily for three days. Drug doses were given according to body weight range. Treatments for first episodes were supervised and unsupervised for subsequent ones. ECG and audiograms were performed in patients > 12 years of age. Primary outcome was adequate parasitological and clinical response rate after PCR correction on Day 28 for the first episode. A total of 840 patients were screened, 366 patients were enrolled in the two groups (184 for ASAQ and 182 for AL) and followed-up during 2 malaria transmission seasons. In the ITT population, ACPR after PCR correction at D28 for the 1st episode was 98.4% vs 96.2% respectively in the ASAQ and AL groups. A 100% ACPR rate was also obtained at D28 in the 60 and 4 patients who experienced respectively a 2nd and a 3rd episode. Treatment-related AEs were reported in 11.7% of the patients without significant differences between the 2 groups. A better improvement of hemoglobin rate at D28 was noted in the ASAQ group. No sign of ototoxicity was demonstrated. A widening of the QTc interval was observed in both groups during treatment with no clinical consequence. Study results confirmed the satisfactory efficacy and safety profile of ASAQ and AL. Moreover, in patients who were treated at least twice, repeated administration of ASAQ or AL did not result in any significant safety issue.

404

COGNITIVE FUNCTIONING AFTER CEREBRAL MALARIA IN UGANDAN CHILDREN BELOW FIVE YEARS: A PROSPECTIVE STUDY

Paul Bangirana[1], Michael J. Boivin[2], Robert O. Opoka[1], Richard Idro[1], Justus Byarugaba[1], Chandy C. John[3]

[1]Makerere University, Kampala, Uganda, [2]Michigan State University, East Lansing, MI, United States, [3]University of Minnesota, Minneapolis, MN, United States

Earlier studies in African children aged 5 to 12 years have shown cognitive deficits mainly in attention and memory after an episode of cerebral malaria (CM). We present preliminary results of cognitive function after CM in children less than 5 years of age. Sixty nine Ugandan children aged 18 months to 4.9 years admitted with CM at Mulago Hospital were assessed for fine and gross motor skills, receptive and expressive language skills, memory and visual spatial skills a week after discharge and six months later. Test scores were compared to 67 community controls recruited from the families of the CM group or other similar families. Children with CM more frequently had impairment in one or more of the areas tested as compared to community children at baseline (17% vs 9%; p=0.15) and at 6 months (14% vs 8%; p=0.48), but these differences were not statistically significant. Children in the CM group having cognitive impairment at 6 months had a lower WAZ score than those not impaired (-3.28 vs -1.29; p=0.001). No other factor was associated with impairment at 6 months. In conclusion, in children < 5 years of age with CM, malnutrition at admission may play a role in the persistent cognitive impairment. Analysis of tests of attention, the area found most impaired in studies of children over 5 years of age, is currently ongoing. Final analysis to assess risk of cognitive impairment in children <5 years of age with CM will be performed when study cohort enrollment is complete.

405

SELECTION AGAINST THE EL TOR ALLELE OF *CTX*B IN *VIBRIO CHOLERAE* O139 AND O1 REVEALS THEY HAVE DISTINCT NICHES

O. Colin Stine[1], Jason Bailey[1], T. Ramamurthy[2], G. Balakrish Nair[2]

[1]University of Maryland, Baltimore, MD, United States, [2]National Institute of Cholera and Enteric Disease, Kolkata, India

Vibrio cholerae causes cholera, a severe diarrheal disease causing an estimated 3 to 5 million cases and c. 120,000 deaths per year worldwide. *V. cholerae* has two epidemic serogroups: O1 and O139, the latter arose from the former by lateral gene transfer of a novel serogroup encoding region. Our working paradigm is that the two serogroups are a single disease entity. Recently new variants have arisen that are clinically more severe and have the 'classical' allele at *ctx*B (cholera toxin-coding) locus. Ninety isolates of O139 collected systematically from patients with cholera admitted in to the Infectious Diseases Hospital in Kolkata between 1992 and 2000 were genotyped at the *ctx*B locus and at the five loci used in MLVA (multilocus variable tandem repeat analysis). Our MLVA produced a network of genetic relatedness consistent with the genotypes evolving over time. The *ctx*B genotypes correlated with the MLVA genotypes. The 'El Tor' allele of *ctx*B was replaced selectively by the 'classical' allele. The selection coefficient was estimated to be greater than 10-6. A similar selection coefficient was estimated among O1 *V. cholerae* where the 'El Tor' allele of *ctx*B was replaced selectively by the 'classical' allele. The selective sweeps occurred at different times in *V. cholerae* O139 and O1 serogroups. Our findings are consistent with the idea that the two serogroups have two distinct niches and should be thought of not as one disease, but rather as two distinct disease entities. Like different pathogroups of diarrheagenic *Escherichia coli* (EPEC, EAEC, etc), we should think of *V. cholerae* O1 and O139 as independently evolving distinct diseases.

406

NORTH AMERICAN PARAGONIMIASIS FOLLOWING INGESTION OF RAW CRAYFISH IN THE MISSOURI OZARKS

Michael A. Lane, Ericka V. Hayes, Samuel Z. Davila, Lee Z. Demertzis, Nur F. Onen, Thomas C. Bailey, **Gary J. Weil**

Washington University School of Medicine, St. Louis, MO, United States

Seven autochthonous cases of North American paragonimiasis due to *Paragonimus kellicotti* were published between 1969 and 2007. This presentation will describe 6 cases seen at a single medical center in Missouri over the past 3 years. The patients (5 M, 1F) ranged in age from 12 to 32 years. All patients reported having ingested raw crayfish during camping or canoe trips along float streams in SE Missouri. Patients presented with fever and cough associated with eosinophila 3 weeks to 3 months after crayfish ingestion. The diagnosis of paragonimiasis was made weeks to many months after the onset of symptoms, generally after several failed therapeutic trials of antibiotics and/or steroids. All patients had pulmonary infiltrates and/or pleural effusions with eosinophilia (>10% in peripheral blood). Other clinical manifestations included pericardial effusion (2 patients), migratory subcutaneous nodules (2 patients), and visual changes with an occipital lobe lesion present on MRI (1 patient). Numerous procedures were performed on these patients including thoracentesis, pleural biopsy, bronchoscopy with lavage and lung biopsy, pericardiocentesis, and laparascopic cholecystectomy. *Paragonimus* ova were not identified in stool or sputum in these patients, and only 3 of 6 patients had positive serology tests for antibodies to P. westermani. All patients promptly responded to treatment with praziquantel (75 mg/kg/d in three divided doses for 2 days). It was distressing for us to see patients who had suffered so much from this easily preventable and treatable infectious disease. Therefore, we worked with the Missouri Department of Health and Senior Services to develop a health advisory letter for physicians (to shorten the time to diagnosis and treatment) and a warning poster for campgrounds and canoe rental businesses (to warn people not to eat raw crayfish). Physicians should consider the diagnosis of paragonimiasis in patients with pulmonary symptoms, fever and eosinophilia.

407

THE EFFECT OF GENITAL *SCHISTOSOMA HAEMATOBIUM* INFECTION ON FEMALE FERTILITY

Eyrun F. Kjetland[1], Edith Nyaradzai Kurewa[2], Takafira Mduluza[2], Nicholas Midzi[3], Exnevia Gomo[2], Henrik Friis[4], Svein Gunnar Gundersen[5], Patricia D. Ndhlovu[2]

[1]Oslo University Hospital, Oslo, Norway, [2]University of Zimbabwe, Harare, Zimbabwe, [3]Blair Research Institute, Harare, Zimbabwe, [4]University of Copenhagen, Copenhagen, Denmark, [5]Agder University Hospital, Kristiansand, Norway

A cross-sectional study in an *Schistosoma haematobium* endemic area of rural Zimbabwe was done in order to examine the association between genital schistosomiasis and fertility. In a community-based study all resident women of Mupfure Ward, between the ages of 20 and 49, not pregnant, not virgins, and not passed menopause, who had lived in the schistosomiasis endemic area for more than 3 years were invited into the study. Four hundred and eighty three women were interviewed about reproductive health issues and underwent a gynecological investigation. Genital, urinary and, faecal specimens were examined for parasite ova, analyses were done for sexually transmitted diseases. Logistic regression was used to control for the influence of sexually transmitted diseases and HIV on the association between schistosomal infection and fertility. Women with genital schistosomasis had fewer children. The presence of *S. haematobium* ova in genital tissue was found to be significantly associated with infertility (Adj. OR 3.6, 95% CI 1.05-12, p=0.034). We have previously published that *S. haematobium* was not associated with abnormal menstruation. Furthermore, the uterus rarely harbours *S. haemtobium* ova. However, many case reports have shown partial or fully blocked Fallopian tubes where ova are deposited. Previous reports indicate that anti-schistosomal treatment may reverse infertility, however this study could not confirm this. Larger studies are needed to determine the mechanism of infertility and if these women are more prone to abortion.

408

TOWARDS ENHANCED SURVEILLANCE FOR MONKEYPOX: APPLICATION OF A ROBUST CLINICAL CASE DEFINITION

Andrea M. McCollum[1], Marcel P. Balilo[2], Elizabeth Pukuta[3], Jean J. Muyembe[3], Inger K. Damon[1], Mary G. Reynolds[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Ministry of Health, Tshuapa District, The Democratic Republic of the Congo, [3]Institut National de Recherche Bio-Medicale, Kinshasa, The Democratic Republic of the Congo

Monkeypox virus is endemic in central and western Africa. Surveillance data from January through May 2009 in Tshuapa District, Democratic Republic of the Congo, yield an estimated annual incidence of 3 per 10,000; a five fold increase from the 1988 reported disease incidence. Misdiagnosis and confusion with varicella infection occurs. Adequate detection of cases and valid contemporary incidence measures of monkeypox will help to understand whether the disease is reemerging in the Congo Basin and to inform vaccine utilization policy. A surveillance case definition was developed based on published clinical symptoms of human monkeypox infection. Using this case definition, 33 suspect monkeypox cases from multiple African countries submitted to the US Centers for Disease Control and Prevention's (CDC) Poxvirus Team for consultation were evaluated by an independent observer using the data collected by healthcare workers, including photographs for each case. Using descriptive clinical information from six cases where there was laboratory testing for Orthopoxvirus or monkeypox virus, the specificity of the surveillance case definition was 0.8 and the sensitivity was 1.0. Using photographic evidence only, the specificity and sensitivity of the case definition were both 1.0. Photographic evidence classified 14 of 33 suspect cases (42.4%) as monkeypox, 4 (12.1%) as possible monkeypox or indeterminate, and 15 (45.5%) as not monkeypox. Healthcare workers misdiagnosed as many as 57% of those examined visually and 90% by clinical description as monkeypox. The concise surveillance case definition performed well and is expected to be useful in a clinical setting. Diagnosis of human monkeypox is easily confused with other vesiculopapular rash illnesses, particularly with varicella infections. Application of rigorous enhanced surveillance methods including a case definition, adequate case form and data collection, photographs of the patient, and laboratory testing of samples, and continuing training in their use, will help define the incidence and epidemiology of human monkeypox.

409

PROSPECTIVE COHORT STUDY ON THE BURDEN OF LEPTOSPIROSIS AND ITS TRANSMISSION IN THE URBAN SLUM ENVIRONMENT

Guilherme S. Ribeiro[1], Ridalva D. Felzemburgh[2], Federico Costa[2], Renato B. Reis[2], Debora B. Fraga[2], Adriano Queiroz[2], Balbino L. Santos[2], Andréia C. Santos[2], Elsio A. Wunder[2], Wagner S. Tassinari[3], Marília S. Carvalho[3], Mitermayer G. Reis[2], Albert I. Ko[4]

[1]Institute of Collective Health, Federal University of Bahia, Salvador, Brazil, [2]Gonçalo Moniz Research Center, Oswaldo Cruz Foundation, Brazilian Ministry of Health, Salvador, Brazil, [3]National School of Public Health, Oswaldo Cruz Foundation, Brazilian Ministry of Health, Rio de Janeiro, Brazil, [4]Department of Epidemiology and Public Health, Yale School of Medicine, New Haven, CT, United States

Leptospirosis has emerged as an urban slum health problem worldwide. However prospective studies have not been performed to evaluate its disease burden and obtain information on transmission factors which is needed to design effective interventions. We enrolled 9,862 inhabitants from a slum community in Salvador, Brazil in 2004 and followed the

cohort for a three-year period by: 1] active hospital-based surveillance to identify leptospirosis cases and 2] annual serosurveys of a sample of 2,003 subjects to identify *Leptospira* infection, defined as seroconversion in the microscopic agglutination test. Generalized estimation equation model was used to account for the time dependence of repeated measure data in risk factor analysis. The mean annual incidence for severe leptospirosis and *Leptospira* infection was 24 (95% CI, 3-73) cases and 2,690 (1,870-,3739) infections per 100,000 inhabitants, respectively. The infection-to-severe disease ratio was 114 (53-290). The annual incidence of secondary infections during the follow-up period was 8,475 (4,139-15,033) per 100,000 inhabitants. Independent risk factors for *Leptospira* infection were increasing age, male gender (RR 1.99; 95% CI 1.32-3.02), low per capita daily household income (0.83; 0.71-0.97 per US$1) and elevation of residence above the lowest point in the valley (0.98; 0.97-1.00 per m; *P*=0.02), a proxy for flooding risk. In conclusion, our findings showed that 2.7% of the urban slum residents were infected each year with *Leptospira*. Severe disease occurred in a small proportion (<1%) of infections, indicating that the disease burden for leptospirosis may be larger than previously believed. Re-infection was a frequent phenomenon, suggesting the presence of high-risk groups within slum communities who are repeatedly exposed to the pathogen. Furthermore, our findings emphasize the need for public health responses which address the social factors that produce unequal health outcomes among slum residents, as well as the infrastructure deficiencies, such as lack of closed drainage and sanitation systems, which serve as source for exposure to contaminated floodwater.

410

IMMUNOMODULATION BY *SCHISTOSOMA MANSONI* BY IMPAIRMENT OF HOST PURINERGIC SIGNALING PATHWAYS

Rita Bhardwaj, Patrick J. Skelly

Tufts Cummings School of Veterinary Medicine, North Grafton, MA, United States

Schistosomes are human parasitic flatworms that constitute an important public health problem globally. The parasites live for years, sometimes decades, in what is putatively a very hostile environment - the blood of vertebrates - yet they seem to elicit little if any protective reaction from two of the host's major defensive systems: the hemostatic system and the immune system. We hypothesize that this is because schistosome nucleotide metabolizing ecto-enzymes (NMEEs, alkaline phosphatase (SmAP), ecto-phosphodiesterase (SmPDE) and ecto-ATP-diphosphohydrolase (SmATPDase)), among a small subset of proteins expressed on the parasite surface membranes, dampen host pro-inflammatory and pro-thrombotic purinergic signaling mechanisms. In this way, these surface enzymes attenuate the host's ability to focus damaging thrombotic and immunological mediators in the parasite's vicinity, as reported previously. In this work, we show that the expression of all 3 NMEE genes is upregulated following vertebrate host invasion and that all are located in the tegument, by immunofluorescence and immuneEM. RNAi treatment targeting each NMEE gene results in potent suppression of gene expression, as determined by quantitative real-time PCR and by western analysis. The viability of suppressed versus control parasites is similar in culture but is significantly diminished *in vivo*. We show that, unlike parasites whose SmAP and SmPDE genes are suppressed, parasites whose SmATPDase gene is suppressed are significantly impaired in their ability to catabolize the potent pro-inflammatory molecule, ATP. We also show that parasites whose SmAP gene is suppressed, unlike parasites whose SmPDE and SmATPDase genes are suppressed, generate the potent anti-inflammatory molecule adenosine by catabolizing AMP. These data are consistent with the idea that some NMEEs provide an important immunomodulatory role for schistosomes within their hosts by impairing host purinergic signaling pathways.

411

AN INVESTIGATION OF POLYMORPHISM IN THE TETRASPANIN-2 GENE OF *SCHISTOSOMA MANSONI* FIELD ISOLATES

Pauline M. Cupit[1], Charles Cunningham[1], Bradley W. Tonnessen[1], Gerald M. Mkoji[2]

[1]University of New Mexico, Albuquerque, NM, United States, [2]Center for Biotechnology Research and Development, Nairobi, Kenya

Tetraspanin-2 (tsp-2) is a four transmembrane-domain protein located in the tegument of Schistosoma mansoni. As yet, its function is unknown though mammalian homologues are thought to mediate signal transduction events that play a role in the regulation of cell development, activation, growth and motility. In addition to the four transmembrane domains, the protein is characterized by two extracellular loops. A recombinant version of the large extracellular loop 2 (LEL2) has been used by Loukas and colleagues, as reported previously, to vaccinate mice that were subsequently challenged with S. mansoni resulting in reductions of 57 % and 64 % in the mean adult worm burden and liver egg burden over two independent trials. Clearly, tsp-2 has the potential to be an effective vaccine against S. mansoni, however, this potential may only be realized if tsp-2 proves to have a limited level of non-consequential polymorphisms.

Using S. mansoni field isolates obtained from 5 people who live and/or work close to infected water sources in Kisumu, Western Kenya we are studying the nucleotide and inferred amino acid sequences of the gene and transcript encoding tsp-2 in at least 8 worms from each individual. Our preliminary data suggests the presence of an indel prior to the sequence encoding LEL2 that results in a frame shift and premature stop codon in a significant proportion of the transcript sequences. As this indel appears after a short polyA tract we are trying to determine if it is a result of an amplification or sequencing error. Several other polymorphisms have also been noted. We also hope to present data that sheds further light on the issue of tsp-2 polymorphism in S. mansoni obtained from a wider population of African and South American field isolates.

412

INNATE IMMUNE PRIMING OF ADAPTIVE RESPONSES TO SCHISTOSOME INFECTION

Christine E. Ferragine, Stephen J. Davies

Uniformed Services University of the Health Sciences, Bethesda, MD, United States

Schistosomes are intravascular helminths affecting approximately 200 million people throughout the tropics and subtropics. Upon infection, previous models proposed that an early Th1 response to schistosomes is replaced at roughly 6 weeks post infection by a Th2 response initiated by egg deposition. However, our data show that, in addition to IFN-γ, $CD4^+$ T cells produce IL-4 and IL-10 in response to worm antigens during early infection. We hypothesize that production of IL-10, a regulatory cytokine, creates an immunomodulatory milieu permissive for parasite establishment and development. To test this hypothesis, we attempted to establish the identity of the IL-10-producing $CD4^+$ T cells and elucidate how this response is induced. To determine whether $CD4^+CD25^+Foxp3^+$ natural T regulatory (nTreg) cells are an important source of IL-10, wild type mice were treated with monoclonal antibodies that deplete nTreg cells by inhibiting IL-2 signaling. This approach demonstrated that depletion of nTreg cells did not significantly reduce IL-10 production, suggesting an inducible $CD4^+$ T cell population, rather than nTreg cells, are the predominant source of IL-10. Our analysis of the innate APC response to schistosome infection supports a model whereby schistosome worms induce a population of myeloid suppressor cells, which subsequently interfere with primary activation of naïve T cells. The precise identity of this myeloid suppressor population and their effects on T cell priming are currently under investigation.

413

TRANSCRIPTIONAL PROFILING OF *SCHISTOSOMA JAPONICUM*-STIMULATED ALTERNATIVELY ACTIVATED MACROPHAGES

Melissa L. Burke[1], Sheila Donnelly[2], Donald P. McManus[1], John P. Dalton[3], Geoffrey N. Gobert[1]

[1]Molecular Parasitology Laboratory, Queensland Institute of Medical Research, Brisbane, Australia, [2]Institute for the Biotechnology of Infectious Diseases, University of Technology Sydney, Sydney, Australia, [3]Institute of Parasitology, McGill University, Montreal, QC, Canada

Alternatively activated macrophages (AAMϕs) play important roles in a number of Th2 driven pathologies including asthma and allergy and a number of parasite infections. In *Schistosoma mansoni* infections AAMϕs have been associated with profibrotic and immunomodulatory roles. However, the molecular mechanisms involved in the activation of AAMϕs and their function are not well understood. Our own studies and those of others investigating *Schistosoma japonicum* infection strongly suggest the presence of AAMϕs in *S. japonicum*-infected tissues. However, the effect of *S. japonicum* antigens on macrophage activation and the role of AAMϕs in *S. japonicum* infection have not been investigated. In the present study we demonstrate, for the first time, that *S. japonicum*-secreted egg antigens are able to induce the alternative activation of macrophages as characterised by the significant induction of *Chi3l3* and *Arg1* expression. *Retnla* was not significantly induced in these macrophages suggesting that the specific function of these cells may differ to those induced by *S. mansoni* and other parasites. Closer examination of the gene expression profile of these cells identified other pathways that may confer immunomodulatory activity, independent of *Retnla* expression, including modulated expression of T-cell co-stimulatory molecules and chemokines. *S. japonicum*-stimulated alternative activation of macrophages was additionally associated with deactivation of classical activation pathways and altered expression of cell surface receptors and complement components that may alter phagocytic activity. There was no evidence of direct profibrotic activity. Together these data significantly enhance our understanding of the mechanisms associated with alternative activation of macrophages, highlight the importance of the context of activation in directing AAMϕ phenotype and function, and provide significant insight into the role of these cells in schistosomiasis japonica.

414

DEVELOPMENT AND IMMUNOSCREENING OF AN IMMUNOMICS PROTEIN MICROARRAY TO INVESTIGATE SCHISTOSOMIASIS

Patrick Driguez[1], Magda Ellis[1], Hamish McWilliam[2], Angela Trieu[1], Soraya Gaze[3], Malcolm K. Jones[1], Geoff Gobert[1], Alex Loukas[3], Denise L. Doolan[1], Philip L. Felgner[4], Donald P. McManus[1]

[1]Queensland Institute of Medical Research, Herston, Australia, [2]Monash University, Clayton, Australia, [3]James Cook University, Cairns, Australia, [4]University of California, Irvine, Irvine, CA, United States

Schistosomiasis causes significant human morbidity and mortality and, despite mass chemotherapy programs, reinfection rates remain high and long-term transmission is undiminished. Mathematical modelling has demonstrated that an anti-schistosome vaccine could assist in the elimination of this disease. For this purpose we have developed a 289 feature protein microarray for *Schistosoma japonicum* and *S. mansoni* as a vaccine antigen discovery tool. Proteins were selected on the basis of known or predicted surface localisation, secretion/excretion, life-stage specificity, and potential antigenicity. This study represents the first use of an immunomics protein microarray in schistosome research. The schistosomiasis-resistant brown rat, an excellent model of resistant human immunity, has demonstrated consistent antibody recognition of ~10 antigens including calcium-binding proteins, a CutA-related precursor, a protease inhibitor and *S. mansoni* homologs. These serum antibodies will be compared with tissue and life-stage specific responses in draining lymph nodes, spleen and BAL fluid, particularly after repeat infections. We will complement this animal model data with the screening of human sera from patients in schistosomiasis-endemic regions of China. In excess of 100 samples have been collected from putative-resistant and susceptible individuals as well as acute, severe and chronic disease states. These individuals were identified from large-scale field surveys and during treatment in established clinics. Using this data we will identify a subset of proteins for future animal vaccine trials in mice (susceptible model). By simultaneously examining two species of human schistosomes we hope to discover cross-reactive antigens that may lead to vaccine candidates and diagnostic/susceptibility markers for disease. This project will further our understanding of resistant host immune responses and parasite immune evasion and potentially identify future vaccine candidates.

415

IMBALANCE OF REGULATORY AND ACTIVATED T CELLS IN HUMAN *SCHISTOSOMA HAEMATOBIUM* INFECTIONS

Norman Nausch[1], Nicholas Midzi[2], Takafira Mduluza[3], Rick M. Maizels[1], Francisca Mutapi[1]

[1]University of Edinburgh, Edinburgh, United Kingdom, [2]National Institute of Health Research, Harare, Zimbabwe, [3]University of Zimbabwe, Harare, Zimbabwe

Acquired immunity against helminths is characterised by a complex interplay between Th1 and Th2 immune responses, and is often manifest only with increasing age. Data from experimental models suggest that immunity is also influenced by regulatory T cells (Treg), but as yet studies on Treg in human schistosome infections are limited. We therefore characterized regulatory and activated T cell (Tact) populations in Zimbabweans (aged 8-60 years) exposed to *Schistosoma haematobium* parasites. Activated T cells were classified as CD4+CD25+FOXP3- while Treg were defined as CD4+(dim)CD25+(high)FOXP3+CD127low. The participants were partitioned into two age groups, young children (8-13 years) in whom schistosome infection levels were rising to peak and older people (14+ years) with declining infection levels. Treg proportions rose significantly with increasing infection in the younger age group resulting in an increase of the Treg:Tact ratio with level of infection. In contrast Treg were negatively correlated to infection intensity in the older age group. The balance between regulatory and effector responses differ significantly between young individuals in whom high infection is associated with an enhanced regulatory phenotype and older infected patients in whom the regulatory response is attenuated. This may reflect different stages of the development of protective schistosome acquired immunity.

416

WHOLE BLOOD CYTOKINE RESPONSES IN URINARY SCHISTOSOMIASIS: THE EFFECT OF PRAZIQUANTEL TREATMENT ON NATURAL IMMUNE RESPONSES

Claire Bourke

University of Edinburgh, Edinburgh, United Kingdom

Urinary schistosomiasis is a debilitating disease caused by chronic Schistosoma haematobium infection. 1/3 of sub-Saharan Africans are currently infected with the most severe cases aggregated in school-age children. Praziquantel is the drug of choice to clear infection, but fails to prevent re-infection in endemic communities. Our study investigates the changes to the natural human cytokine response following praziquantel treatment. Whole blood was collected from 255 Zimbabweans (aged 3-84 years) inhabiting an S. haematobium-endemic region. Blood was cultured with crude parasite (cercariae, egg and adult worm homogenates) and purified vaccine candidate (GST and Sh13) antigen preparations for 48 hours. Culture supernatants were harvested and a panel of 13 cytokines (IFNγ, TNFα, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17A, IL-21 and IL-23) was measured via Enzyme-linked Immunosorbent Assay (ELISA). S.haematobium infection was quantified by urine filtration.

Schistosoma mansoni, soil-transmitted helminth and malaria positive cases were excluded. Following initial sampling, participants were treated with a single dose of praziquantel. Serology and parasitology was repeated 6 weeks and 6 months post treatment. Multivariate data was analysed by ANOVA and uncorrelated variables were grouped using principal components analysis (PCA). Prior to treatment S. haematobium prevalence was 51.44% and mean infection intensity was 28.5 eggs/10ml urine and both peaked at age 11-14 years. Both infection intensity and prevalence were significantly reduced at 6 weeks post-treatment. Pre and post-treatment cytokines showed distinct patterns according to antigen stimulation. The age distribution of cytokine production was altered by treatment, suggesting long-term effects of treatment on host immunity to S. haematobium.

417

SERO-PREVALENCE AND DISTRIBUTION OF KALA-AZAR IN POKOT COUNTY, AMUDAT DISTRICT, EASTERN UGANDA

Walter D. Odoch, Joseph Konde-Lule, Nazarius M. Tumwesigye

Makerere University College of Health Sciences, School of Public Health, Kampala, Uganda

Visceral Leishmaniasis(Kala Azar) is the only clinical manifestation of leishmaniases so far reported in Uganda and has been largely confined in Pokot County. Here, it is caused by *Leishmania donovani* and transmitted by the sand fly Phlebotomus martini. Records of 2006 from Amudat hospital indicates that KA accounted for about 17% of hospital in-patients, but actual prevalence of this disease is not known in Pokot County. This study sought to determine KA prevalence using Direct Agglutination Test (DAT) and and describe its distribution in Pokot County in order to inform control measures in Amudat district, Karamoja region, Uganda. A cross-sectional study was conducted in February to March 2010. The study participants were children aged ≥5years and adults ≥18 years randomly selected from the various strata in the selected clusters (Manyattas) obtained using Bennett's formula. A structured questionnaire was used to elicit the demographic profile and other characteristics of the participants. Standard operating procedures were performed for DAT using blood samples collected from participants on blotting papers at Amudat Hospital laboratory. Data was entered in EPIINFO and exported to STATA, used to produce frequencies and cross tabulation of DAT outcome and independent variables. Multivariable logistic regression was used to find association between the DAT outcome and potential selected risk factors. A total of 285 respondents were interviewed, response rate, 100%. The prevalence of Kala azar in Pokot County was 17.2% (49/285). The prevalence of clinical KA cases in the community was 2.5%. KA occurs commonly in children 10 years and below and is more common in males than females (1.33:1). DAT positive participants were more in sub counties with more clinical cases. Loroo Sub County had the highest number of DAT positive participants followed by Karita and Amudat at 30, 14 and 5 of the 49 positive participants respectively. In conclusion, Kala Azar is common in Pokot County and is most likely to spread to other parts of the county unless the ministry of health makes deliberate efforts and spearheads making available critical resources necessary to control and/or eliminate Kala Azar in Pokot.

418

IS SUDANESE VISCERAL LEISHMANIASIS DIFFERENT FROM VL ON THE INDIAN SUBCONTINENT?

Ed E. Zijlstra[1], Ahmed M. Musa[2], Eltahir A. Khalil[2], Muntasser E. Ibrahim[2], Moawia M. Mukhtar[2], Ahmed M. El-Hassan[2]

[1]Erasmus Medical Centre, Rotterdam, The Netherlands, [2]Institute of Endemic Diseases, Khartoum, Sudan

Globally, there are two major foci of visceral leishmaniasis (VL): on the Indian subcontinent, in particular Bihar state in India, and eastern and southern Sudan. In Sudan VL occurs both endemic and epidemic. Since the 1990s important observations have been made that indicate that Sudanese VL has unique characteristics in epidemiology, clinical presentation, diagnosis and treatment that contrast with reports from the Indian subcontinent. Transmission in Sudan is believed to be both anthroponotic and zoonotic with dogs and rodents as potential reservoirs, while in India VL is thought to be exclusively anthroponotic. More than 50% of VL patients in Sudan develop Post Kala-azar Dermal Leishmaniasis (PKDL) after treatment for VL compared with 5% in India, although higher PKDL rates are now reported from Nepal and Bangladesh, probably the result of improved reporting. The interval between VL and PKDL in Sudan is 0-6 months, while this is longer (often several years) in the Indian subcontinent. While PKDL causes considerable additional morbidity, it may have important implications for transmission. In addition, other post-kala-azar manifestations such as mucosal and ophthalmic leishmaniasis have been reported that have not been described elsewhere. Serological tests have different performance; while the rK39 striptest in India has sensitivity of nearly 100%, in Sudan this is less than 75%. Response to treatment is also different; on average the response to stibogluconate is good, while in India important resistance exists probably the result of inadequate dosing and compliance. In contrast, in a recent trial, the efficacy of paromomycin was considerably less in Sudan compared with what was found in India. Parasites circulating in Sudan are genetically more diverse (heterogeneic) through recombination that may explain at least some of these differences mentioned. Understanding the basis of these differences is important as this may lead to a different approach in clinical management of VL.

419

EQUITY IN CUTANEOUS LEISHMANIASIS TREATMENT ACCESS: CHALLENGES AND OPPORTUNITIES FROM KABUL, AFGHANISTAN

Elena Vuolo

World Health Organization - Eastern Mediterranean Regional Office, Cairo, Egypt

This presentation analyzes some of the determinants at the primary healthcare level in Kabul (Afghanistan) affecting the access to treatment among cutaneous leishmaniasis (CL) patients. The analyzed determinants include two major levels of assessment. First, the socio-economic status of the patients seeking for treatment at the primary healthcare level, addressing both refugee and civilian population in Kabul. Second, the features of the leishmaniasis service delivery system: the availability, access and use of the anti-leishmaniasis drugs; the operational status of the centres providing healthcare management; the healthcare workers policies applied to medical and no-medical national staff providing treatment to cutaneous leishmaniasis patients; the coordiantion and governance dynamics among different strategic and implementing partners operating in Kabul in the CL control services provision (UN, national program for control of leishmaniasis and NGOs). The analysis will address these elements among the national centres in Kabul, comprising the period 2009-2010 and illustrating how the differently combined determinants to access ultimately impact on the capacity of the public health sector to ensure equity among patients. The analysis aims at recognizing the operational and strategic challenges posed to the access to leishmaniasis control activities in Kabul among patients, with the ultimate goal to provide a way forward to share with decision and policy makers.

420

HOW CAN TSETSE POPULATION GENETICS CONTRIBUTE TO AFRICAN TRYPANOSOMIASIS CONTROL?

Philippe Solano[1], Sophie Ravel[2], Dramane Kaba[3], Jeremy Bouyer[4], Issa Sidibé[5], Mamadou Camara[6], Gérard Cuny[2], Thierry de Meeûs[1]

[1]Institut de Recherche pour le Développement (IRD)/Centre International de Recherche pour le Développement en zone Subhumide (CIRDES), Bobo-Dioulasso, Burkina Faso, [2]Institut de Recherche pour le Développement (IRD), Montpellier, France, [3]Institut Pierre Richet, Abidjan, Côte D'Ivoire, [4]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD)/Institut Sénégalais de Recherche Agricole (ISRA), Dakar, Senegal, [5]Programme de Création de Zones Libérées Durablement de la mouche tsé-tsé (PATTEC-PCZLD), Bobo-Dioulasso, Burkina Faso, [6]Programme National de Lutte contre la THA, Conakry, Guinea

In sub-Saharan Africa, tsetse transmitted Trypanosomiases have an enormous impact on both human health and economic development. Both the World Health Organisation and African countries through the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) have recently asserted their determination to rid the sub-continent of these diseases, and it is increasingly recognised that vector control should play an important role. This review mainly focuses on population genetics of tsetse of the palpalis group, the main vectors of sleeping sickness, and reports recent results on tsetse population structure and on measures of gene flow between populations in different countries (Burkina Faso, Senegal, Guinea, Ivory Coast). Implications of these studies for large-scale tsetse control programmes being undertaken in West Africa are important, particularly regarding the definition of control strategies (suppression or eradication).

421

EVIDENCE THAT THE TYPE IIA STRAIN OF *TRYPANOSOMA CRUZI* IS ADAPTED TO CONGENITAL TRANSFER

Jennifer Patonay, **Chris A. Hall**

Berry College, Mount Berry, GA, United States

It is well known that *Trypanosoma cruzi* represents a genotypically diverse family of organisms. Although some studies have suggested that the pathological outcome to infection may be associated with specific isolates, no correlation between strain and modification in transmission strategy has been identified. We have previously demonstrated in mice that the Type IIa strain of *T. cruzi* found in the southeastern United States is transferred congenitally at a significantly higher rate than the Type I strain from the same region. Using an *in vitro* cell culture model for human placental syncytial trophoblasts, we have tested whether the Type IIa strain has an enhanced ability to invade and replicate in these cells. Cultures of BeWo cells were exposed to either a Type I or Type IIa isolate of *T. cruzi* and assessed microscopically at 48, 72, and 96 hours for the percentage of cells infected and the average number of intracellular amastigotes. Cultures exposed to Type IIa isolate had significantly higher percentages of infected cells, as well as increased average numbers of intracellular amastigotes. Control infections carried out in DH-82 canine macrophage cells found that the Type I isolate was at least equal to the Type IIa strain in the ability to invade and replicate under these non-placental cells. Our results confirm that significant differences exist in the ability of these two isolates to invade syncytial trophoblast cells, suggesting adaptations in the Type IIa strain toward congenital transmission. This study not only provides the first *in vitro* evidence of strain-associated tissue tropism for *T. cruzi*, but also supports previous hypotheses for the evolution of the Type II strain in placental animals.

422

A NEW APPROACH TO IDENTIFYING DRUG LEADS FOR CHAGAS' DISEASE: HIGH THROUGHPUT SCREEN AGAINST AN INTRACELLULAR PATHOGEN

Juan C. Engel

University of California at San Francisco-Sandler Center for Drug Discovery, San Francisco, CA, United States

Trypanosoma cruzi is the parasitic agent of American trypanosomiasis or Chagas' disease, a neglected infectious disease affecting around 10 million people and an overwhelming human and economic burden throughout Latin America. A surge of patients identified in developed countries in recent years has highlighted its importance in global health. Discovery of new chemotherapy without the severe side effects associated with nifurtimox or benznidazole is essential. It is becoming evident that multi-drug therapy can prevent or significantly delay the onset of Chagas' disease pathology. To facilitate the rapid screening of large drug-like libraries, we have recently developed and validated an image-based high throughput screening assay for the pathogenic amastigote stage of *T. cruzi*. Our assay can be used with a variety of *T. cruzi* isolates and host cells and simultaneously measure trypanocidal efficacy and drug cytotoxicity to mammalian host cells. We can use various parasites strains with different biological characteristics (*e.g. T. cruzi* resistant to nifurtimox and benznidazole, clinical strains) and a range of host cells from primary human cell cultures to established cell lines (e.g. muscle cells, macrophages, hepatocytes). Our high content assay can be easily adapted to screen drugs against other intracellular pathogens such as *Leishmania* and *Toxoplasma gondii*. We are currently exploring large libraries of compounds by high through put screening to identify hits with trypanocidal efficacy and drug-like properties.

423

MOLECULAR GENETIC STUDIES OF *GLOSSINA FUSCIPES FUSCIPES* AND *TRYPANOSOMA BRUCEI RHODESIENSE* IN EASTERN UGANDA

Richard Echodu[1], John C. Enyaru[2], Elizabeth Opiyo[1], Loyce M. Okedi[3], Jon S. Beadell[4], Serap Aksoy[5], Gisella Caccone[4]

[1]Gulu University, Faculty of Science, Gulu, Uganda, [2]Makerere University, Faculty of Science, Kampala, Uganda, [3]National Livestock Resources Research Institute, Tororo, Uganda, [4]Yale University, Department of Ecology and Evolutionary Biology, New Haven, CT, United States, [5]Yale University School of Public Health, New Haven, CT, United States

Tsetse flies (Diptera: Glossinidae) are vectors of several species of pathogenic trypanosomes in sub-Saharan Africa causing Human African Trypanosomiasis (HAT) and African Animal Trypanosomiasis. Uganda has two forms of parasites, *Trypanosoma brucei rhodesiense* and *T. b. gambiense* causing HAT. Tsetse flies infest two thirds of Uganda with *Glossina fuscipes fuscipes*, predominating followed by *G. pallidipes* and *G. morsitans*. Genetic studies indicate genetic differentiation of *G. f. fuscipes* into Southern and Northern as separated by Lake Kyoga with co-occurrence of the two populations in central Uganda. Studies have indicated high dispersal rates in *G. f. fuscipes*. Such dispersal rates need monitoring local patterns and stability of genetic homogeneity over time at spatial scales to provide useful information for designing effective control programs. Little is known about the genetic stability of *G. f. fuscipes* populations and the genetic changes associated with temporal changes. These regions also span the historical disease foci caused by *T. b. rhodesiense* parasites. No information is available on the fine scale differentiation of parasite populations resident in distinct flies, animal reservoirs and humans. This project is to analyze: (1) The spatial and temporal stability of the genetic structure of *G. f. fuscipes* spanning southern and northern tsetse populations and (2) Parasite genotypes in infected flies, animal reservoirs, and in humans along the same transect. We are using nuclear and mitochondrial DNA markers to assess *G. f.*

fuscipes populations and *T. b. rhodesiense* isolates from the same regions. Data are being collected on nine populations with 600 tsetse flies and 200 cryo-preserved *Trypanosoma* isolates from infected tsetse, vertebrates and humans. We will discuss the results of these analyses in light of the previous population level genetic data and their potential impact in providing insights on control measures. In addition, we will discuss the genetic differentiation observed among lineages of trypanosomes collected in the same region.

424

ROSIGLITAZONE ADJUNCTIVE THERAPY IMPROVES THE OUTCOME OF EXPERIMENTAL CEREBRAL MALARIA IN *PLASMODIUM BERGHEI*-INFECTED MICE TREATED WITH ARTESUNATE

Lena Serghides, Ziyue Lu, Sarah Higgins, Kevin C. Kain

McLaughlin-Rotman Centre for Global Health, UHN and University of Toronto, Toronto, ON, Canada

Evidence is accumulating for the emergence of artemisinin resistant parasites. Treatments that modulate the host response to malaria may be useful adjunctive therapies that could potentiate clinical outcomes for artemisinin-based therapies. We have previously shown that rosiglitazone, an FDA approved PPARγ agonist, improved survival in an experimental model of cerebral malaria, and given as adjunctive therapy, improved parasite clearance times in Thai adults with uncomplicated malaria. Here we investigated whether rosiglitazone given in combination with artesunate would improve disease outcome in a model of *Plasmodium berghei* experimental cerebral malaria. Mice infected with *P. berghei* were given a sub-curative dose (10mg/kg) of artesunate for 4 days starting on day 3 post infection, with or without rosiglitazone (2.5mg/kg). Mice receiving artesunate in combination with rosiglitazone had a significant improvement in survival over mice receiving artesunate only (100% vs 50% respectively; P <0.0001), and were completely protected from cerebral malaria. Although both artesunate and combination-treated mice had similar levels of sequestered parasites in their brains, combination-treated mice had significantly less blood brain barrier permeability, as determined by Evan's blue staining, than artesunate-treated mice. Further, combination-treated mice had higher plasma levels of angiopoeitin 1 and lower levels of soluble ICAM-1 throughout infection, indicating less endothelium activation compared to artesunate-treated mice. In summary, we have shown that rosiglitazone, a compound that modulates the host response to infection, improved the outcome of experimental cerebral malaria when administered in combination with artesunate.

425

MOLECULAR DETERMINANTS OF EXPERIMENTAL CEREBRAL MALARIA IN THE BRAIN AND CIRCULATION

Miranda S. Oakley[1], Victoria Majam[1], Babita Majajan[1], Thomas McCutchan[2], L. Aravind[2], Sanjai Kumar[1]

[1]Food and Drug Administration, Rockville, MD, United States, [2]National Institutes of Health, Rockville, MD, United States

Cerebral malaria (CM) is a primary cause of deaths caused by *Plasmodium falciparum* with the majority of cases occurring in young children living in sub-Saharan Africa. Improved methods for early prognosis and differential diagnosis will help in reducing the high mortality rate of CM. To better understand the host molecules that mediate the pathogenesis of CM, we identified over 200 host biomarkers of experimental cerebral malaria (ECM) caused by infection with *P. berghei* ANKA parasites by performing microarray analyses in the brain tissue of moribund, non-moribund, and three type of resistant mice infected with *P. berghei* ANKA parasites. We next assessed the biological relevance of CD14 and galectin-3, two biomarkers significantly over expressed in brain tissue of mice with ECM, and found that both CD14 and galectin-3 deficient mice were significantly protected from ECM. Next, we identified over 300 potential prognostic/diagnostic indicators of ECM in the circulation by comparing the whole blood transcriptional profiles of resistant (BALB/c) mice to two susceptible strains (C57BL/6 and CBA/CaJ) of mice during ECM. A panel of ECM associated genes detectable in the peripheral blood has been selected to create a diagnostic signature of ECM by real time PCR. Bioinformatics analysis of this dataset has indicated that during ECM, erythropoiesis is dysfunctional, platelet and blood clotting related genes are down-regulated, and cell surface glycosylation is modified. Furthermore, computational analysis of immunity related genes suggests that distinct mechanisms of immunopathogenesis may operate in susceptible C57BL/6 and CBA/CaJ mice. The biological relevance of a few selected circulatory biomarkers of ECM is currently being assessed in biochemical and immunological studies in mice. Finally, circulatory biomarkers of ECM will be tested in human studies to identify prognostic/diagnostic markers of CM in African children.

426

TOWARD A RHESUS G-6PD DEFICIENT MODEL

Hans H. Wei[1], Gregory Deye[1], Jacob Johnson[1], Hiroshi Ishida[1], Lalaine Anova[1], Hla Myint[1], Aruna Sampath[1], Alan Magill[1], Larry Walker[2], Colin Ohrt[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]University of Mississippi, Oxford, MS, United States

Glucose-6-phosophate dehydrogenase (G6PD) deficiency is one of the most prevalent and well characterized enzymopathies found in about 400 million people worldwide. Presently, there is no validated model to predict G6PD deficiency related hemolytic potential for drugs, which limits development of antimalarial drugs in the 8-aminoquinolone class. We present results of preliminary steps in the development of a rhesus model of G6PDD that could be used to evaluate hemolytic potential of drugs. Healthy rhesus monkeys were phlebotomized. Glutathione (GSH) was depleted from erythrocytes by incubation with diethylmaleate (DEM) and buthionine sulfoximine (BSO) *ex vivo*. After labeling treated and untreated cells with separate fluorescent dyes they were transfused back to the donor animals. Animals then received primaquine 4mg/kg (n=2) or vehicle 2ml/kg (n=2) daily for 9 days. Daily flow cytometry was used to measure cell life-span. Concentrations of primaquine and its major metabolite, carboxyprimaquine were measured by chiral selective LC-MS. Methemoglobin and complete blood cell counts were measured. Comparison of % cells remaining, and of the ratio of treated to untreated cells showed a trend toward faster clearance of GSH depleted cells when exposed to primaquine than either untreated cells exposed to primaquine or depleted cells exposed to vehicle control. The greatest clearance of these cells is within two days after drug exposure. After racemic primaquine treatment, there is no difference in parent drug absorption, distribution and elimination between primaquine enantiomers. However, plasma concentration of the metabolite (-)carboxyprimaquine is 10 times higher than either the parent compound or (+)carboxyprimaquine. In conclusion, preliminary results show promise in the ability of a rhesus GSH depletion model to detect G6PDD-related hemotoxicity. Further validation of the model is required and is on-going.

427

ISOLATION OF VIABLE *PLASMODIUM FALCIPARUM* MEROZOITES TO DEFINE ERYTHROCYTE INVASION EVENTS AND ADVANCE VACCINE AND DRUG DEVELOPMENT

Michelle J. Boyle[1], Danny W. Wilson[1], Jack S. Richards[1], David T. Riglar[1], Kevin K. Tetteh[2], David J. Conway[3], Stuart A. Ralph[4], Jake Baum[1], James G. Beeson[1]

[1]Walter and Eliza Hall Institute, Parkville, Australia, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom, [3]Medical Research Council Laboratories, Fajara, Gambia, [4]Bio21 Institute, University of Melbourne, Parkville, Australia

During blood-stage infection by *Plasmodium falciparum,* merozoites invade red blood cells (RBCs). Currently there is limited knowledge of

cellular and molecular invasion events, the kinetics of invasion and no established assays to readily measure and quantify invasion-inhibitory antibodies or compounds for vaccine and drug studies. This is in part due to the technical limitations of isolating viable merozoites from parasite cultures *in vitro* due to their short half-life. We have developed novel methods to isolate merozoites, at high yield and purity that retain their invasive potential and viability. Using these methods we have made important insights into the biology of invasion, defining the kinetics of and requirements for merozoite invasion of RBCs. Using purified merozoites, we have developed and optimized an assay to measure the invasion-inhibitory activity of antibodies and compounds distinct from other mechanisms of growth inhibition of asexual stage parasites. Interestingly, the assay was more sensitive for detecting inhibitory activity than established growth-inhibition assays. Furthermore, it was possible to fix merozoites at different stages of invasion for visualization by immunofluorescence microscopy and electron microscopy. Using this we demonstrate that processing of the major merozoite antigen MSP1 occurs at the point of RBC invasion. These findings have important implications for defining invasion events and molecular interactions, understanding immune interactions, and for the identification and evaluation of inhibitors to advance vaccine and drug development.

428

PLASMODIUM FALCIPARUM FIELD ISOLATES USE COMPLEMENT RECEPTOR 1 (CR1) AS A RECEPTOR FOR INVASION OF ERYTHROCYTES IN A COMPLEMENT-INDEPENDENT MANNER

Gordon A. Awandare[1], Carmenza Spadafora[2], J. Kathleen Moch[1], Sheetij Dutta[1], J. David Haynes[1], José A. Stoute[3]

[1]Division of Malaria Vaccine Development, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Instituto de Investigaciones Científicas y Servicios de Alta Tecnología-AIP (INDICASAT-AIP), Ciudad del Saber, Clayton, Panama, [3]Department of Medicine, Division of Infectious Diseases, Pennsylvania State University College of Medicine, Hershey, PA, United States

The malaria parasite *Plasmodium falciparum* invades erythrocytes using complex and incompletely understood mechanisms. A major invasion pathway relies on sialic acid (SA) residues of glycophorins present on the erythrocyte surface. However, some *P. falciparum* strains have the ability to invade neuraminidase-treated erythrocytes which lack SA. We recently reported that complement receptor 1 (CR1, CD35) is a SA-independent invasion receptor for many laboratory strains of *P. falciparum*. To determine the role of CR1 in the invasion of erythrocytes by *P. falciparum* field isolates, we tested eight isolates obtained from Western Kenya. In addition, we determined whether C3 plays a role in the CR1-dependent invasion of erythrocytes by laboratory and field isolates. All the parasites examined demonstrated an ability to invade erythrocytes in a SA-independent manner, although invasion rates varied among different isolates. Anti-CR1 and soluble CR1 (sCR1) partially inhibited invasion of intact erythrocytes in a majority of isolates tested. In addition, invasion of neuraminidase-treated erythrocytes was nearly completely blocked in the presence of sCR1 and anti-CR1, confirming that CR1 is the major erythrocyte receptor that mediates sialic acid-independent invasion in these field isolates. Sequence analysis of the hypervariable region of the *P. falciparum AMA-1* gene showed considerable diversity among the isolates tested, suggesting that the use of CR1 as a receptor is likely widespread in fields parasites. Although CR1 is a receptor for C3b, CR1-dependent invasion was not affected by heat-inactivation or by C3 depletion of plasma, suggesting that parasite ligands may be interacting directly with CR1. Taken together, the data demonstrate that CR1 is an important mediator of both SA-dependent and SA-independent erythrocyte invasion by *P. falciparum* field isolates. The identification of this receptor should facilitate the search for parasite ligands that interact with it and the formulation of an effective blood stage vaccine.

429

MULTIPLE ROUTES TO HOSTS - COMPARATIVE ANALYSIS OF PROTEIN TRAFFICKING IN APICOMPLEXAN PARASITES

Rays H. Jiang[1], Daniel E. Neafsey[1], Manoj Duraisingh[2], Matthias Marti[2]

[1]The Broad Institute of MIT and Harvard, Cambridge, MA, United States, [2]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA, United States

Apicomplexan parasites such as Haemosporea and Piroplasmea cause blood diseases world wide. Amongst these, *Plasmodium* is the causal agent of the devastating diseases of malaria. Apicomplexa parasitize their host intracellularlly, many are enclosed within a membranous parasitophorous vacuole (PV). To colonize host cells across the membrane barrier, Apicomplexa secrete an estimated 10-20% of their total proteins. These proteins are deployed on the parasite surface, host cellular environment and host cellular surface. We have identified several trafficking signals that can be responsible for targeting different groups of proteins at hosts. We have also analyzed the trafficking machineries that recognize the trafficking signal and deliver the proteins. Trafficking into host is likely to have evolved multiple times within the Apicomplexa parasites. These delivered proteins are presumably involved in manipulation of host cell metabolism and evasion of host immune responses.

430

MOLECULAR ANALYSES OF *PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX* PARASITES INVOLVED IN HEPATIC DYSFUNCTION

Ashis Das[1], Shilpi Garg[1], Amit K. Subudhi[1], Boopathi P.A.[1], Vishal Saxena[1], Deepak P.[1], Dhanpat K. Kochar[2], Sheetal Middha[2], Jyoti Acharya[2], Sanjay Kochar[2], Utpal Tatu[3], Pragyan Acharya[3], Rani Pallavi[3]

[1]Birla Institute of Technology and Science, Pilani, Rajasthan, India, [2]S. P. Medical College, Bikaner, Rajasthan, India, [3]Indian Institute of Science, Banglore, India

Plasmodium falciparum and *P. vivax* contributes to the majority of the human malaria cases throughout the world. In India due to the geo ecological diversity both the parasites are found with variable distribution in different geographical areas. Severe manifestations due to *P. falciparum* has been known for a long time, but the recent reports of severe manifestations due to *P. vivax* like cerebral malaria, hepatic dysfunction, acute renal failure, ARDS, circulatory collapse, severe anemia, hemoglobinurea, abnormal bleeding due to *P. vivax* monoinfections from Bikaner, as reported previously, and other parts of the world has substantiated the need to look into this parasite with a new perspective. The pathogenesis of severe *Plasmodium* malaria is not clear, but is believed to be multi-factorial, due to its diverse clinical nature. We have analyzed the expression profile of the Indian *P. falciparum* and *P. vivax* isolates showing hepatic dysfunction alone as well as in combination with other manifestations like cerebral malaria, acute renal failure, severe anemia etc. Global Expression analysis of few *P. falciparum* and *P. vivax* hepatic dysfunction cases has shown appreciable differences when compared with non severe parasite from the same region. A substantial differential expression in the parasite encoded surface antigens like PfEMP-1, rifins, stevors and surfins (in P.*falciparum*) and vir genes, Duffy Binding Protein, Reticulocyte Binding Protein etc. (in *P. vivax*) has been observed.

431

COMPLEMENT RECEPTOR 1 IS THE "X" RECEPTOR (SIALIC ACID-INDEPENDENT) FOR *PLASMODIUM FALCIPARUM* IN THE HUMAN ERYTHROCYTE

Carmenza Spadafora[1], Gordon Awandare[2], Karen M. Kopydlowski[3], Jozsef Czege[4], J. Kathleen Moch[2], Robert W. Finberg[5], George C. Tsokos[6], José A. Stoute[7]

[1]Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT-AIP), Panama, Panama, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]United States Army Medical Materiel Development Acitivity, Ft. Detrick, MD, United States, [4]The Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [5]University of Massachusetts School of Medicine, Boston, MA, United States, [6]Harvard Medical School, Boston, MD, United States, [7]Pennsylvania State University College of Medicine, Hershey, PA, United States

Plasmodium falciparum is a highly lethal malaria parasite of human red blood cells. The molecular mechanisms of erythrocyte invasion are incompletely understood. *P. falciparum* depends heavily on sialic acid (SA) present on glycophorins to invade erythrocytes. However, a significant proportion of laboratory and field isolates are also able to invade erythrocytes in a SA-independent manner. The identity of the erythrocyte SA-independent receptor has been a mystery for decades. We report that the complement receptor 1 (CR1) is the major SA-independent receptor (X receptor) for the invasion of erythrocytes by *P. falciparum*. Soluble CR1 (sCR1) as well as polyclonal and monoclonal antibodies against CR1 inhibited SA-independent invasion in a variety of laboratory strains and wild isolates. Merozoites were observed interacting directly with CR1 on the erythrocyte surface by immunofluorescent microscopy. Also, the invasion of neuraminidase-treated erythrocytes correlated with the level of CR1 expression. Finally, both sialic acid-independent and dependent strains invaded CR1 transgenic mouse erythrocytes preferentially over wild-type erythrocytes but invasion by the latter was more sensitive to neuraminidase. This suggests that in the normal red cell both SA-dependent and independent strains interact with CR1 in the invasion process. However, only SA-independent strains can do so without the presence of glycophorin sialic acid. Our results close a longstanding and important gap in the understanding of the mechanism of erythrocyte invasion by *P. falciparum* necessary for the development of an effective blood stage vaccine.

432

MODULATION OF INTERFERON REGULATORY FACTORS (IRFS) UNDERLIES THE SUPPRESSION OF MALARIA-SPECIFIC IMMUNE RESPONSES IN HUMAN PATENT FILARIAL INFECTION

Simon Metenou[1], Benoit Dembele[2], Michael Kovacs[1], Siaka Konate[2], Housseini Dolo[2], Siaka Y. Coulibaly[2], Yaya I. Coulibaly[2], Abdallah A. Diallo[2], Lamine Soumaoro[2], Michel E. Coulibaly[2], Dramane Sanogo[2], Salif S. Doumbia[2], Sekou F. Traoré[2], Siddhartha Mahanty[1], Amy Klion[1], Thomas B. Nutman[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Filariasis Unit, Faculty of Medicine, Pharmacy and Dentistry, University of Bamako, Bamako, Mali, Bamako, Mali

Having demonstrated previously that patent filarial infection suppresses the production of malaria-specific IL-12p70, IFN-γ and CXCL-10 (mediated through IL-10) in a malaria/filarial co-endemic region of Mali, we sought to elucidate the mechanisms underlying this suppression. Using reverse transcriptase quantitative PCR to assess the expression levels of malaria antigen-specific IL-12Rβ1, IL-12Rβ2 and IRF-1, molecules known to regulate the IL-12/IFN-γ pathway, in blood obtained from 18 filaria-infected (Fil+) and 17 filaria-uninfected (Fil-) individuals from a malaria-endemic region of Mali, we found that Fil+ individuals had lower expression of IRF-1 ($p = 0.04$) but not IL-12Rβ1, IL-12Rβ2 than did Fil- subjects; this diminished IRF-1 expression could be reversed by neutralizing anti-IL-10 antibody. Because IL-12 in humans is produced primarily by dendritic cells (DCs), we used flow cytometry to assess the frequency of DCs (mDCs and pDCs) producing IL-12 or IFN-β respectively from Fil+ and Fil- subjects. We found that Fil+ subjects had lower frequencies of IL-12+ mDCs ($p = 0.0037$) after malaria antigen stimulation than did the Fil- subjects; there were no differences in the frequencies of IFN-β-producing pDCs between the two groups. Using an in vitro model of DC filaria/malaria co-infection, we found that mDCs pre-exposed to Brugia malayi microfilariae produced lower levels of CXCL-9, CXCL-10, IL-12p35, IL-12p40, IL-12p19 and CXCL-11 ($p = 0.0025$, $p < 0.0001$, $p = 0.0002$, $p = 0.006$, $p = 0.0034$ and $p < 0.0001$, respectively) following malaria antigen stimulation and had markedly downregulated expression of IRF-1, IRF-2 and IRF-3 compared to mf-unexposed mDC ($p = 0.0031$, $p = 0.0001$ and $p = 0.0039$, respectively). Other cytokines (TNF-α, IL-10, IL-1α, IL-1β and IL-6) were upregulated in the context of co-infection.

Thus, our data demonstrate that the suppression of malaria-specific IL-12/INF-γ/CXCL10 appears to be mediated by the modulation of IRFs (IRF-1 particularly) that play key roles in Th1 differentiation.

433

INDUCTION OF AN ENVIRONMENTAL STRESS RESPONSE IN *PLASMODIUM FALCIPARUM* USING HUMAN INNATE IMMUNE CELLS

Krishanthi Subramaniam

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

Infection with *Plasmodium falciparum* accounts for 1-3 million deaths annually, primarily among children in sub-Saharan Africa. The advent of artemisinin-combined therapies has led to a decrease in malaria-associated deaths however, the recent emergence of resistant strains calls for not only the development of more effective vaccines but also a better understanding of the parasite's biology in the human host. Through *in vivo* expression profiling we have previously shown that during the erythrocytic cycle *P. falciparum* has three novel transcriptional states; one of which resembles an environmental stress response (ESR) that has not been observed *in vitro*. Our goal was to elicit this *in-vivo* parasite ESR *in-vitro* by using human immune cells that are likely involved in the immunologic response during the erythrocytic cycle. We incubated *P. falciparum* 3D7 with physiologic levels of human PBMCs and PMNs and then isolated parasite RNA at different life stages in order to evaluate their gene expression. Our results show that we were able to elicit the *in-vivo* parasite stress state *in-vitro*, and that certain gene families involved in both stress and virulence were differentially expressed. Our *in-vitro* stress state yielded changes in the expression of a number of heat-shock proteins; an effect that was significantly correlated with the *in-vivo* ESR. Moreover, we also found induction of stress-related genes such as spermidine synthase. Our results also demonstrate that the parasite can alter genes encoding surface targeted proteins that function in immune evasion/virulence specifically proteins of the *var* and *rif* gene families. The differential regulation of the rifin proteins in our *in-vitro* stress state may help to shed light on the role of these proteins in the host response. Overall, these results illustrate for the first time an *in-vitro* parasite stress state induced by host immune cells and provide evidence of specific gene usage that may accurately reflect what occurs physiologically in the circulation of an infected host and play a role in pathogenesis.

434

RESVERATROL, A COMPONENT OF RED WINE, IMPAIRS THE CYTOADHERENCE OF *PLASMODIUM FALCIPARUM*-INFECTED RED BLOOD CELLS BY REDUCING THE EXPRESSION OF PFEMP-1

Jordan A. Zuspann[1], Mary Y. Sedegah[1], Neida K. Mita-Mendoza[1], Chanaki Amaratunga[1], Jeanette G. Tse[1], Gordon Langsley[2], Yaakov Pollack[3], Rick M. Fairhurst[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Institut Cochin, Universite Paris Descartes, Paris, France, [3]Ben Gurion University of the Negev, Beer-Sheva, Israel

Sequestration of *Plasmodium falciparum*-infected red blood cells (IRBCs) is critical to parasite survival and is centrally involved in the pathogenesis of malaria. Adherence of IRBCs to microvascular endothelial cells (MVECs) enables parasites to avoid clearance from the bloodstream by the spleen. Cytoadherence is also implicated in microvascular inflammation and endothelial dysfunction. Rosetting is also believed to contribute to obstruction and ischemia-induced inflammation in microvessels. Both cytoadherence and rosetting are associated with severe and fatal *falciparum* malaria. *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1), a family of parasite-encoded antigenically-variant proteins, mediates cytoadherence and rosetting and is encoded by *var* genes. The expression of *var* genes is regulated by parasite-encoded sirtuin 2 (PfSir2), a histone deacetylase. The polyphenol resveratrol (RV) activates PfSir2 and was recently shown to transcriptionally repress, in a differential manner, all three major sub-families of *var* genes. We thus hypothesized that RV impairs the cytoadherence and rosetting of IRBCs. To test this, we infected RBCs with the HB3 and FCR-3 *P. falciparum* lines in the presence of increasing concentrations of RV. After one cycle of parasite invasion and development to the trophozoite stage expressing PfEMP-1, we found that RV impaired (up to 57%) adherence to MVECs in a dose-dependent manner. Using the rosetting *P. falciparum* line 'varO', we found that RV also reduced (up to 40%) rosette frequencies in a dose-dependent manner. These findings were associated with moderate reductions in the levels of PfEMP-1 on the surface of IRBCs detected by flow cytometry. These reductions in cytoadherence, rosetting, and PfEMP-1 levels were not associated with decreased parasite viability. These data suggest the possibility that commercially-available RV - a component of red wine - may attenuate the virulence of *P. falciparum* by impairing cytoadherence and rosetting *in vivo*. Our findings thus provide a rationale investigating whether RV, in combination with antimalarial chemotherapy, could improve the survival of patients with severe malaria.

435

PLASMODIUM FALCIPARUM-INFECTED ERYTHROCYTES CONTAIN URIC ACID PRECIPITATES THAT ARE HIGHLY INFLAMMATORY

Diana L. van de Hoef[1], Jamie Orengo[1], Isabelle Coppens[2], Ana Rodriguez[1]

[1]New York University School of Medicine, New York, NY, United States, [2]Johns Hopkins Malaria Research Institute, Baltimore, MD, United States

Malaria life-threatening pathology is caused or exacerbated by excessive inflammatory responses in the host. Further understanding of the mechanisms involved in this process is needed to develop more effective therapies against malaria-induced pathology. We previously defined uric acid (UA) as a mediator of malaria-induced inflammation in mouse and human cells as reported previously. We have now discovered that *Plasmodium falciparum*-infected erythrocytes contain UA precipitates. Using both immunofluorescence with specific antibodies and lysate fractionation, we have detected UA precipitates in P. *falciparum*-infected erythrocytes in all cycle stages. UA precipitates are localized in the Plasmodium cytosol and are released into the medium upon schizont rupture. The inflammatory properties of UA precipitates (also named crystals) are well known because they are the causative agent of gout and are also considered a danger signal for the immune system. Direct release of UA precipitates in the blood upon schizont rupture may cause strong inflammatory responses during malaria infection. We found that addition of UA inhibitory drugs, allopurinol and uricase, reduced secretion of inflammatory cytokines (TNF, IL-1β and IL-6) from human peripheral blood mononuclear cells in response to Plasmodium-infected erythrocytes, suggesting that a decrease in UA levels in vivo may reduce the host inflammatory response and pathology. We obtained intracellular UA precipitates derived from Plasmodium-infected erythrocytes. These precipitates caused increased expression of the dendritic cell activation markers, CD40, CD80 and CD86 in vitro. This inflammatory effect was sensitive to uricase treatment, confirming their identity. This suggests that Plasmodium-derived UA activates the host inflammatory response and may contribute towards malaria pathology. Inhibiting UA formation may therefore decrease malaria-induced pathology, and this will establish the basis for developing specific therapies against this devastating disease.

436

VAR2CSA ELICITS BROAD REACTIVE ANTI-ADHESIVE ANTIBODIES

Daniel T. Minja[1], Pamela A. Magistrado[2], John P. Lusingu[1], Nicaise Tuikue-Ndam[3], Christentze Schmiegelow[2], Mayke Oesterholt[4], Stephanie Bostrom[5], Davis John[6], Mafalda Resende[2], Tina Dobrilovic[2], Lea Barfod[2], Vera Pinto[2], Madeleine Dahlback[2], Thomas Lavsten[2], Marita Troye-Blomberg[5], Phillip Deloron[3], Adrian J.F. Luty[4], Martha M. Lemnge[1], Lars Hviid[2], Ali Salanti[2], Morten Nielsen[2], Thor Theander[2]

[1]National Institute for Medical Research, Tanga Medical Research Centre, Tanga, United Republic of Tanzania, [2]Centre for Medical Parasitology Department of International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark, [3]Institut de Recherche pour le Développement (IRD), Paris, France, [4]Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, [5]Wenner-Gren Institute, University of Stockholm, Stockholm, Sweden, [6]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania

Pregnancy Associated Malaria (PAM) has harmful consequences for both the mother and foetus, primarily due to the accumulation of infected erythrocytes (iE) in the placenta. A member of the *Plasmodium falciparum* erythrocyte membrane protein 1 (*Pf*EMP-1) family, called VAR2CSA, is a variant surface antigen (VSA) which mediates adherence of iE to a placental receptor - chondroitin sulphate A (CSA). Women with PAM-related placental infection develop VSAPAM-specific anti-CSA adhesive antibodies after successive pregnancies that protect them from the severe consequences of PAM. Identifying which part of the VAR2CSA protein elicits broad reactive anti-CSA adhesive antibodies will provide a breakthrough for developing an anti-PAM vaccine. A cohort of 1000 pregnant women, recruited before 24weeks of pregnancy, was followed until delivery with the aim of accurate quantification of the effects of PAM on foetal and maternal health in Korogwe, North-eastern Tanzania. The overall aim of this longitudinal study is to optimize strategies for preventive intermittent treatment and facilitate development of a vaccine against PAM. Parasite isolates collected from pregnant women were cultured to late trophozoite and schizont stages and then tested for their ability to transcribe and express VAR2CSA by using qRT-PCR and flow cytometry, respectively. Antibodies raised in rats against different VAR2CSA Duffy binding like (DBL) domains of the FCR3 strain were assessed for their ability to inhibit adhesion of the PAM-derived *P. falciparum* iE to CSA *in vitro* using a static inhibition of binding assay (IBA). Based on qRT-PCR and flow cytometric analyses, we show that parasite isolates from pregnant women transcribe and express VAR2CSA on the surface of iE. The IBA results show that antibodies targeting particular VAR2CSA DBL domains inhibit the adhesion of most clinical isolates tested. In conclusion, immunization of rats with particular recombinant VAR2CSA protein domains based on the FCR3 sequence elicits broad reactive anti-adhesive

antibodies. Our findings bring us closer to identifying which part of the VAR2CSA protein may be used as a basis for developing an anti-PAM vaccine candidate.

437

ANTIBODIES AGAINST VAR2CSA OF PFEMP1 DBL2X AND DBL3X DOMAINS INHIBITED ADHESION OF IE TO CHONDROITIN SULFATE A

Harold T. Obiakor[1], Marion Avril[2], Yanling Zhang[1], Nicholas MacDonald[1], Karine Reiter[1], Richard Shimp, Jr.[1], Prakash Srinivasan[3], Megan Cartwright[2], Lynn Lambert[1], Michal Fried[2], Patrick Duffy[1], Joseph Smith[2], David L. Narum[1], Louis H. Miller[3]

[1]LMIV/National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States, [2]Seattle Biomedical Research Institute, Seattle, WA, United States, [3]Laboratory of Malaria and Vector Research/National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States

Over 500 million cases of clinical malaria occur annually. Malaria is the leading cause of infant mortality in under-developed countries. During pregnancy, *Plasmodium falciparum* infected erythrocytes (IE) bearing a preferentially expressed VAR2CSA surface protein sequester on placental syncytiotrophoblast by binding to Chondroitin Sulfate A (CSA). This phenomenon occurs mostly in primigravidae resulting in maternal anemia, low birth weight and in severe cases death of the fetus. However, after multiple pregnancies, multigravidae women develop blocking antibodies against VAR2CSA protein. VAR2CSA is a member of PfEMP1; a family of structurally related proteins with its extracellular portion made up of six Duffy-Binding-Like (DBL) domains and four Cysteine-rich Inter-Domain Regions. We refolded and purified recombinant DBL2X, DBL3X, and CSA binding sub-domain 3 of DBL3X (DBL3X-S3) and sub-domain 3 of DBL2X (DBL2X-S3) from *E. coli* inclusion bodies. DBL2X-S3 and DBL3X-S3 bind with higher specificity and lower affinity to CSA expressed on CHO-K1 cells compared to DBL2X and DBL3X which bind with lower specificity and higher affinity, respectively. Rat and rabbit antibodies raised against the DBL domains recognized homologous parasite IE and some heterologous parasite IE expressing alternative alleles of VAR2CSA. Preliminary results obtained with combinations of antibodies against these DBL domains suggest additive inhibition of IE binding to CSA expressed on CHO-K1 cells. Several of the rat and rabbit antibodies raised against these DBL domains showed limited inhibition of maternal field isolate binding to CSA, however, further studies are required. Taken together, these individual DBL domains of approximately 25-30 kDa can be produced in large quantities and scale in *E. coli*, hence favoring them as viable vaccine candidates for PAM.

438

DISPARITIES IN ACCESS TO SANITATION IN BOLIVIA

Alexandra D. Huttinger[1], William E. Oswald[2], Christine L. Moe[1]

[1]Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, GA, United States, [2]Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, United States

Bolivia is the only country in Latin America that is falling short of Millennium Development Goal #7 target for sanitation. Understanding where access to sanitation is the lowest, and the socio-economic factors associated with lack of access to sanitation, aid in identification of the populations most in need. Bolivia's population is estimated to be up to two-thirds indigenous Amerindian, and these groups dominate the rural population. Among the rural population, 57% (≈1,894,000 people) do not have access to a toilet or latrine. Previous studies have demonstrated that children in rural Bolivia are at greater risk of morbidity, malnutrition and impaired development associated with diseases linked to inadequate water, sanitation and hygiene. This analysis provides an in-depth assessment of disparities in access to sanitation by comparing the relative influence of location, socioeconomic factors (household construction materials, number of household members), educational status and gender for major ethnic groups in Bolivia using the most recent data from the nationally representative Demographic and Health Survey (DHS). The language that the head of household reported as learning to speak first was selected to indicate ethno-linguistic group. Across the 3 major indigenous ethno-linguistic groups of Bolivia, the primary correlates with access to sanitation differ: among the Aymara people (20% of total population, 46% household sanitation coverage within group), rural location is the strongest correlate with low sanitation coverage; among the Quechua (27% of total population, 48% household sanitation coverage within group), rudimentary household construction materials are most strongly associated with lack of household sanitation; and among the Guaraní and other Llano region groups (1% of total population, 53% household sanitation coverage within group), larger household size is associated with less access to sanitation. These differences in the primary correlates with lack of household sanitation across the ethno-linguistic groups of Bolivia can inform regional sanitation programs by identifying the populations with the greatest need and helping implementers to better target population selection and sanitation intervention strategies to be more effective for the geographic and social context of their programs.

439

AN EVENT-BASED MODEL FOR ENVIRONMENTAL TRANSMISSION OF *GIARDIA* INFECTION

Kyle S. Enger, Joan B. Rose

Michigan State University, East Lansing, MI, United States

Diarrheal illness is a major burden in the developing world, with a median of ~3.2 episodes per year in children under 5 years. Giardia is a major contributor to this burden. Where resources are insufficient for distribution of clean drinking water and removal of human waste, many less expensive antidiarrheal interventions have been investigated. These include latrine construction, handwashing with soap, and various methods for household water treatment (HWT). Published trials of these interventions often claim reductions in diarrheal illness of 30% or more. However, most trials are short-term, and nearly all are subject to bias. Furthermore, characteristics of interventions and communities vary greatly, and interventions are seldom maintained after the trial is over. Since long-term trials require much time and money, a simulation approach may be helpful for assessing the effectiveness of interventions in various contexts. A simulation model describing Giardia transmission in an isolated, underdeveloped community was programmed using Octave 3.0. It uses the Gillespie event-based algorithm to stochastically track susceptible, exposed, and infectious individuals and the number of cysts in the water source. Dose-response modeling determines exposure outcomes from ingestion of contaminated water. The model also includes an HWT intervention that reduces the number of cysts in drinking water for community members who use it.

Results (preliminary): If there is no intervention, the model equilibrates at a hyperendemic state, with ~90% of the population infected. If the entire community uses an HWT intervention that reduces cysts in drinking water by 99.0%, the prevalence gradually declines to ~8% after 1 year. If only 75% of the population uses the intervention, ~36% of the population is infected after 1 year. If everyone in the community uses the intervention on 95% of their drinking water, but continues to drink untreated water 5% of the time, the model equilibrates at ~67% infected after ~150 days. Highly consistent use of HWT may be necessary to control giardiasis in hyperendemic communities. Further refinements of the model may alter these conclusions. Household structure and additional transmission routes (e.g., contaminated hands) will be included in future versions of the model, allowing simulation of additional interventions (e.g., handwashing and sanitation).

440

CRYPTOSPORIDIUM CONTAMINATION OF SURFACE AND WATER SUPPLIES IN HAITI

Philippe M. Brasseur[1], Ketty Balthazard-Accou[2], Patrice Agnamey[3], Evens Emmanuel[2], Michel Vaillant[4], Christian Raccurt[5]

[1]IRD, Dakar, Senegal, [2]Université de Quisqueya, Port-au-Prince, Haiti, [3]CHU, Amiens, France, [4]CRP-Santé, Luxembourg, Luxembourg, [5]Laboratoire National de Santé Publique, Port-au-Prince, Haiti

Cryptosporidiosis is one of the most frequent causes of diarrhoea in Haiti. Transmission in children less than five years-old, HIV-infected individuals, and people living in low socio-economic conditions is frequently due to consumption of water or food contaminated by *Cryptosporidium* oocysts. This study examined the circulation of *Cryptosporidium* oocysts in surface waters and in public water supplies in the district of Port-au-Prince and in the surface water and groundwater used by the population of Les Cayes (Haiti). Data were gathered in 37 sample sites in Port-au-Prince and in 15 sites in les Cayes and in surroundings of the city (bathing water, household waste water, spring water, boreholes, water supply, domestic wells). Each sample of 100 litres of water was collected and immediately filtered using a polyethersulfone capsule. Oocysts were isolated using an immuno-magnetic method and counted under fluorescence microscopy after labelling with a monoclonal antibody. In the district of Port-au-Prince, 24/37 (65%) of water samples collected were contaminated by *Cryptosporidium* oocysts and the number of oocysts per 100L ranged from 4 to 1,274. In the reservoirs used by people living in peripheral areas, 10/11 (91%) of samples collected were contaminated with a mean number of 140 oocysts per 100L. In water samples from public standpipes provided by Camep, the public company of water distribution in Port-au-Prince, 7/13 (54%) were contaminated. All surface water 4/4 collected in Port-au-Prince or in peripheral areas was highly contaminated. In Les Cayes 8/15 (53%) samples contained *Cryptosporidium* oocysts and the number detected varied from 5 to 100 (mean 29) / 100 L of water filtered. In conclusion, a commitment to environmental improvement in Port-au-Prince and in Les Cayes is required to improve the quality of drinking water and to limit the risk of human transmission of cryptosporidiosis.

441

DETERMINANTS OF HOUSEHOLD WATER QUALITY IN PERI-URBAN SETTINGS

Robert S. Dreibelbis[1], April Davies[2], Richard Rheingans[2]

[1]Department of International Health, Bloomberg School of Public Health, Baltimore, MD, United States, [2]Center for Global Safe Water, Emory University, Atlanta, GA, United States

Public standpipes providing access to treated drinking water form municipal distribution systems are an increasingly common approach in urban environments; however, little data is available on the quality of drinking water at the household-level in urban and peri-urban environments. In February 2009, we assessed the determinants of household water contamination in a peri-urban settlement in Kisumu, Kenya. Data collection included: water quality measures at all drinking water sources; a population-based survey of 1,000 households, water source selection, and water handling practices; and water quality measures (fecal coliform and *E.coli* concentrations) of all household stored drinking water. Socio-economic position was assessed through an inventory of household goods and respondents divided into wealth quintiles. Logistic regression models were developed to determine the association between drinking water contamination and household behaviors and socio-economic characteristics. A total of 88 potential drinking water sources were identified, including 25 municipal standpipes and 63 shallow wells. Three of the municipal taps tested positive for *E.coli* contamination. Over 91% of respondents reported collecting drinking water from a municipal tap; and 47.9% of household stored drinking water samples tested positive for *E.coli* contamination. Significant predictors of *E. coli* contamination included: ever using a well as a drinking water source (OR=2.6), having a water treatment product in the house (OR=0.74), storing water in a narrow-mouthed container (OR=0.68). There was a marginally significant reduction in the odds of contamination among wealthier households when compared to poorer households. In conclusion, findings suggest that efforts to provide clean drinking water through public standpipes are not sufficient to guarantee clean drinking water at the household level. Even when clean drinking water is provided from municipal distribution systems, household water contamination is mediated by a variety of household-level behavioral factors.

442

EVALUATION OF POT-CHLORINATION OF WELLS DURING A CHOLERA OUTBREAK, BISSAU, GUINEA-BISSAU, 2008

Elizabeth Cavallaro[1], Julie Harris[1], Mauricio Goia[2], Jean Carlos dos Santos Barrado[3], Aglaer Alves da Nobrega[3], Inacio Carvalho de Alvarenga[4], Jeremy Sobel[1], Eric Mintz[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Department of Water and Sanitation, Bissau, Guinea-Bissau, [3]Field Epidemiology Training Program, Brasilia, Brazil, [4]World Health Organization, Bissau, Guinea-Bissau

Waterborne cholera epidemics are a major public health problem in sub-Saharan Africa. Guinea-Bissau has experienced five cholera epidemics since 1994. The most recent epidemic occurred in 2008, causing >14,000 cases and 225 deaths. In the capital city, Bissau, UNICEF-designed pot-chlorinators were used to disinfect shallow wells, a common source of drinking water. We evaluated the ability of pot-chlorinators to achieve free residual chlorine (FRC) levels in well water adequate to inactivate *Vibrio cholerae*. Thirty wells were randomly selected from six neighborhoods. Pot-chlorinators - bottles filled with gravel, sand, and calcium hypochlorite granules - were placed in each well. FRC was measured before and 24, 48, and 72 hours after placement and compared with WHO-recommended levels of ≥1-5 mg/L during cholera outbreaks and 0.2-5mg/L in non-outbreak settings. Water turbidity, presence of well covers, distance from wells to latrines, and rainfall were noted and pH was measured at each well 24, 48, and 72 hours post-chlorination. Complete post-chlorination data were collected from 26 wells; 15 (58%) were <2 meters deep, with well volumes of 0.6-8.0 m3. Twenty-four (92%) wells were <30 meters from a latrine. Four (15%) wells were covered on all observation days; rain fell on the second night at all wells. Four to 15% of wells had turbid water over the observation period; rainfall and presence of a lid did not appear to affect water turbidity. All wells had a pH <8 at baseline, 24, and 48 hours post-chlorination; one well had a pH >8 at 72 hours. At baseline, no wells had FRC >0.09 mg/L. Four (15%), one (4%), and no wells had FRC ≥1 mg/L and 16 (62%), 4 (15%), and 1 (4%) wells had FRC between 0.2-5 mg/L at 24, 48, and 72 hours post-chlorination, respectively. Pot-chlorinators failed to achieve WHO-recommended FRC levels in wells during a cholera outbreak, and may convey a false sense of security to local residents. Pot-chlorination should be discouraged and alternative approaches to well-water disinfection promoted.

443

CERAMIC WATER FILTERS REDUCE DAYS OF DIARRHEAL ILLNESS IN HIV-INFECTED INDIVIDUALS IN LIMPOPO PROVINCE, SOUTH AFRICA

Lydia Abebe[1], Sophia Narkiewicz[2], Mmbangiseni Mashao[3], Alukheti Singo[3], Julia Brant[1], Amidou Samie[3], Vinka Craver[2], James Smith[1], Rebecca A. Dillingham[1]

[1]University of Virginia, Charlottesville, VA, United States, [2]University of Rhode Island, Providence, RI, United States, [3]University of Venda, Thohoyandou, South Africa

Individuals infected with HIV frequently suffer from diarrheal illness transmitted by water-borne pathogens. Locally produced ceramic water filters impregnated with colloidal silver can be a sustainable solution to purify water in resource-limited settings. This work investigates if these

filters can reduce the rates of diarrheal illness in individuals being treated for HIV in rural South Africa. This randomized, controlled trial recruited HIV-infected individuals receiving anti-retroviral therapy (ART) from a private clinic. After randomization, individuals either received a ceramic water filter along with training on its use (intervention) or received routine clinical care which included recommendations about drinking treated water (control). Participants in both groups completed daily diarrhea diaries and submitted the diaries weekly for 40 weeks. Influent and effluent water samples were tested using the membrane filtration method to evaluate the number of colony-forming units of fecal coliforms. Stool samples were collected at enrollment and evaluated for *Cryptosporidia sp*. and enteroaggregative *E. coli* (EAEC) by PCR. 65 participants completed the study with 35 in the intervention arm and 30 in the control arm. 90% of participants were female. Average age was 41.5 years. All participants were receiving chronic ART. 18/71 (25%) participants reported diarrhea within the month prior to enrollment in the study. At baseline, 27/76 (35.5%) had *Cryptosporidia sp*. and 16/76 (21%) had EAEC in their stool samples. Influent water samples yielded an average of 6,863 CFU/100 mL. Following filtration, the effluent samples showed 0 CFU/100 mL. After 40 weeks of follow-up, the participants in the control arm have reported a total of 176 days of diarrhea, and those in the intervention arm have reported 68 days. These findings represent rates per person-year of 7.6 days and 2.6 days respectively. (p=0.011). In conclusion, silver-impregnated ceramic water filters significantly reduce the number of days of diarrhea in HIV-infected patients taking ART in rural South Africa who have high levels of fecal contamination intheir drinking water and high prevalence of enteric pathogens.

444

POST-IMPLEMENTATION ASSESSMENT OF CERAMIC WATER FILTERS DISTRIBUTED TO TSUNAMI-AFFECTED HOUSEHOLDS IN SRI LANKA

Lisa M. Casanova[1], Adam Walters[1], Grant Ligon[1], Ajith Nagahawatte[2], Mark Sobsey[1]

[1]University of North Carolina Chapel Hill, Chapel Hill, NC, United States, [2]University of Ruhuna, Galle, Sri Lanka

This study was a post-implementation assessment of tsunami-affected households in Sri Lanka that received ceramic water filters (CWF) during a distribution program in 2008. The research design was a cross-sectional study to determine the number of households still using the filter, factors associated with filter use and disuse, and the microbiological effectiveness of the filters. Data was collected by in-person oral interview from September to December 2009. Based on self-reported results, 76% of recipient households were still using the filter at the time of survey. At the time of survey, filters in user households had been in use from 6 months to >2 years, depending on the time of distribution. The data suggest that the main drivers of filter use and disuse are household water source, filter breakage, filter flow rate, and perception of water quality. Breakage was the most frequently cited reason for stopping filter use; this includes both filter breakage and storage container breakage. Logistic regression modeling showed that the variables with the greatest effect on continued filter use were having tap or well water, perceiving water as dirty, and perceiving water as unsafe. Households that had tap water were more likely to discontinue filter use, while households that had wells were significantly less likely to discontinue filter use. Source water quality in many survey households was fairly good; ~50% of filter households had <1 E. coli/100 mL in their water, as did ~70% of non-filter households. Analysis of E. coli levels in untreated and filtered water indicates that the microbial quality of water is improved by filters. These results suggest that filters improve water quality and have high levels of user satisfaction; sustained filter use needs to be maintained by the establishment of supply chains for replacement filters and user education about water quality.

445

DEVELOPMENT OF A MULTIPLEX PCR-BASED SUSPENSION ARRAY ASSAY FOR THE SIMULTANEOUS IDENTIFICATION OF FIVE *ENTAMOEBA* SPP. COMMONLY FOUND IN HUMAN STOOLS

Helena L. Santos[1], Kakali Bandyopadhyay[2], Rebecca Bandea[2], Alexandre J. da Silva[2], Jose M. Peralta[1]

[1]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States

Six species of the genus *Entamoeba*, i.e., *E. histolytica, E. dispar, E. moshkovskii, E. polecki, E. coli, and E. hartmanii* can be found in human stools. Of these only *E. histolytica* is considered pathogenic causing intestinal and extra-intestinal disease. *E. histolytica*, *E. dispar* and *E. moshkovskii* are morphologically identical. *E. polecki*, *E. coli*, and *E. hartmanii* can be differentiated morphologically from *E. histolytica*, but some of their diagnostic morphologic features may overlap creating issues for the differential diagnosis. Nevertheless, all these species can be differentiated using DNA-based approaches. The objective of this study was to develop a rapid, high-throughput screening method using a suspension array technique for the simultaneous detection and differentiation of *Entamoeba* species. PCR amplification was performed with byotinilated *Entamoeba* sp 18 S rRNA gene primers JVF and EntaRev, designed to amplify a fragment of approximately 360 bp of the *Entamoeba* spp studied. Regions of this fragment that had could differentiate among *E. histolytica*, *E. moshkovskii, E. dispar, E. hartmanni* and *E.coli* were selected to design hybridization probes to link to Luminex beads. The assay was standardized with cloned DNA and evaluated with ten DNA extracts from samples obtained from individuals that had these amebas in their stools. Using these approach we were able to correctly identify *E. histoltyica*, *E. dispar*, *E hartmanni*, *E. coli* and *E. moshkovskii* in all studied. These results show that this method could be used in the future for diagnostic detection of *Entamoeba* spp in fecal samples.

446

HUMAN PRIMARY EPITHELIAL CELL MODEL FOR *CRYPTOSPORIDIUM* INFECTION

Alejandro Castellanos-Gonzalez[1], Miguel Cabada[1], Dann Sara[1], Valerie Bauer[2], Guillermo Gomez[2], Joan Nichols[1], Arthur Clinton White, Jr.[1]

[1]Infectious Diseases Division, Department Internal Medicine, University of Texas Medical Branch, Galveston, TX, United States, [2]Department Surgery, University of Texas Medical Branch, Galveston, TX, United States

Cryptosporidiosis is caused by protozoan parasites that infect human intestinal epithelial cells. Studies of human infection have been limited by *In vitro* and *in vivo* models (no untransformed cells). In previous studies we have used human explants to study *Cryptosporidium* infection; however the model has been limited by the time because the cells go quickly to apoptosis. We hypothesized that by using anti apoptotic proteins and specific environments is possible to culture intestinal primary cells. Therefore the objective of this work was to develop a human primary epithelial cell model for studying *Cryptosporidium* infection. We obtained epithelial cells from human small intestine that would otherwise have been discarded after small intestinal surgeries. The cells were cultured in matrigel using specific enterocyte medium supplemented with growth and anti-apoptotic molecules. After 1 week, the cells formed organoids-like structures. The organoids were obtained, dissociated and seeded in culture chambers. The cells were characterized by IF, EM, ELISA and PCR. Cells remained viable and growing for >1 month. The cell population included intestinal stem cells, but also matures cells (with microvilli, tight junctions, and surface alkaline phosphatase). Cultured cells were infected with *Cryptosporidium* sporozoites and different stages of *Cryptosporidium* including oocysts were observed. Real time-PCR studies were performed to show an increase in the number of parasite during the time of

infection. Those results suggest the parasite is completing the life cycle in our system. This should provide an improved tool to study host-parasite interactions in intestinal infections as cryptosporidiosis.

447

CRYPTOSPORIDIUM IN NIGERIA

Alex D. Acholonu[1], Vitalis I. Njoku[2], A. J. Njoku[2], C. N. Ukuga[1]

[1]Alcorn State University, Alcorn State, MS, United States, [2]Imo State University, Owerri, Nigeria

Epidemiological study to determine the association of *Cryptosporidium* and other enteric protozoan parasites with diarrhea in Owerri and its environs of Imo- State, Nigeria was carried out between September 2002 and May 2005. Further determined was the relationship between these enteric parasites, especially *Cryptosporidium,* with HIV/AIDS. A total of 3054 stool samples from patients attending various health institutions in the study area was examined. Of these, 1204 (39.4%) were diarrheic while 1850(60.6%) were non-diarrheic. Enteric parasites were detected in 572 (47.5%) of the diarrheic stool samples. Enteric parasites identified in diarrheic stool samples include, protozoans (28.7 %), helminths (4.9%), and mixed infections (1.8%).The enteric protozoans identified include; *Entamoeba histolytica* (10.1%), *Giardia duodenalis* (7.9%), *E. coli* (5.8%) and *Cryptosporidium* species (4.9%). *Cryptosporidium* infection was higher in children aged <1-5 years and higher in those of 21-60. Age related protozoan infections showed significant variations ($p<0.05$) between children aged <1-20 years and subjects aged 21 years and above. Of the 3054 stool samples examined, 356 were from HIV positive patients. 52 (14.6%) had *Cryptosporidium* oocysts in their stools while 14 (0.5%) of 2698 stool samples from non-HIV patients had *Cryptosporidium* oocysts. *Cryptosporidium* associated diarrhea showed significant difference ($p<0.05$) among HIV positive (17.8%) and HIV negative (1.4%) diarrhea patients. Protozoan infections of HIV positive diarrhea patients showed significant difference ($p<0.05$) from similar infections of HIV negative diarrhea patients. Cryptosporidiosis had a high pathogenicity and was found in association with diarrhea and only rarely in non-diarrhea samples. From this study *Cryptosporidium* is associated with diarrhea as much as *Giardia lamblia* and *E. histolytica* especially in HIV patients and children. Since diagnosis of *Cryptosporidium* species is possible with simple staining technique, it is suggested that routine examination for *Cryptosporidium* be part of the parasitological routine especially with AIDS patients. The epidemiological significance of these results is discussed, especially in the context of controlled measures.

448

THE EFFECT OF MATERNAL BREAST FEEDING ON TIME TO FIRST CRYPTOSPORIDIAL INFECTION AMONG CHILDREN IN A SEMI-URBAN SLUM IN SOUTH INDIA

Rajiv Sarkar[1], Bhuvaneswari Thangaraj[1], Arun Kannan[1], Sitara S. Ajjampur[1], Elena N. Naumova[2], Honorine Ward[3], Gagandeep Kang[1]

[1]Christian Medical College, Vellore, India, [2]Tufts University School of Medicine, Boston, MA, United States, [3]Tufts Medical Center, Boston, MA, United States

Cryptosporidium spp. are a major cause of parasitic diarrhea in children worldwide. Although breast feeding protects children from gastrointestinal illnesses, there is no conclusive evidence on the degree of protection conferred by exclusive breast feeding on acquisition of cryptosporidiosis. As part of an ongoing cohort study on cryptosporidial transmission among children in south India we ascertained the effect of exclusive breast feeding on time to first cryptosporidial infection. Over a 9-month period we recruited 150 children from households using packaged or municipal drinking water (75 children in each cohort) for weekly follow-up from the time of introduction of supplementary feeding until the first identified cryptopsoridial infection. Surveillance stool samples were examined monthly and during diarrheal episodes for the presence of *Cryptosporidium* spp. by PCR PFLP at the 18S rRNA locus. The mean (SD) age at introduction of supplementary feeding was 19.8 (6.1) weeks. Over 4195 child-weeks of observation, 46 children developed cryptosporidial infection 21.9 (9.2) weeks after stopping exclusive breast feeding. The first symptomatic infection occurred earlier than asymptomatic infection (16.4 vs. 24.1 weeks post-weaning, $P=0.01$; for 33 symptomatic and 13 asymptomatic cases, respectively). The duration of exclusive breast feeding was inversely related to the time to first infection (Spearman's rho=-0.47, $P=0.02$) in the municipal water cohort, even after adjusting for symptomatic infections ($P=0.01$). Such an association was not observed in the packaged water cohort (Spearman's rho=-0.08, $P=0.73$). In conclusion, these preliminary data suggest a complex relationship between duration of exclusive breast feeding and the time to first cryptosporidial infection. Immune responses, household hygiene & water handling practices might also play an important role and will be further examined.

449

POPULATION GENOMICS OF THE SEXUALLY TRANSMITTED HUMAN PATHOGEN *TRICHOMONAS VAGINALIS*

Melissa D. Conrad[1], Zuzana Zubacova[2], Julia Schillinger[3], William E. Secor[4], Jacqueline Upcroft[5], Jorge Gonzalez[6], Rossana Arroyo[7], Steven Sullivan[1], Jane M. Carlton[1]

[1]Department of Medical Parasitology, New York University Langone Medical Center, New York, NY, United States, [2]Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic, [3]New York City Department of Health and Mental Hygiene, Bureau of STD Control, New York, NY, United States, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]Queensland Institute of Medical Research, Brisbane, Australia, [6]Molecular Parasitology Unit, Health Sciences Faculty, University of Antofagasta, Antofagasta, Chile, [7]Departamento de Infectomica y Patogenesis Molecular, Centro de Investigacion y de Estudios Avanzados del IPN (CINVESTAV-IPN), Mexico City, Mexico

Trichomonas vaginalis, the causative agent of human trichomoniasis, is the most prevalent non-viral sexually transmitted infection with over 174 million new global cases occurring every year. Historically it has been considered a "self-clearing female nuisance infection", but more recently it has been associated with increased risk of HIV transmission, making detection of the parasite and treatment of the disease an important part in the fight against AIDS. Currently little is known about the genetic diversity and population structure of the species, but with the publication of the *T. vaginalis* genome in 2007, new molecular tools are becoming available. We have developed and validated a panel of 21 microsatellite and six single copy gene markers to evaluate the population genomics of new clinical isolates collected from female patients attending New York City STD clinics, as well as extant isolates collected from around the world. Using an array of population genomic tools, we have detected significant genetic diversity within the species and have found that it is maintained across global regions. We have also found evidence of a two-clade population structure that may be correlated with parasite virulence. These findings will be important in understanding the spread of drug-resistance, in determining virulence factors, and in understanding why many individuals remain asymptomatic while others have severe manifestations of disease.

450

DIFFERENTIAL ABILITIES OF *TOXOPLASMA GONDII* AND *NEOSPORA CANINUM* TO DEVELOP RESISTANCE AGAINST THE ACTION OF PHENYLATED PENTAMIDINE-DERIVATIVES (ARYLIMIDAMIDES) *IN VITRO*

Andrew Hemphill[1], Christian Kropf[1], Karim Debache[1], Chad E. Stephens[2], David Boykin[3]

[1]University of Berne, Berne, Switzerland, [2]Augusta State University, Augusta, GA, United States, [3]Georgia State University, Atlanta, GA, United States

Toxoplasma gondii causes a variety of clinical diseases in man, is one of the most common opportunistic infections in immuno-compromised patients, and also represents a major veterinary medical and economic burden. Neospora caninum is a major cause of abortion in cattle and neuromuscular disease in dogs, and is closely related to T. gondii. We have earlier shown that a phenylated pentamidine derivative, DB750, inhibited the proliferation of *T. gondii* and *N. caninum* tachyzoites *in vitro*, with IC50 values of 0.16µM and 0.24µM, respectively. By *in vitro* culture and step-wise increase of drug concentration, *T. gondii* (ME-49) tachyzoites were able to adapt to DB750 treatment, up to a max. conc. of 1.2µM, and the adapted strain was named T. gondii_DB750. In contrast, *N. caninum* (Nc-1 isolate) failed to adapt to the drug. Screening of a range of other pentamidine derivatives against *T. gondii* lead to the identification of DB745, a compound that is structurally related to DB750, with highly improved IC50 (0.03µM). DB745 did not notably affect host cell (human foreskin fibroblast, HFF) proliferation nor host cell integrity at concentrations up to 3µM. Short term pretreatment of HFF with 1µM DB745 prior to infection and subsequent removal of the drug severely reduced the capacity of infecting *T. gondii* to proliferate intracellularly. In contrast to DB750, DB745 also inhibited invasion of tachyzoites into HFF. The DB750-adapted strain showed reduced susceptibility towards DB745, since T. gondii_DB750 exhibited an IC50 for DB745 of 0.07µM, which is more than 2 times higher than the one originally reported for DB745 in the non-adapted parasites, but still well below the IC50 values originally reported for DB750. Since *N. caninum* was unable to adapt to increasing DB750 concentrations, Db750 was applied in a mouse model for acute cerebral neosporosis, and showed promising results and reduced cerebral parasite burden and viability. Thus, arylimidamides might represent useful treatment options against neosporosis, but, due to ability of Toxoplasma to readily adapt to drug pressure, probably less against toxoplasmosis.

451

DEVELOPMENT AND EVALUATION OF CHIMERIC ANTIGENS FOR THE VACCINATION AGAINST NEOSPOROSIS

Thierry Monney, Karim Debache, **Andrew Hemphill**

University of Berne, Berne, Switzerland

Neospora caninum (Apicomplexa: Eimeriina: Sarcocystidae) is reported as the leading cause of bovine abortion, thus the disease represents an important veterinary health problem and is of high economical significance, as reported previously. Currently, only one vaccine against bovine neosporosis is available on the market (Bovilis Neoguard®, Intervet). This vaccine is based on tachyzoite protein extract but confers only partial protection against the disease, as reported previously. Basically, an efficient vaccine against *N. caninum* infection in cattle (or other animals) should prevent tachyzoite proliferation and dissemination in pregnant dams to avoid transplacental transmission to the fetus, and prevent tissue cyst formation in animals that have been infected with oocysts or tissue cysts. This could be achieved by a vaccine that stimulates protective cellular immune responses as well as antibody responses at both mucosal sites and systemically. It is very unlikely that this could be achieved by a single antigen, but most likely by a mixture of parasite antigens or a vaccine that contains a number of relevant antigenic domains of different proteins. The overall goal of our investigations on *N. caninum* is to develop a vaccine that limits both the cerebral infection and the transplacental transmission. Since promising results were obtained with a combination of the recombinant forms of three secreted proteins, NcMIC1, NcMIC3 and NcROP2 in the reduction of cerebral infection and vertical transmission in infected mice (as reported previously), we focused on the use of these proteins for further vaccination strategies. In order to increase the immunogenic potential of these antigens, the production of different chimeric proteins based on their putative antigenic domains was investigated. Antibodies against these proteins were raised in mice and tested for their inhibitory effect on the host cell invasion by *N. caninum in vitro*. Their capability to recognize the native proteins was also assessed by Western blot and immunofluorescence. A vaccination trial in mice is currently under investigation and the survival rate and health of the challenged mice as well as an assessment of the parasite burden in brain will be performed. Moreover, the cellular and humoral immune responses will be assessed. A summary of the results achieved so far will be presented.

452

DEVELOPMENTAL EXPRESSION OF FUCOSYLATED CARBOHYDRATES IN MIRACIDIA AND PRIMARY SPOROCYSTS OF *SCHISTOSOMA MANSONI*: CHARACTERIZATION OF THE FUCOSYLATION MACHINERY

Nathan A. Peterson, Timothy P. Yoshino

University of Wisconsin, Madison, WI, United States

Fucosylated carbohydrate epitopes (glycotopes) of the parasitic flatworm *Schistosoma mansoni* are key determinants in its development and immunobiology. Importantly, studies indicate that glycotope expression is developmentally and gender-specifically regulated, however the mechanism of differential expression is not well understood. Ongoing research seeks to identify and functionally characterize the enzymatic machinery that contributes to their production, specifically the enzymes involved in fucoconjugation, GDP-L-fucose synthesis, and GDP-L-fucose transport. A homology-based bioinformatics approach for gene discovery identified several schistosome genes that are putatively involved in fucosylation, including α3- and α6-fucosyltransferases, GDP-D-mannose-4,6-dehydratase (GMD), GDP-4-keto-6-deoxy-D-mannose-3,5-epimerase-4-reductase (GMER), and GDP-L-fucose transporter (GFT). At present, gene transcription has been confirmed and full-length transcript sequences have been determined via RT-PCR and 5′/3′ RACE. Interestingly, most genes exhibit alternative splicing. Current analyses include Southern and northern hybridizations, antibody production for western blotting and immunolocalization, quantitative real-time PCR to assess relative gene transcription amongst developmental stages, and functional assays such as RNAi-mediated gene silencing in conjunction with phenotypic screening in snail-associated schistosome larvae. Additionally, the enzymatic function of heterologously expressed GMD, GMER, and GFT will be assessed using canonical bioassays, including the *in vitro* reconstitution of GDP-L-fucose synthesis and transport. Of significance, the identification and characterization of these genes may provide novel targets for drug discovery, which could be useful for the control of schistosomiasis in snail and mammalian hosts.

453

TRANSCRIPTIONAL PROFILING OF THREE DIFFERENT MODELS OF RESISTANCE TO TREMATODE INFECTION IN *BIOMPHALARIA GLABRATA*

Michelle A. Forys, Patrick C. Hanington, Coen M. Adema, Eric S. Loker

Center for Theoretical and Evolutionary Immunology (CETI), University of New Mexico, Albuquerque, NM, United States

As the intermediate host for the trematode *Schistosoma mansoni*, the freshwater snail *Biomphalaria glabrata* plays a significant role in

the transmission of schistosomiasis to human populations. In the lab, *B. glabrata* has demonstrated its ability to defend against trematode infections by employing specific defense strategies to counteract parasite evasion or immuno-suppression. We here compare three different forms of resistance of *B. glabrata* to trematode infection: age-based, strain-based, and acquired resistance. To make these comparisons, we used a *B. glabrata* oligo-based microarray (1152 features) emphasizing stress and immune-response factors. We monitored the transcriptional profiles of *B. glabrata* from 0.5 up to 32 days post-exposure. The age-based array compared susceptible juvenile M-line snails (4-8mm) to adult, resistant snails (10-14mm), both exposed to the trematode *Echinostoma paraensei.* The strain-based array compared the responses to *S. mansoni* of resistant BS-90 snails with those of susceptible M line snails. Finally, our acquired resistance array examined the response of M-line snails that were first exposed to irradiated miracidia of *E. paraensei* and then 8 days later, challenged with viable miracidia. The three treatments each revealed a unique transcriptional profile, with each highlighting potential resistance-associated transcripts. We discovered a common pattern in which susceptible snails, at 2 days post-exposure, displayed a significant down-regulation of certain immune-associated transcripts (FREP3, C1q-like lectin, Dermatopontin, and others). In contrast, resistant snails at the same time point up-regulated many of the same transcripts and lacked the marked overall pattern of down-regulation associated with susceptibility. We hypothesize that this up-regulation has a significant impact on the ability of the snail to resist infection, and we are now looking further into the individual, functional roles of these molecules in resistance.

454

SOMATIC DIVERSIFICATION OF FREP3, AN ANTI-PARASITE RESPONSE FACTOR IN HEMOCYTES OF THE SNAIL *BIOMPHALARIA GLABRATA*

J.W. Dragoo, S.D. Stansbury, S.K. Buddenborg, C.M. Adema

University of New Mexico, Albuquerque, NM, United States

Trematode infection causes the snail *Biomphalaria glabrata* to respond with increased expression of fibrinogen-related proteins (FREPs), parasite-reactive lectins with N-terminal IgSF domains and a downstream FBG domain. Several mechanisms contribute to FREP (DNA and mRNA) sequence diversity in individual snails: presence of several FREP gene subfamilies each with a number of loci; retro/pseudogenes; alternative splicing; and a combination of point mutations and gene conversion drives somatic diversification. The underlying system for diversification of these innate-type immune factors in an invertebrate was explored by investigating the genomic architecture of FREP genes using BAC clones generated with DNA from B. glabrata. Medium throughput sequencing and SSCP was used to study diversity of genomic FREP sequences within subpopulations of bloodcells (hemocytes) of individual B. glabrata, in controls and following exposure to the digenetic trematode parasite Echinostoma paraensei. Two full-length FREP3 genes and several incomplete FREP3 gene subfamily-like sequences cluster within the 120kB genomic insert from *B. glabrata* in BAC clone 125N01. This tandem configuration is amenable to gene conversion. High fidelity PCR and DNA template derived from 20-40 hemocytes from single *B. glabrata* yielded diverse genomic sequences from exon 5 from FREP3. These results imply that *B. glabrata* hemocytes are functionally diverse.

455

ANTI-PATHOGEN RESPONSES IN *BIOMPHALARIA GLABRATA* SNAILS HARBORING LONG-TERM SCHISTOSOME INFECTIONS

C.M. Adema, S.K. Buddenborg, S.D. Stansbury, P.C. Hanington, E.S. Loker, J.W. Dragoo

University of New Mexico, Albuquerque, NM, United States

Investigations of schistosome parasite- snail host interactions usually focus on early stages of infection. However, colonization by *Schistosoma mansoni* of *Biomphalaria glabrata* is merely the start of a long term, intimate association in which the parasite modulates host immunity, physiology and reproduction to benefit parasite survival, growth and development. Some consider long term infected snails as an extended phenotype of the parasite. The survival of the immune-inhibited snail host is critical for continuation of the parasite's life cycle, and questions arise as to how immune function is organized in the "parasite/immuno-modulated host" entity to protect against other parasites and pathogens. Are snail defenses completely or selectively inhibited by S. mansoni, or does the parasite provide compensatory immuno-surveillance? Transcriptomic responses of *B. glabrata* to long term *S. mansoni* infection (up to and including patency) were recorded with an in-house developed *B. glabrata* oligo microarray (1152 features, emphasizing immune and stress factors). After initial upregulation, extensive downregulation of many (immune-relevant) transcripts was evident from infected snails starting at day 4. Of the immune genes, only FREP (fibrinogen-related protein)4 and galectin7 remained at increased levels, other features (including other FREPs) returned to control value or decreased.

456

CONTROLLING TRANSMISSION OF *SCHISTOSOMA JAPONICUM* IN SICHUAN PROVINCE, CHINA: CONTROL APPROACHES, EPIDEMIOLOGIC TRENDS, AND CHALLENGES

Bo Zhong[1], Edmund Seto[2], Dongchuan Qiu[1], Robert Spear[2], Song Lang[3]

[1]Institute of Parasitic Disease, Sichuan Center for Disease Control and Prevention, Chongdu, China, [2]University of California, Berkeley, CA, United States, [3]The Ohio State University, Columbus, OH, United States

In Sichuan Province, schistosomiasis is endemic in 62 counties. With extensive efforts, schistosomiasis control in Sichuan achieved a milestone in 2008 - 39 counties achieved transmission interruption (i.e., elimination of transmission), while the remaining 23 counties achieved transmission control (i.e., human and cattle infection prevalences were below 1%, no infected snails were found in the past two years). Since then, an ambitious plan was instituted to eliminate the transmission of the disease throughout the province by 2015. Here, we present a systematic review of epidemiology and control of schistosomiasis in Sichuan, emphasizing epidemiologic trends, control experience, lessons learned, and challenges faced in moving towards elimination of the disease. Schistosomiasis control program in Sichuan started in the mid-1950s during which a snail control oriented strategy was implemented with modest success. In the mid-1980s, the introduction of praziquantel as a major chemotherapeutic agent for schistosome caused a major shift in strategy from snail to morbidity control, which resulted in a significant reduction in infected cases of humans and cattle. This progress was furthered through the support of the World Bank Loan Program (WBLP) during 1992-1996, over which time 47% and 62% reductions were observed in humans and cattle, respectively. However, an upsurge of 93.2% human cases was observed in 1999 compared to 1996 in the province and the transmission even re-emerged in some previously controlled area after the completion the WBLP Program. In 2004, an integrated control program that coupled extensive chemotherapy with snail control, and to a lesser extent, environmental modification was initiated. This integrated program yielded remarkable results - a 91% reduction in human cases observed in 2008 vs. 2004, bringing the overall human prevalence of infections below 1%. In spite of this achievement, many challenging questions emerged.To address these questions and to inform strategies moving forward, we are actively conducting epidemiologic studies and exploring disease modeling scenarios.

457

AN EVALUATION OF SURVEILLANCE METHODS FOR DETECTING SCHISTOSOMIASIS REEMERGENCE

Elizabeth J. Carlton[1], Michael N. Bates[1], Edmund Y. Seto[1], Bo Zhong[2], Robert C. Spear[1]

[1]University of California, Berkeley, Berkeley, CA, United States, [2]Institute of Parasitic Diseases, Sichuan Center for Disease Control and Prevention, Chengdu, China

Schistosomiasis has reemerged in Sichuan Province, China, highlighting the challenges of sustaining reductions in this parasitic infection. Surveillance methods that rapidly identify areas where human infections have returned can direct the deployment of interventions to treat infections and prevent their further spread. We evaluated two surveillance methods commonly used in low prevalence and controlled areas, acute schistosomiasis case reports and surveys for *Schistosoma japonicum* infected snails, as well as alternative methods for detecting reemergence. Residents in 53 villages were tested for *S. japonicum* infection using the miracidial hatch test and the Kato-Katz thick smear procedure in a region where reemergence had been documented. We conducted surveys for *S. japonicum*-infected snails, tested cows and water buffalo for *S. japonicum* infection and examined county and provincial surveillance records for reports of acute schistosomiasis. The sensitivity and specificity of surveillance methods were estimated using the human infection surveys as the gold standard: villages were classified as positive if at least one human *S. japonicum* infection was detected. Human infections were detected in 35 villages. Acute schistosomiasis reporting and surveys for *S. japonicum* infected snails grossly underestimated the number of villages where human infections were present (sensitivity <10% for each method). Surveys for the presence of the snail host or *S. japonicum*-infected bovines had moderate sensitivity (69% and 59%, respectively) and specificity (44% and 67%, respectively). Limiting testing to adults age 30 to 49, the age group with the highest infection intensities, yielded higher sensitivity. Surveillance systems that rely on the detection of *S. japonicum* infected snails and reporting of acute schistosomiasis are ill-equipped to detect lapses in schistosomiasis control. While labor intensive, direct sampling of high-risk human populations defined by demographic characteristics or local environments should be considered.

458

CAUTIONING THE USE OF DEVELOPMENTAL MODELS FOR CLIMATE CHANGE PREDICTIONS: PREDICTING *SCHISTOSOMA JAPONICUM* INTERMEDIATE HOST DISTRIBUTION IN A FUTURE CLIMATE IN CHINA

Julia L. Moore, Justin Remais

Emory University, Atlanta, GA, United States

Research establishing quantitative relationships between climate and diseases carried by vectors or intermediate hosts often relies on degree-day functions, which incorporate temperature-dependence into development processes such as progression through an instar stage or reproductive maturation. These degree-day functions measure the vector or host developmental response to temperature in units of degree-days, which are accumulated only when the temperature exceeds a minimum threshold, Tmin. Development is complete once the accumulated degree-days reach a certain threshold, Ddays. These models are commonly used to predict the impact of future climate change on disease intensity, distribution, and timing. Though the simplicity of these models is appealing, little work has been done to analyze their ability to make long-term, regional, or global predictions of vector or intermediate host distributions given the influence of a changing climate. To assess the reliability of these models for such an application, we used a developmental model for *Oncomelania hupensis*, the intermediate snail host for the parasite *Schistosoma japonicum*, to investigate the sensitivity of host range predictions to degree-day model specification and parametric uncertainty. The model included a temperature-dependent recruitment process, and used predicted snail densities as an estimate of population viability at each grid cell. Uncertainty in Tmin and Ddays strongly influenced *O. hupensis* range predictions, and significant bias was identified when degree-day models were misspecified or were applied to temperatures outside the range for which the model parameters were estimated. Range predictions based on degree-day models should be considered reliable only for the populations and temperature ranges used to estimate model parameters. This conclusion has important implications for predictions of the impact of global climate change on vector- and intermediate host-borne diseases.

459

THE EFFECT OF MASS DRUG ADMINISTRATION OF IVERMECTIN TO HUMANS ON WILD *ANOPHELES GAMBIAE S.S.* SURVIVORSHIP

Kevin C. Kobylinski

Colorado State University, Fort Collins, CO, United States

Ivermectin is an anthelmintic drug that is mass drug administered (MDA) to humans for the control of onchocerciasis and lymphatic filariasis in sub-Saharan Africa. We have shown in the laboratory that ivermectin reduces the survivorship of colonized *Anopheles gambiae* s.s. G3 strain, at concentrations that are present in human venous blood post-ingestion of 150µg/kg of ivermectin. Our hypothesis is that wild *An. gambiae* s.s. survivorship will be reduced post-ivermectin MDA of humans. In southeastern Senegal, there are abundant *An. gambiae* s.s. populations, high levels of malaria transmission, and ivermectin MDA. In 2008 and 2009 blood fed, *Anopheles* mosquitoes were aspirated from the insides of villagers' huts before and after ivermectin MDA in southeastern Senegal. Mosquitoes were held in an insectary for five days post collection and survivorship was monitored daily. Mosquitoes were also captured by CDC light traps hung next to bed nets in randomly selected huts before and after MDA. Mosquitoes were identified to species morphologically and molecularly if applicable, blood meals were identified, and *Plasmodium* spp. sporozoite infection was determined. *Anopheles gambiae* s.l., *An. funestus*, and *An. nili* were the primary malaria vectors captured in the area and other vectors such as *An. coustani* and *An. rufipes* were abundant. Statistical analysis of aspirated *An. gambiae* s.s. from treated villages demonstrates that there was a drop in survivorship post ivermectin MDA from up to six days post MDA. Data on the molecular species identification, sporozoite rates and blood meal identification is currently being analyzed. Given the effects of ivermectin on *Anopheles gambiae* s.s. survivorship, more frequent administration of ivermectin MDA may be used to interrupt malaria transmission.

460

THE AUTO-DISSEMINATION OF A POWERFUL MOSQUITO LARVICIDE AND CHEMOSTERILANT UNDER FIELD CONDITIONS: A NEW VECTOR CONTROL TOOL

Elvira Zamora Perea[1], Nicole L. Achee[2], Daniel L. del Cuadro Hidalgo[1], **Gregor J. Devine**[3]

[1]Laboratorio de Salud Publica, Iquitos, Peru, [2]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [3]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania

Recent proof-of-principal studies show that the natural behaviours of adult mosquitoes can be exploited for the highly efficient targeting of an insect juvenile hormone analogue (pyriproxyfen or PPF) to breeding sites. This is potentially far more efficient than conventional larviciding. The optimization and standardization of this method in the field would represent an exciting step in the development of a powerful new vector control tool. A commercial mosquito trap was adapted so that it could be used to 1) expose a natural population of *Aedes aegypti* mosquitoes to PPF and 2) release exposed individuals unharmed in order to facilitate the autodissemination of PPF. This "expose and release" tool was treated with a pulverised solid formulation of PPF and deployed in the field. Its effect

on the development of larvae and pupae in sentinel aquatic habitats was noted. Under experimental field conditions, 95% of larvae and pupae developing at sentinel aquatic sites failed to develop to adulthood. In comparison, less than 10% of larvae and pupae failed to develop during control periods (when no PPF was deployed). In a parallel set of field experiments, the exposure of adult females to PPF by these standardized tools was also seen to have a profound effect on the fecundity of the mosquito population. Only 48% of eggs laid at sentinel sites by the exposed population hatched. Almost 100% of eggs laid during control periods eclosed successfully. PPF is a known insect chemosterilant, but its effects on mosquitoes in the field have not previously been documented. The combined effects of autodissemination and auto-sterilization, demonstrated here using a standardized "expose and release" tool, a WHO-approved insecticide (PPF), and a naturally-occurring Aedes population, have enormous potential for mosquito control. Models show that both chemosterilant and auto-disseminative effects on this scale are likely to have profound impacts on mosquito abundance and, by implication, on disease transmission.

461

CLUSTER RANDOMIZED TRIALS OF INSECTICIDE TREATED MATERIALS (ITMS) FOR DENGUE VECTOR CONTROL IN LATIN AMERICA AND SOUTHEAST ASIA

Audrey Lenhart[1], Carmen Elena Castillo[2], Yuwadee Trongtokit[3], Elci Villegas[2], Chamnarn Apiwathnasorn[3], Neal Alexander[4], Philip McCall[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Universidad de los Andes, Trujillo, Bolivarian Republic of Venezuela, [3]Mahidol University, Bangkok, Thailand, [4]London School of Hygiene and Tropical Medicine, London, United Kingdom

Prevention of dengue fever relies on control of its vectors, *Aedes aegypti* and *Ae. albopictus*, to prevent transmission. Prior studies in Latin America indicated that pyrethroid treated ITMs impact on dengue vector populations and potentially on dengue virus transmission, but this was the first large scale trial of ITMs for dengue control in SE Asia. The trials, in Venezuela (6000 households in 75 clusters) and Thailand (2000 households in 26 clusters), together offer the most comprehensive body of evidence to date on the potential and limitations of ITMs for dengue prevention. Novel aspects of the trials variously included: ITMs deployed as window curtains or container covers were tested alone or in combination; householders could choose their own ITMs; spill-over effects of the interventions into neighboring control clusters areas were monitored; coverage-dependent impact was assessed; the effect on both *Ae. aegypti* and *Ae. albopictus* (SE Asia) was analysed. Entomological indices were high at baseline in both trials and, although the types of ITMs differed between trials (Venezuela: window curtains and water storage container covers; Thailand: indoor door, wardrobe and window curtains), ITMs were adopted and maintained by the populations similarly (*i.e.* high initial acceptance, dropping to around 70% after 9 months). In Venezuela, results showed trends similar to those seen in previous trials, with an immediate drop in entomological indices post-intervention and an overspill effect in adjacent control clusters; impact on vector populations by each intervention was sustained throughout the trial but was most pronounced in the clusters which received both curtains and jar covers. In contrast, results from Thailand showed no measurable impact of ITMs on entomological indices. Reasons for this apparently dramatic difference in effect between both trials and the implications for the applicability of ITMs to dengue vector control initiatives, and the potential use of ITMs where pyrethroid resistant vector populations occur will be discussed.

462

WHOLE GENOME TRANSCRIPTIONAL PROFILING OF A HIGHLY INSECTICIDE RESISTANT POPULATION OF *ANOPHELES GAMBIAE* MOSQUITOES

Sara N. Mitchell[1], Martin J. Donnelly[1], Pie Muller[2]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Swiss Tropical and Public Health Institute, Basel, Switzerland

Anopheles gambiae populations from southern Ghana, Africa are known to be highly resistant to pyrethoid insecticides; the class used for bednets and increasingly for indoor residual spraying. The pyrethroid resistance phenotype was shown to be mediated by a combination of target site insensitivity (*kdr*) and metabolism via over expression of a cytochrome P450 (*Cyp6M3*). This resistance may, in due course, necessitate a switch to other insecticidal classes so in the present study we describe the patterns of resistance to two likely candidate compounds bendiocarb and DDT. Mosquitoes, collected from several sites in Accra, were exposed to either 0.1 % bendiocarb resulting in an LT50 of 1hr or 4 % DDT where a 6 hr exposure produced only 33% mortality; reflecting high levels of resistance to both compounds. Phenotyped specimens were subsequently screened for known target site insensitivity mechanisms and differentially expressed genes. To date studies of differential expression in mosquitoes have used a small candidate array approach. However this approach may not objectively screen all known transcripts and key resistance mediators could remain undetected. We describe the design and application of two whole-genome microarrays; a 4x44K and an 8x15K array were used to screen for genes differentially expressed in bendiocarb and DDT resistant mosquitoes respectively. Members of the three major enzyme families previously linked to resistance were represented in both experiments with candidates in the bendiocarb study including cytochrome P450s, GSTs and carboxylesterases, while P450s and GSTs were differentially expressed in the DDT experiments. However a number of novel candidates were also uncovered. Genes putatively linked to insecticide transport were up regulated in both the bendiocarb and DDT resistant mosquitoes. In addition a structural cuticular gene and a number of novel proteases were represented in the DDT result set. Real-time qPCR and recombinant protein expression systems have been employed to validate expression differences and confirm function *in vitro*.

463

EFFECTS OF CHEMICAL EXPOSURE ON *AEDES AEGYPTI* RECAPTURE RATES USING THE BG-SENTINEL™ TRAP UNDER SCREENHOUSE AND FIELD CONDITIONS

Ferdinand V. Salazar[1], Theeraphap Chareonviriyaphap[1], Suppaluck Polsomboon[1], John Grieco[2], Lars Eisen[3], Nicole Achee[2]

[1]Kasetsart University, Bangkok, Thailand, [2]Uniformed Services University of Health Sciences, Bethesda, MD, United States, [3]Colorado State University, Fort Collins, CO, United States

As part of a larger research program focused on quantifying the effects of spatial repellents (SR) and contact irritants (CI) to reduce indoor densities of host-seeking *Aedes aegypti*, the BG-Sentinel™ trap is being evaluated as a tool for removing chemically repelled *Ae. aegypti* from the peridomestic environment and monitoring potential diversion of mosquitoes to untreated locations. This requires understanding the potential effects of chemical exposure on host-seeking behaviours of the female *Ae. aegypti* mosquito and subsequent trapping success. Screenhouse studies were performed to quantify trap recapture rates in the absence and presence of mosquito exposure to chemical. Effects of chemical were evaluated by exposing cohorts of female *Ae. aegypti* mosquitoes, positioned within sentinel cages, to candidate compounds inside treated and chemical-free experimental huts. Following exposure, cohorts were released inside the screenhouse and recapture rates monitored for two days. Further, BG-Sentinel™ traps were positioned at various locations near the treated and chemical-free experimental

huts and cohorts of *Ae. aegypti* females were released into the outdoor environment to quantify diversion based on recapture rates by trap and hut location. Results from these experiments indicate similar total numbers of *Ae. aegypti* recaptured under screenhouse conditions for both non-exposed and chemical-exposed mosquitoes. Further, there was no evidence of significant diversion from treated to control (chemical-free) huts in outdoor trials. This information will serve to better understand the role of a trapping device to augment a SR and CI vector control strategy and guide the optimization of the BG-Sentinel™ trap to serve as a complementary component of a Push-Pull vector control strategy currently in the proof-of-principle stage of development in Thailand.

464

PRINCIPAL VECTORS OF MALARIA AND FILARIASIS IN PAPUA NEW GUINEA (*ANOPHELES PUNCTULATUS* SIBLING SPECIES) ARE SUSCEPTIBLE TO STANDARD INSECTICIDES USED IN LONG-LASTING INSECTICIDE-TREATED NETS

John B. Keven[1], Cara Henry-Halldin[2], Edward Thomsen[1], Ivo Mueller[3], Peter Siba[3], Peter Zimmerman[2], Lisa Reimer[1]

[1]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [2]Case Western Reserve University, Cleveland, OH, United States, [3]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea

Pyrethroids and dichlorodiphenyltrichloroethane (DDT) affect insects by interfering with voltage-gated sodium channel proteins in neurons. In many parts of the world, mosquitoes have developed resistance to these insecticides. This has threatened to impede insecticide-based vector control programs. The primary mechanism of resistance is the knockdown resistance (*kdr*) allele, a mutation in the insects' voltage-gated sodium channel gene (*vgsc*) that inhibits binding of DDT and pyrethroids to the protein channel. Physiological resistance to DDT causes cross resistance to pyrethroids. Papua New Guinea (PNG) has a history of both DDT and pyrethroid use for the control of malaria vectors. The Global Fund is currently supporting the distribution of long-lasting pyrethroid-treated nets in the country for disease control. However, the status of pyrethroid resistance in the local vectors has never been determined. This study investigated the status of pyrethroid resistance in the major malaria and filariasis vectors, the *Anopheles punctulatus* group, in areas of PNG where DDT or pyrethroids have been used. The study employed World Health Organization standard susceptibility bioassays to detect kdr phenotypes in 2 to 5 day old female *Anopheles*. In the cone assay, mosquitoes were exposed to deltamethrin-treated netting (55mg/m2) for 3 minutes and the rate of knock-down was measured within 60 min post exposure. In the tube assay, mosquitoes were exposed to lambdacyhalothrin-treated paper (18.35mg/m2) for 60 min during which time knock-down rate was measured. Mortality status was measured 24 hr post exposure for both assays. The *kdr* allele was diagnosed using a novel nested polymerase chain reaction amplification of a *vgsc* region that contains the mutation site. This was followed by a restriction digest using *Dde*I restriction enzyme. 100% knockdown and 100% mortality were observed in all populations. 100% mortality indicates a pyrethroid susceptible population according to the WHO percentage mortality index. All the mosquitoes that were genotyped were wild-type at the *kdr* locus.

465

RESISTANCE TO ORGANOPHOSPHORUS/CARBAMATES INSECTICIDES AND ACE-1 DUPLICATION IN *ANOPHELES GAMBIAE*: A CHALLENGE FOR MALARIA CONTROL

Luc S. Djogbénou[1], Pierrick Labbé[2], Fabrice Chandre[3], Nicole Pasteur[2], Mylène Weill[2]

[1]Institut Régional de Santé Publique /IRD, Cotonou, Benin, [2]Université Montpellier 2 - CNRS, ISEM, Montpellier, France, [3]LIN/Institut de Recherche pour le Développement, Montpellier, France

Insecticide resistance is a rapid and recent evolutionary phenomenon with serious economic and public health implications. In the mosquito *Anopheles gambiae* s.s., main vector of malaria, organophosphates and carbamates resistance is mainly due to a single amino-acid substitution in acetylcholinesterase 1 (AChE1). This mutation entails a large fitness cost. However, a resistant duplicated haplotype (ace.1D) of the gene encoding AChE1 (ace-1) recently appeared in *A. gambiae*. In an upstream study, the duplicated haplotype was detected at molecular level in a framework of distribution study of ace.1R allele (resistant allele against carbamate and organophosphate) in natural populations of *A. gambiae* from West Africa. Using molecular phenotype data collected from natural populations from West Africa, we investigated the frequency of this duplicated haplotype by statistical inference. This inference is based on the departure from Hardy-Weinberg phenotypic frequency equilibrium caused by the presence of this new haplotype. The duplicated allele, Ag-ace-1D, reaches a frequency up to 0.65 in Ivory Coast and Burkina Faso, and is potentially present in Benin. This allele was recorded in both M and S molecular forms of *Anopheles gambiae* s.s. in different West Africa countries. It was generated by a single genetic event and present distribution suggests that this new allele is currently spreading. Unfortunately, the spread of this less costly resistance haplotype is potentially a major threat to public health, as it may impede *A. gambiae* control strategies, and thus increases the risk of malaria outbreaks.

466

ACUTE FEBRILE ILLNESS SURVEILLANCE IN A TERTIARY HOSPITAL EMERGENCY DEPARTMENT: COMPARISON OF INFLUENZA AND DENGUE INFECTIONS

Kay M. Tomashek[1], Carlos Garcia-Gubern[2], Olga D. Lorenzi[1], Ivonne E. Galarza[3], Héctor Acosta[2], Yiraima E. Medina Blasini[2], Luis M. Cintrón[2], Elizabeth Hunsperger[1], Jorge L. Munoz-Jordan[1], Duy M. Bui[4], Steve Oberste[4], S. Peñaranda[4], Luis M. Santiago[1], Christopher Gregory[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]Department of Emergency Medicine San Lucas, Ponce School of Medicine Ponce, PR, United States, [3]Department of Pediatrics, Ponce School of Medicine Ponce, Puerto Rico, Ponce, PR, United States, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States

Dengue infections are often difficult to distinguish clinically from other acute febrile illnesses (AFI), including influenza. In 2009, an increased proportion of suspected dengue cases reported to the passive surveillance system in Puerto Rico were laboratory-negative in dengue-specific assays. As a result, enhanced AFI surveillance was initiated at the Emergency Department of a tertiary care hospital in southern Puerto Rico. From September to December 2009, 284 patients who presented with fever for 2-7 days and no identified source of infection were tested for influenza, leptospirosis, and enteroviruses, in addition to dengue. Thirty-one patients were confirmed as having dengue, 136 had influenza, 1 had leptospirosis, 3 had enterovirus, and 2 had dual infections; 111 had no infectious etiology identified. Median patient age was 17.9 years (range 0.5-82) and 55% were female. The majority were from Ponce (128, 45%) or neighboring Villalba (40, 14%) and Juana Diaz (38, 13%). Dengue patients were more likely than influenza patients to be residents of Villalba (58.1% versus 6.6%) and less likely to be from Ponce (3.2%

versus 54.4%). Nearly half (15, 48.4%) of all dengue patients met criteria for influenza (i.e., fever with cough or sore throat), and the majority (78.7%) of influenza patients met criteria for dengue fever. Dengue patients were more likely than influenza patients to have bleeding (80.6% vs. 26.5%), rash (38.7% vs. 8.8%), and positive tourniquet test (51.6% vs. 18.1%). Mean platelet count was 74,484 ± 58,000 for dengue patients and 189,639 ± 57,400 for influenza patients while mean white blood cell count was 3,400 ± 1,400 and 5,800 ± 2,800, respectively. Clinical diagnosis can be especially difficult when outbreaks of other AFI occur during dengue season. Our findings highlight the focal nature of dengue outbreaks and suggest that physician notification to public health officials should be encouraged. With many dengue patients meeting the case definition for influenza and vice versa, complete blood count and tourniquet test may be useful to differentiate dengue from other AFIs.

467

SEVERE CO-INFECTIONS OF DENGUE AND PANDEMIC INFLUENZA A H1N1 VIRUSES

Aubree Gordon[1], Maria Angeles Pérez[2], Felix Sanchez[2], Federico Narvaez[2], Gamaliel Gutierrez[3], Oscar Ortega[3], Andrea Nuñez[4], Eva Harris[1], Angel Balmaseda[4]

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

Dengue and influenza are both acute-onset viral illnesses that can initially present with similar symptoms. Epidemics of influenza and dengue generally do not overlap in Nicaragua, and virus co-infections have not been documented. However, in September 2009, simultaneous high rates of transmission of pandemic influenza and dengue in Nicaragua resulted in co-infections. Here we report on four hospitalized patients with dengue-influenza virus co-infections. All patients were RT-PCR positive for dengue virus serotype 3 and for pandemic influenza A H1N1. Clinical findings at presentation ranged from influenza-like illness to severe dengue. The clinical progression of the infections varied by case, but all developed classic dengue symptoms and had interstitial and/or alveolar infiltrates. Three cases required intensive care including mechanical ventilation, and one was fatal. All of the cases requiring mechanical ventilation had asthma, and the fatal case was also obese. Thus, dengue-influenza virus co-infections may lead to severe disease and can be fatal. Due to the varied clinical presentation and difficulties differentiating dengue-influenza virus co-infections from single infections, especially early after symptom onset, it is advisable that testing for both viruses be performed when they are co-circulating.

468

DETERMINANTS OF RISK FOR CARDIOVASCULAR SHOCK AND MORTALITY IN HOSPITALIZED DENGUE PATIENTS IN HO CHI MINH CITY, VIETNAM

Katherine Anders[1], Minh Nguyet Nguyen[1], Vinh Chau V. Nguyen[2], Thanh Hung Nguyen[3], Thi Thuy Tran[4], Bich Lien Le[3], Jeremy Farrar[1], Bridget Wills[1], Tinh Hien Tran[1], 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]Children's Hospital Number 1, Ho Chi Minh City, Vietnam, [4]Children's Hospital Number 2, Ho Chi Minh City, Vietnam

Dengue represents a growing global public health challenge. Understanding trends in disease burden and epidemiology is important for vector control, allocation of health services and planning the introduction of vaccines and therapeutic drugs. We analysed clinical and demographic trends in the dengue case burden in Ho Chi Minh City, Vietnam, between 1996 and 2009, and assessed risk factors for dengue shock syndrome (DSS) and mortality among 102,494 dengue patients admitted between 2000 - 2009. The dengue caseload across the three hospitals increased over the study period, to a peak in excess of 20,000 cases in 2008. Adults represented an increasing proportion of cases over time. The vast majority (13,595/14,079; 96.6%) of patients with DSS were children, with those aged 6 - 10 at higher risk of DSS than younger or older children. In contrast, the risk of mortality was highest in younger children and decreased with age (OR 0.52, 95% CI 0.36 - 0.75 in 6 - 10 year olds and OR 0.27, 95% CI 0.16 - 0.44 in 11 - 15 year olds, compared with 1 - 5 year olds). Overall mortality was low (0.20%) and progressively decreased during the study period (estimated change per year = -0.04%, 95% CI -0.06% - -0.02%). Males were overrepresented among dengue cases, suggesting a gender difference in healthcare seeking behaviour and/or susceptibility to disease. Strikingly however girls had a higher risk of DSS (OR 1.19, 95% CI 1.14 - 1.24) and death (OR 1.57, 95% CI 1.14 - 2.17) than boys. This hospital caseload indicates a startlingly high dengue disease burden in Ho Chi Minh City, with at least 1 in 400 people and 1 in 140 children admitted to one of the three study hospitals with dengue in 2008. In conclusion, the risk of DSS and death is highest in young female children. Young children are at greatest risk of death and this population should be targeted in clinical trials of dengue vaccines and therapeutics. The increased risk of severe outcomes in girls warrants further attention both in studies of dengue pathogenesis and of health-seeking behaviour, and in clinical care.

469

OLDER AGE IS A RISK FACTOR FOR SYMPTOMATIC DENGUE VIRUS INFECTION IN NICARAGUAN CHILDREN

Aubree Gordon[1], Guillermina Kuan[2], Oscar Ortega[3], Juan Carlos Matute[4], Juan Carlos Mercado[4], Josefina Coloma[1], William Aviles[3], Angel Balmaseda[5], Eva Harris[1]

[1]University of California, Berkeley, Berkeley, CA, United States, [2]Socrates Flores Vivas Health Center, Ministry of Health, Managua, Nicaragua, [3]Sustainable Sciences Institute, Managua, Nicaragua, [4]National Center for Diagnosis and Reference, Managua, Nicaragua, [5]National Center for Diagnosis and Reference, Ministry of Health, Managua, Nicaragua

The Nicaraguan Pediatric Dengue Cohort Study is a prospective cohort study, established in August 2004, to examine the incidence and clinical manifestations of dengue virus (DENV) infection in children 2-14 years old in Managua, Nicaragua. Children were enrolled prospectively, with yearly participation of 3,693-3,795 children. Participants are encouraged to come to the study Health Center at first sign of illness and all medical care is provided free-of-charge. Participants with suspected dengue or undifferentiated fever are tested for dengue by RT-PCR, virus isolation, and serological assays. Additionally, yearly blood samples from all cohort members are collected to determine the incidence of inapparent DENV infection. Univariate and multivariable generalized estimating equations (GEE) with a Poisson model were used to examine risk factors for symptomatic disease given DENV infection. Variables included in the multivariate models were: year of study, immune status, sex, and age. In the first 4 years of the study, 159 acute dengue cases and 9 DHF/DSS cases were detected, yielding an incidence rate of 11.2 (95% CI 9.6, 13.1) acute dengue cases and 0.65 DHF/DSS cases per 1000 person-years. During the same period, 1,047 DENV infections (symptomatic and inapparent) were detected, yielding an incidence of 78.9 (95% CI 74.2, 83.8) DENV infections per 1000 person-years. The incidence of cases and infections as well as the ratio of cases to infections varied substantially year-to-year. Incidence of cases varied markedly by age, with the highest incidence rate of symptomatic dengue in 10 year-olds. In contrast, the incidence of DENV infection was more constant across ages, with the highest incidence observed in the youngest one-year age groups. In multivariable models, age group (9-12 years old) was a significant predictor of symptomatic disease given infection (incidence rate ratio (IRR) 1.9; 95% CI 1.2-2.9), but immune status was not (IRR 1.3; 95% CI 0.9-1.8). Stratifying by immune status revealed that age is an important risk factor for developing symptomatic infection among primary DENV infections (IRR 4.0; 95% CI

1.7-9.7). Multiple children experienced two or more DENV infections. We are currently examining the effect of nutrition on risk for symptomatic or severe disease and investigating serial DENV infections in the cohort. This study is providing critical data on the epidemiology and transmission of dengue in the Americas.

470

A LONGITUDINAL ANALYSIS OF MATERNAL DENGUE ANTIBODY KINETICS AMONG INFANTS IN BANGKOK

Willem G. Van Panhuis[1], Christine Luxemburger[2], Krisana Pengsaa[3], Kriengsak Limkittikul[3], Arunee Sabchareon[3], Derek A. Cummings[4], Jean Lang[2], Anna P. Durbin[4]

[1]University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, United States, [2]Sanofi Pasteur, Lyon, France, [3]Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [4]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Maternal dengue antibodies are important factors for dengue pathogenesis and vaccine efficacy in infants. Previous studies have estimated the proportion of infants with detectable antibody levels and single, monophasic decline rates. These rates have been used to extrapolate antibody levels at birth to estimate values at later ages. No longitudinal analysis of the heterogeneity in antibody decline between and within infants has ever been conducted to provide more in depth knowledge on underlying patterns and determinants. Data from a previous cohort study of 140 infants in Bangkok were used to estimate serotype-specific decline rates of maternal, neutralizing dengue antibodies and social-economic determinants. Longitudinal regression methods were used to model average decline rates for different age intervals and to detect atypical patterns that were significantly different from the average pattern in the rest of the cohort. Antibody decline rates between birth and 3 months of age ranged from 51 to 58% per month. For DENV-1, 2 and 4, these rates were significantly different from rates between 3-9 months: 36, 38 and 13% respectively. Decline rates after 9 months for these serotypes were not significantly different from zero. For DENV-3, only two age intervals were identified with a decline of 36% per month between 3-12 months. For DENV-1, a significantly lower decline rate was found for infants with atypical patterns (17%). For DENV-4, a faster decline rate was found in such infants (26%). This is the first study that applied longitudinal methods to estimate maternal dengue antibody decline rates. Single, monophasic rates have been used previously, but based on our data we suggest using age specific decline rates to improve the accuracy of extrapolations. This could be of great use to studies on the optimal age of vaccination and dengue pathogenesis. Atypical decline patterns were found that may imply asymptomatic DENV infections in infancy, which could have implications for the response to vaccination in this age group.

471

IDENTIFICATION AND CHARACTERIZATION OF NOVEL HUMAN ANTI-DENV-2 MONOCLONAL ANTIBODIES THAT DO NOT TARGET DOMAIN III OF THE DENV-2 ENVELOPE GLYCOPROTEIN

William Messer[1], Ruklanthi de Alwis[2], Quang Pham[3], Jeremy Huynh[4], Martina Betramello[5], Federica Sallustro[5], Antonio Lanzavecchia[5], Ralph S. Baric[4], Aravinda de Silva[2]

[1]University of North Carolina School of Medicine, Department of Medicine, Chapel Hill, NC, United States, [2]University of North Carolina School of Medicine, Department of Microbiology and Immunology, Chapel Hill, NC, United States, [3]University of North Carolina School of Medicine, Chapel Hill, NC, United States, [4]University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC, United States, [5]Institute for Research in Biomedicine, Bellinzona, Switzerland

Despite the worldwide importance of dengue virus (DENV) as a pathogen, the basis of both protective immunity and pathogenesis in the human host remains incompletely understood. The principal target of the neutralizing human antibody response is the DENV envelope glycoprotein (E). Epitopes on the E glycoprotein may also play a role in enhancing viral infection through the attachment of cross-reactive, non-neutralizing heterotypic antibodies. The E glycoprotein consists of 3 domains designated EDI, EDII and EDIII. Mouse antibodies that strongly neutralize DENV mainly bind to EDIII. We have previously demonstrated that, unlike mice, humans exposed to DENV mainly have neutralizing antibodies that bind to epitopes outside EDIII, presumably on EDI or II. The goal of this study was to use human mAbs to map neutralizing epitopes on EDI and EDII. Human mAbs were isolated from memory B cells of a donor with a history of DENV2 infection. To identify the location of epitopes on EDI and II, DENV was passaged serially in the presence of excess neutralizing antibody to select for escape mutants. Neutralizing mAb escape was confirmed by plaque reduction neutralization test. Antibody escape mutants were plaque-purified and their E genes sequenced and mapped onto the crystal structure of the E glycoprotein dimer. These studies demonstrate that naturally infected persons develop memory B-cells that produce neutralizing mAbs directed to epitopes on EDI and II of DENV.

472

DENGUE VIRUS-SPECIFIC T CELL RESPONSES MORE THAN SIXTY YEARS AFTER INFECTION

Alexandra Grurary[1], Janet Meeks[1], Munkhzul Sukhbaatar[1], Allison Imrie[2]

[1]University of Hawaii at Manoa, Honolulu, HI, United States, [2]University of Western Australia, Perth, Australia

We assessed long term dengue virus (DENV)-specific memory T cell responses in individuals exposed more than 60 years previously, during the 1940s Pacific DENV epidemics. We compared these data to responses in 10 individuals infected in 2001 during a DENV-1 epidemic in Hawaii, and to 10 control subjects with no serological evidence of prior DENV infection. PBMC were collected from 7 individuals more than 60 years after they experienced a dengue-like illness in Hawaii or in the Pacific. PRNT90 analysis confirmed previous exposure to DENV-1 in 4 of 7 individuals (reciprocal titres > 1:160) with low level responses (1:10) in one other subject. Proliferative responses to DENV-1, DENV-2, DENV-3, and DENV-4, and memory markers, were assessed by FACS, and IFN-gamma responses measured by ELISPOT and ICS. DENV-specific CD4+ T cell responses were long-lived and detectable after 60 years in most subjects, whereas DENV-specific CD8+ responses declined over time (Multiparameter ANOVA; $p < 0.001$) and were not measurable in 2 subjects with robust CD4 responses. DENV-1-specific memory T cells were primarily of the CD4+ central memory CD45RA-CCR7+CD62L+ phenotype in contrast to the predominantly CD8+ effector CD45RA-CCR7-CD62L- phenotype of the 2001 cohort up to 5 years after infection. DENV1-specific memory T cells were highly cross-reactive with DENV-2, DENV-3, and/or DENV-4 in both groups, and to a higher degree in the 1940s group. We have identified and characterized DENV-specific immune responses more than six decades after infection. These findings may contribute to our understanding of DENV pathogenesis, and to the design of safe and effective dengue vaccines.

473

NO EVIDENCE THAT ARTEMISININ-RESISTANT MALARIA HAS SPREAD TO SOUTH ASIA

Peter Starzengruber[1], Hans-Peter Fuehrer[1], Paul Swoboda[1], Verena Hofecker[1], Anja Siedl[1], Markus Fally[1], Wasif A. Khan[2], Emran B. Yunus[3], Shah M. Hossain[4], Pascal Ringwald[5], Harald Noedl[1]

[1]Medical University of Vienna, Vienna, Austria, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]Chittagong Medical College, Chittagong, Bangladesh, [4]Ministry of Health and Family Welfare, Dhaka, Bangladesh, [5]World Health Organization, Global Malaria Programme, Geneva, Switzerland

Virtually all malaria-endemic countries have officially adopted artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated *falciparum* malaria. However, recent data indicate that the first cases of genuine artemisinin resistance have already emerged along the Thai-Cambodien border.

We conducted an open-label, randomized, controlled 42-day clinical trial in southeastern Bangladesh to investigate the potential spread of clinical artemisinin resistance from SE-Asia. A total of 126 uncomplicated *falciparum* malaria patients were randomized to one of 3 treatment arms (artesunate monotherapy with 2 or 4 mg/kg/day once daily for 7 days or quinine plus doxycycline TID). Treatment response and safety parameters were closely monitored throughout the study. In vitro drug sensitivity was assessed by HRP2 assay and samples for genotyping were collected on admission and in case of re-emergence of parasitemia. Only cases fulfilling all of the following criteria were defined as being artemisinin-resistant: recrudescence during follow up; prolonged PCT; exclusion of re-infection; pharmacokinetic parameters confirming adequate drug levels; in vitro data or genetic makers indicating reduced drug susceptibility. The 42-day cure rates in the artesunate monotherapy (2 and 4 mg/kg) and quinine/doxycyline arms were 97.8%, 97.7% and 100%, respectively. A single case of re-infection was seen in each of the artesunate arms, not a single case of recrudescence was observed during this trial. No differences in median PCT and FCT were found between the 2 artesunate arms (29.8 hrs and 17.9 hrs vs. 29.5 hrs and 19.1 hrs). No serious adverse events were observed. The parasite phenotype seen in Bangladesh is likely to be representative of Asian Plasmodium *falciparum* populations before the introduction of artemisinins. Not a single case fulfilled our criteria of artemisinin resistance. PCTs were considerably shorter and in vitro results indicate significantly higher susceptibility to artemisinins as compared to SE-Asia. There was also no indication of compromised intrinsic drug sensitivity to artemisinins and treatment response was not dose-dependent.

474

PREVALENCE AND SELECTION OF *PLASMODIUM FALCIPARUM* DRUG RESISTANCE MOLECULAR MARKERS UNDER INTERMITTENT PREVENTIVE THERAPY IN BURKINA FASO

Some Anyirekun Fabrice

IRSS-BOBO, Bobo-dioulasso, Burkina Faso

Single nucleotide polymorphisms (SNPs) in the *P. falciparum pfcrt*, *pfmdr1*, *pfdhfr* and *pfdhps* genes are associated with decreased response to aminoquinoline and antifolate antimalarials and have been shown to be selected by use of these drugs. The degree of selection by intermittent preventive therapy (IPT) regimens is unknown. We assessed the baseline prevalence and selection of common SNPs by IPT in children in Bobo-Dioulasso, Burkina Faso. We studied 1500 children (aged 3-59 months) randomized to receive monthly dihydroartemisinin-piperaquine (DP) or amodiaquine-sulfadoxine/pyrimethamine (AQ/SP) for 3 months during the malaria transmission season in 2009. The efficacy, safety and tolerability of DP vs. AQ/SP will be described elsewhere. For random samples of 120 children from each arm of the study and for 120 of 250 untreated controls we evaluated the prevalence of key resistance-mediating SNPs. We then assessed the prevalence of the same SNPs in samples collected in November, 1 month after the third of 3 monthly treatments with DP or AQ/SP.Before therapy malaria prevalence was 48.1% based on microscopy and 72.5% measured by PCR. Prevalences of SNPs before therapy were 68.2% (178/261) for *Pfcrt* 76T: 24.9% (65/261), 56.3% (147/261) and 8.0% (21/261) for *Pfmdr1* 86Y, 184F and 1246Y, respectively; 58.6% (153/261), 54.8% (143/261), and 55.17% (144/261) for *Pfdhfr* 51I, 59R and 108N, respectively; and 33.7% (88/261) and 57.47% (157/261) for *Pfdhps* 436S and 437G. The SNPs *of Pfmdr1* 1034C and 1042D; *Pfdhfr* 164L; and *Pfdhps* 540E were not seen. SNP prevalences after three monthly treatmens are currently being analyzed. Our results indicate high prevalence of key resistance-mediating polymorphisms. Associations between the prevalence of these SNPs and IPT will be assessed.

475

UNDERRATING MOSQUITOES YET AGAIN: THE EVASION OF SURVEILLANCE BY *PLASMODIUM FALCIPARUM* DRUG RESISTANCE MUTANTS

Sungano Mharakurwa[1], Mulenga Musapa[1], Clive Shiff[2], Philip E. Thuma[1]

[1]The Malaria Institute at Macha, Choma, Zambia, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

By definition, "malaria" was an initial underrating of mosquitoes, ascribing the deadly disease to "bad air". Ironically, present-day surveillance for drug-resistant *Plasmodium falciparum* mutants is primarily based on genotyping microscopy-positive human infections and presumed representative of the surveyed areas. Genotypes in the definitive mosquito host are seldom examined, despite field evidence of association between mosquito control and drug-resistant *P. falciparum* prevalence. In the current study, we captured 796 *Anopheles arabiensis* vector mosquitoes from sleeping rooms of a representative sample of 2279 human residents in Southern Zambia. We examined the cross-sectional composition of *P. falciparum* antifolate resistance polymorphisms in both human and mosquito infections using PCR, allele-specific restriction enzyme digestion and DNA sequencing confirmations. High levels of pyrimethamine resistance mutants were found in human *P. falciparum* infections, with nearly saturated S108N (92.7%) and considerably prevalent N51I (81.5%) and C59R (58.5%). In contrast, the odds of these mutants were up to 101-fold lower in the mosquito phase (OR [95% CI]: 101.3 [34.34 - 299.03], $p < 0.001$). Mosquitoes, instead, exhibited high prevalence of cycloguanil resistance S108T/A16V mutants, which are currently considered rare or absent from natural *P. falciparum* infections, especially in Africa. We initially did not detect these mutants in humans but subsequently found them among submicroscopic infections. Cycloguanil has not been used in the area. One mosquito mid-gut infection carried the rare I164L mutant, while another bore a hitherto undescribed I164R variant. Our data demonstrate that *P. falciparum* exhibits different antifolate resistance allele compositions in human and mosquito hosts, presumably reflecting contrasted drug and (or) immune selection. We show that by dint of such host-dependent distribution, *P. falciparum* mutants apparently evade current surveillance for resistance alleles. An unnoticed role of mosquitoes in drug resistance epidemiology is presented.

476

TARGET GENE DUPLICATION IN ARMD *PLASMODIUM FALCIPARUM* ACQUIRING DRUG RESISTANCE

Jennifer L. Guler[1], Daniel L. Freeman[1], Napawan Ponmee[1], John White[1], Ramesh Gujjar[1], Alka Marwaha[1], Margaret A. Phillips[2], Pradipsinh K. Rathod[1]

[1]University of Washington, Seattle, WA, United States, [2]University of Texas Southwestern Medical Center, Dallas, TX, United States

Drug resistance to commonly used antimalarials has produced major barriers to the treatment of *Plasmodium* infections. Some parasites from Southeast Asia exhibit the ARMD phenotype, an enhanced ability to develop resistance to new, unrelated antimalarials *(*as reported previously). Recently, we selected for 5-fold stable resistance to a novel dihydroorotate dehydrogenase inhibitor (DSM1; as reported previously) in ARMD Dd2 parasites. While direct sequencing revealed no mutations in the target gene, comparative genomic hybridization showed a 34-95kb amplification event at the dihydroorotate dehydrogenase locus in four independent DSM1-resistant clones. Quantitative PCR and expression analysis confirmed a two- to three-fold increase in copy number of the target and surrounding genes. Theoretically, amplification events up to 100kb would allow for coverage of the entire 25Mb *Plasmodium* genome with as few as 250 parasites. We propose that this process serves as a critical, early event in the accelerated acquisition of resistance and that extra copies of the target gene may facilitate the accumulation of mutations in a way that is missed by conventional sequencing. Adaptive amplification followed by mutagenesis could be a general strategy that ARMD parasites use to survive and evolve resistance during lethal selection.

477

MEASURING MALARIA PARASITE CLEARANCE USING REAL-TIME PCR ON BLOOD-SPOT DERIVED PARASITE DNA

Khalid B. Beshir[1], Teun Bousema[1], Rachel Hallet[1], Spencer Polley[2], Colin Sutherland[1]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]Hospital for Tropical Diseases, London, United Kingdom

Early detection of slow clearing malaria parasites enables adequate countermeasures to be taken to prevent the emergence of drug resistance. One way of achieving this is by measuring change in peripheral parasitemia in a sequence of samples taken after treatment, and currently this requires microscopic examination of blood films. However, microscopic estimation of parasite clearance time requires considerable expertise; false negatives can easily be read especially at low-level parasitemia; it is labour intensive and suffers from low throughput. We report the development and use of a new multi-plex quantitative PCR (qPCR) assay to measure parasite clearance after treatment. The method is based on the Delta Delta Cycle Threshold (ΔΔCT) method to estimate the relative abundance of two amplification targets, one of parasite origin and one of human origin. The assay was tested on patient blood samples taken before and after treatment and the results were compared to microscopy. Field samples were also evaluated, and individuals harbouring malaria parasites with slower responses to artemether-lumefantrine were successfully identified. This new method is quicker, less laborious, more sensitive and requires less training compared to microscopy. We are planning to incorporate the assay as an endpoint in large scale clinical trials of Artemisinin Combination Therapy (ACT) efficacy in Africa. Filter-paper based identification of slow responding parasites is a valuable surveillance tool for early warning of the emergence of drug resistance.

478

DEFINING THE ROLE OF MUTATIONS IN *PLASMODIUM VIVAX* DIHYDROFOLATE REDUCTASE-THYMIDYLATE SYNTHASE USING A *PLASMODIUM FALCIPARUM* EXPRESSION SYSTEM

Alyson M. Auliff[1], John H. Adams[2], Michael T. O'Neil[3], Qin Cheng[1]

[1]Australian Army Malaria Institute, Enoggera, Australia, [2]Department of Global Health, University of South Florida, Tampa, FL, United States, [3]Division of Experimental Therapeutics, Walter Reed Army Institute of Reseach, Silver Spring, MD, United States

With the high prevalence of *Plasmodium vivax* resistance to antifolates throughout Australasia, it is critical to understand the determinants for resistance and for development of new treatments. Like *P. falciparum*, resistance to antifolates such as pyrimethamine and cycloguanil in *P. vivax*, are caused by point mutations within the parasites dihydrofolate reducatase (DHFR)-thymidylate synthase genes. However several unique mutations have been reported in *P. vivax* DHFR and their roles in resistance to classic and novel antifolates are not entirely clear. We have assessed the *in vitro* expression of the *P. vivax* wild- type and various mutant dhfr alleles using both episomal and piggyBac transposon integrated *P. falciparum* expression systems and compared the effect of these alleles to susceptibility to antifolates. We show that the *P. falciparum* parasites transfected with wild-type pvdhfr, in both expression systems, is as susceptible to classic and novel antifolates as the *P. falciparum* with wild-type pfdhfr, while *P. falciparum* parasites transfected with mutant pvdhfr are as resistant to classic antifolates as mutant *P. falciparum* and are notably more resistant to a novel antifolate drug WR99210. Our results show that the expression of pvdhfr alleles in *P. falciparum*, a closely related biological system, help identify the role and importance of specific mutations against current and new antifolate treatments and provide a system for the quick assessment of the potency of new antifolate drugs against *P. vivax* with different dfhr alleles.

479

IMPLICATIONS OF THE PHARMACOLOGICAL PROFILE OF ACT FOR FUTURE TREATMENT EFFICACY

Zulfiqarali Gulamhussein Premji

Muhumbili University of Health Sciences, Dar es Salaam, United Republic of Tanzania

We examine the specific pharmacological profiles of the different artemisinin-based combination therapies (ACT) and discuss the implications of these properties on their suitability for malaria treatment now and in the future. The half-life of the artemisinin partner drug has a significant effect on the efficacy of ACT and the potential for development of resistance. A shorter half-life may not eliminate parasites, while a longer half-life may expose parasites to sub-therapeutic drug levels, increasing the risk of resistance development. The emergence of resistance to a companion drug leaves the artemisinin derivative exposed as an unprotected monotherapy, which may jeopardize the longevity of the whole ACT class. With partner drugs that have a relatively short terminal half-life, such as lumefantrine, the risk for the emergence of resistance is lower than other agents such as mefloquine. However, a longer half-life may protect the patient against re-infection for longer. An increase in the development of cross-resistance between chloroquine and companion drugs bearing a structural similarity (artesunate and piperaquine) is also a cause for concern. While a once-daily dosing schedule is more likely to encourage compliance, a twice-daily schedule maintains the blood concentration of the artemisinin derivative above the minimum effective concentration for at least two asexual parasite lifecycles. This ensures that parasites are exposed to high levels of artemisinin derivative at the point in their lifecycle when they are most susceptible to antimalarials. In addition, a progressive increase in concentration of the partner drug means that any residual parasites continue to be exposed to high drug levels, reducing recrudescence. With the artemisinin derivatives being the

only antimalarials to which parasite resistance has not yet been reported in Africa, consideration of pharmacological factors will be important in preserving their effectiveness.

480

MULTIPLEX DIAGNOSTIC PROTOCOL FOR ENTEROPATHOGENS

Mami Taniuchi[1], Jie Liu[1], Jean Gratz[1], Happy Nduma[2], Athanasia Maro[2], Gibson Kibiki[2], Rashidul Haque[3], Jaco Verweij[4], **Eric R. Houpt**[1]

[1]University of Virginia, Charlottesville, VA, United States, [2]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania, [3]International Centre for Diarrhoeal Diseases and Research, Dhaka, Bangladesh, [4]Leiden University, Leiden, The Netherlands

Detection of enteropathogens typically requires multiple diagnostic modalities with inherently different sensitivities. We sought to develop a pan-PCR approach for enteric viral, bacterial, protozoal, and helminthic targets. First we adapted our real time PCR assays for the major intestinal parasites *Cryptosporidium*, *Giardia*, *Entamoeba histolytica*, *Ascaris*, *Ancylostoma*, *Necator*, and *Strongyloides* into a single protocol involving two multiplex PCR reactions, one with specific primers for the protozoa and one with specific primers for the helminths, after which PCR product is hybridized to beads linked to a specific internal oligonucleotide probe. Detection occurs on a Luminex platform. The assays exhibited equal or better analytic sensitivity than the parent multiplex real-time PCR assays, and yielded an average sensitivity of 91 12% and specificity of 97% 2% for the analytes on 228 clinical specimens from Bangladesh and The Netherlands. Next we developed PCR and RT-PCR assays for enteric viruses, including Norovirus GI and GII, Rotavirus, Astrovirus, Sapovirus and Adenovirus. The assay yielded a tight correlation with Ct values from real time RT-PCR assay (R2=0.94), indicating quantitation, and exhibited > 95% sensitivity and specificity on 229 fecal samples from inpatients with diarrhea from Tanzania. Internal controls for both DNA and RNA are spiked into each fecal sample before nucleic acid extraction to better quantitate by normalizing for the efficiency of nucleic acid extraction and amplification. We are now adding bacterial targets. This multiplex PCR-bead assay will afford sensitive quantitative detection of all major enteropathogens in a single protocol usable for epidemiologic and clinical studies.

481

ROTAVIRUS DIARRHEA IN YOUNG CHILDREN IN BHARATPUR, NEPAL

Ladaporn Bodhidatta[1], Sanjaya Shrestha[2], Sasikorn Silapong[1], Bhola Ram Shrestha[3], Shanti Regmi[3], Orntipa Sethabutr[1], **Carl J. Mason**[1]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Walter Reed/Armed Forces Research Institute of Medical Sciences Research Unit Nepal, Kathmandu, Nepal, [3]Bharatpur Hospital, Bharatpur, Nepal

Human Rotavirus causes significant morbidity and mortality among children worldwide. Several effective vaccines have become available and can reduce the disease burden and health care costs of rotavirus-specific diarrhea. We conducted a hospital based surveillance of rotavirus diarrhea in Bharatpur Hospital, Bharatpur, Nepal during December 2006 - December 2008 to describe the epidemiology and genotypic distribution of rotavirus, important information for decision-makers on a future vaccine implementation. Stool samples collected from children under 5 years of age with acute diarrhea and non-diarrhea controls were examined for rotavirus by a real time reverse transcriptase polymerase chain reaction (RT-PCR) using primers and probes targeted on VP6 gene. Samples positive for Rotavirus were genotyped using primers targeted on VP7 and VP4 genes to identify 6 G-types (G1-G4, G9 and G12) and 3 P-types (P[4], P[6] and P[8], respectively, by conventional PCR. Rotavirus was detected in 204/598 (34%) of children with diarrhea and 47/597 (8%) of non-diarrhea controls. G12 was the predominant type (69%), followed by G1 (7%), G9 (6%) and G2 (5%). In regard to P-typing, P[8] was predominant (54%) followed by P[6] (29%) and P[4] (7%). The fraction of the major combination of G and P was G12P[8] (36%) followed by G12P[6] (27%). Approximately 13% and 10% could not be characterized by G-typing and P-typing primers, respectively. Results of the sequence analysis will be reported and the emergence of unusual serotypes described. Rotavirus is clearly a significant cause of acute pediatric diarrhea in Nepal with the most common genotypes being G12P[8] and G12P[6].

482

NOROVIRUS INFECTION IN YOUNG CHILDREN IN THAILAND - A MULTICENTER STUDY

Ladaporn Bodhidatta[1], Pimmnapar Neesanant[1], Siriporn Sornsakrin[1], Vorachet Taecharak[2], Krongkaew Supawat[3], Orntipa Sethabutr[1], Carl J. Mason[1]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Srisangwan Hospital, Maehongsorn Province, Thailand, [3]Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand

Noroviruses (NoVs) are associated with acute viral gastroenteritis in humans worldwide. In Thailand, the epidemiology of NoV has not been well described. Limited prevalence data was previously reported as 14% in hospitalized children in 2002-2004 with most NoV identified as genogroup II. We developed and validated a one step real time Reverse Transcription Polymerase Chain Reaction (rt RT-PCR) assay to detect NoV and distinguish between genotype I and II. This assay was applied to stool samples collected from children with acute gastroenteritis (cases) and children without acute gastroenteritis (controls) in multiple sites in Thailand during October 2004 to December 2006. We tested 3,064 stool samples obtained from 3 month to 5 year old children in four different geographical areas of Thailand. 1,502 samples were collected from diarrhea cases and 1,562 samples were from controls with no recent history of diarrhea. NoV was detected in 211/1,502 (14 %) of cases and in 77/1,562 (5 %) of controls (Odds Ratio = 3.2, 95% Confidence Interval 2.4-4.1). GII was the major genotype accounting for approximately 96% and 92% of all NoV identified from cases and controls, respectively. The prevalence of NoV was higher in children less than 36 months old, during cooler months and at certain geographical areas. Our data suggests that NoV is an emerging important cause of acute gastroenteritis in young children and GII is the predominant genogroup.

483

PROLIFERATION OF CYTOKINE PRODUCING T CELLS IN RESPONSE TO *VIBRIO CHOLERAE* O1 INFECTION OR VACCINATION IN BANGLADESHI ADULTS

Alison L. Kuchta[1], Taibur Rahman[1], Taufiiqur R. Bhuiyan[1], Mohammad Arifuzzaman[1], Rasheduzzaman Rashu[1], Ashraful I. Khan[1], Fahima Chowdhury[1], Amit Saha[1], Edward T. Ryan[2], Stephen B. Calderwood[2], Firdausi Qadri[1], Jason B. Harris[2]

[1]ICDDR,B, Dhaka, Bangladesh, [2]Massachusetts General Hospital, Boston, MA, United States

Vibrio cholerae O1 causes diarrheal disease that may be life-threatening without appropriate treatment. Natural infection confers over 90% protective immunity for 5-7 years, while oral cholera vaccines confer up to 3 years of protection at varying degrees. The CD4+ T-cell responses to V. cholerae O1 infection has not been well characterized but may contribute to the development of longer lasting B-cell responses seen after natural infection. In this study, to examine the profile of cytokine secreting CD4+ T cells following either cholera infection or vaccination, we enrolled V. cholerae O1 infected Bangladeshi adult cholera patients and Dukoral vaccine recipients. CD4+ T-cell responses were assessed by intracellular cytokine staining following stimulation of peripheral blood mononuclear cells with V. cholerae specific antigens. CD4+ T cells producing IFN-γ, IL-

13, IL-10 and IL-17 were analyzed at multiple time points after infection or vaccination and compared with responses in healthy adult controls. In patients, IFN-γ response was significantly higher following stimulation with V. cholerae membrane protein (MP) at the acute (day 2) and early convalescence stages (day 7) of cholera infection. In the same group, there was no increase in IL-13 producing T-cells, suggesting a Th1 type of polarization. In the vaccinated population, a significant increase in both IFN-γ and IL-13 cytokine secreting cells was observed, indicating a mixed Th1 and Th2 type response. An increase in IL-17 secreting CD4+ T cells in response to antigenic stimulation was also observed in both patients and vaccinees. These results show that, in a cholera endemic population, CD4+ T-cell responses are demonstrable at acute and early convalescence stages after infection and following vaccination in adults, and that there are significant differences in the characteristics of patient and vaccinee responses. Further studies are needed to address whether differences in the initial CD4+ T-cell response in patients and vaccinees contribute to differences in the subsequent duration of protective immunity.

484

MUCOSAL IMMUNOLOGIC RESPONSES IN CHOLERA PATIENTS IN BANGLADESH

Taher Uddin[1], **Jason B. Harris**[2], Taufiqur Bhuiyan[1], Tahmina Shirin[1], M. Ikhtear Uddin[1], Ashraful I. Kahn[1], Fahima Chowdhury[1], Nur Haq Alam[1], Edward T. Ryan[2], Stephen B. Calderwood[2], Firdausi Qadri[1]

[1]ICDDR,B, Dhaka, Bangladesh, [2]Massachusetts General Hospital, Boston, MA, United States

Vibrio cholerae O1 causes dehydrating diarrhea with a high mortality rate. However, the infection elicits long-term protective immunity against subsequent disease. Since *V. cholerae* is noninvasive, mucosal immunity is likely important for protection. In this study, we characterized cellular and humoral immune responses in the duodenal mucosa and in blood of patients with cholera in an endemic setting. Duodenal biopsies were obtained from eighteen adult Bangladeshi patients 2, 30 and 180 days after presentation with cholera. Five healthy adults evaluated in a study of asymptomatic *H. pylori* infection were used as a comparator group. Mucosal and systemic immune responses were assessed by ELISPOT, ELISA and flow cytometry. In the acute stage of cholera, we observed selective recruitment of CD3+CD4+ T-cells to the lamina propria, but no significant increase in the proportion of CD3+CD8+ or B-cells, suggesting CD4+ T-cells in particular may play an important role in the generation of subsequent adaptive immune responses. Systemic immune responses peaked early after infection and returned to baseline levels by six months after infection. Duodenal IgA antibodies directed against cholera antigens also peaked early after infection, on day 30, and returned to baseline levels by six months after infection. However, a significant increase in IgA antibody secreting cell (ASC) responses to lipopolysaccharide (LPS) in lamina propria lymphocytes (LPL) compared to healthy controls, was found on all study days ($P<0.05$ for days 2, 30 and 180). These mucosal ASC responses peaked on day 30, but were also evident in patients only 2 days after onset of illness, suggesting that in an endemic area, patients mount an anamnestic mucosal immune response to *V. cholerae* antigens. Although duodenal ASC responses waned by day 180, they remained significantly elevated compared to healthy controls, even when serum antibody responses had returned to control levels. These data suggest an early CD4 T-cell response in the gut mucosa may be associated with a subsequent influx of antigen-specific antibody secreting cells that may help mediate protection at the mucosal surface.

485

QUANTITATIVE REAL TIME PCR (QRT-PCR) FOR ENTHEROPATHOGENIC *E. COLI* (EPEC) IN STOOL SAMPLES FROM CHILDREN WITH AND WITHOUT DIARRHEA

Francesca Barletta[1], Erik Mercado[1], Theresa J. Ochoa[1], Lucie Ecker[2], Ana I. Gil[2], Claudio F. Lanata[2], Thomas G. Cleary[3]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Instituto de Investigación Nutricional, Lima, Peru, [3]University of Texas School of Public Health, Houston, TX, United States

EPEC is a main pathogen in children with diarrhea, however it can be identified also in a significant percentage of healthy controls. The aim of this study was to compare the bacterial load of EPEC in stool samples from children with and without diarrhea. We have analyzed 143 stool samples positive for EPEC detected by PCR from Mac Conkey plates; 53 from diarrhea cases and 90 from healthy controls in children under 2 years of age in Lima, Peru. DNA was isolated from stool specimens by a cetyltrimethylammonium bromide (CTAB) extraction method. Primers and probes were designed to amplify and quantify the intimin (*eaeA*) gene of EPEC2348/69 in a single reaction by qRT-PCR. To standardize the method, a direct correlation was determined between the fluorescence threshold cycle (CT) and the copy number of the *eaeA* gene. The standard curve was constructed by using known quantities of genomic DNA from 1.04 x 100 to 1.04 x 108 fg (1 molecule of DNA = 5.44fg of DNA genbank NC011601). A mixture of all PCR reagents without any DNA was used as a negative control. The detection limit of this PCR assay was 5 copies of the *eaeA* gene per mg of stool. The geometric mean of the bacterial load on the diarrhea group was 299.5 bact/mg (95%CI: 77.1-1,163.9) vs. 28.8 bact/mg (95%CI: 9.5-87.3) in the control group ($p=0.016$). Among children younger than 12 months of age the bacterial load in the diarrhea group was higher than the controls (177.6 vs 5.1 bact/mg, $p=0.006$); there were no significant differences among older children. Among children with EPEC as a single infection the bacterial load in the diarrhea group was higher than the controls (463.5vs 24.5 bact/mg, $p=0.006$); there were no significant differences among children with co-infections. In conclusion, the bacterial load of EPEC, measured by qRT-PCR on stool samples, is higher in children with diarrhea than in healthy controls. qRT-PCR is a potential useful tool to study the relation between disease and colonization by enteric pathogens.

486

IMMUNOGENICITY AND PROTECTIVE EFFICACY OF A FIMBRIAL ADHESIN-BASED VACCINE AGAINST ENTEROTOXIGENIC *ESCHERICHIA COLI* IN *AOTUS NANCYMAAE*: EVALUATION OF DOSING AND ROUTE OF VACCINATION

Ryan C. Maves[1], **Michael J. Gregory**[1], David Cepeda[1], Gladys Nuñez[1], Ana Cevallos[1], Nereyda Espinoza[1], Aisling O'Dowd[2], Stephen J. Savarino[2]

[1]United States Naval Medical Research Center Detachment, Lima, Peru, [2]United States Naval Medical Research Center, Silver Spring, MD, United States

Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of morbidity and mortality worldwide. Previous studies have demonstrated the protective efficacy of a 4-dose intranasal (IN) regimen of CfaE, the tip adhesin of CFA/I, admixed with the B subunit of cholera toxin (CTB) to orogastric challenge with ETEC strain H10407 (CFA/I, O78:H11, LT+/ST+). In this study, we replace CTB with the B subunit of ETEC heat-labile toxin (LTB) and compare the 4-dose IN regimen to a 3-dose IN regimen and to a novel 3-dose intradermal (ID) route. 32 adult *Aotus* were assigned to 4 groups of 8 animals each. Group I received 4 vaccine doses by the IN route on days 0, 14, 28, and 84. Groups II and III received 3 vaccine doses by the IN and ID route, respectively, on days 0, 14, and 28. Group IV control animals received IN phosphate-buffered saline (PBS) on days 0, 14, 28,

and 84. Doses contained 200 µg dsc19CfaE and 290 µg rLTB (for IN) or 100 µg dsc19CfaE and 145 µg LTB (for ID) in PBS. 14 days after the final vaccine dose, all animals were orally challenged with 5x1011 CFU of ETEC strain H10407. Diarrhea attack rates were 12.5%, 37.5%, 50%, and 75% in groups I, II, III, and IV, respectively. The mean respective durations of diarrhea were 2, 6.7, 4.3, and 5.5 days. Significant protection was seen only in the 4-dose IN group when compared to controls (p=0.041). Serum IgG and IgA antibody titers to CfaE rose after the second or third dose by the IN route and after the first dose for in the ID group. In groups I and III, peak serum IgG and IgA titers were noted the day before challenge, whereas in group II titers peaked after challenge. Serum IgG responses to LTB rose after the second dose in all groups, peaking the day before challenge. In conclusion, an ETEC vaccine comprising CfaE and LTB, given in a 4-dose IN regimen, significantly protects *Aotus nancymaae* from diarrhea following homologous oral challenge with ETEC. The same vaccine given in a 3-dose IN or 3-dose ID regimen is immunogenic but did not significantly reduce the diarrheal attack rate compared to controls.

487

MORTALITY TRENDS OBSERVED IN POPULATION-BASED SURVEILLANCE OF AN URBAN INFORMAL SETTLEMENT, KIBERA, KENYA, 2007-2009

Beatrice Olack[1], Daniel R. Feikin[2], Leonard O. Cosmas[1], Kennedy O. Odero[1], George O. Okoth[1], Robert F. Breiman[2]

[1]Kenya Medical Research Institute/Centre for Disease Control and Prevention, Nairobi, Kenya, [2]International Emerging Infections Program-Kenya, United States Centers for Disease Control and Prevention, Nairobi, Kenya

Rapid urbanization in sub-Saharan Africa has led to new and expanded informal settlements, where dense populations with sub-standard hygiene and sanitation threaten health of residents. Mortality among residents of such settlements has not been well-characterized. In 2005, we established population-based infectious disease surveillance (PBIDS) within Kibera, the largest contiguous slum in Africa. Surveillance is conducted within two villages with nearly 30,000 participants. Field workers visit each household bi-weekly to identify all illnesses and deaths. Each participant has free access to a field clinic staffed by health care workers trained in PBIDS protocol. We analyzed data from January 2007 to December 2009 on participants who died during this period, focusing on illnesses and health seeking behavior reported in home visits. Person-years of observation (pyo) were based on weekly counts of participants residing within study area. We reported 566 deaths; overall mortality rate was 7.0 (95% CI 6.9-7.0) per 1,000 pyo. Mortality rate for children ≤5 years old was 15.2 (95% CI 15.1-15.2) per 1,000 pyo, 3-fold higher than that for persons >5 years old (5.1 per 1,000 pyo, 95% CI 5.0-5.1).Mortality rate for neonates was 95.3 (95% CI 93.7-96.9) per1000 pyo. Female infants had higher mortality rates than male infants (Rate ratio = 1.60; 95% CI 1.09 - 2.35). In contrast, for persons >5 years old, females had significantly lower mortality rates than males (RR= 0.77; 95% CI 0.62 - 0.95). Most children 74 (82%), ≤5 years old had at least 1 of the following: cough 34 (38%), diarrhea 41 (46 %), or fever 50 (56%); and persons >5 years old; 110(82%) had: cough 43(39%); diarrhea 28(26%) and fever 88(80%). Many of these symptoms overlapped. Accident and injuries accounted for 15(7%) total deaths that occurred within two weeks. Children ≤5 years old were two times more likely to receive clinic or hospital care than persons >5 years during the 2 week period before death. In conclusion, gender differences in mortality by age group require further study, but may reflect differential timing of health care access (for infants) and risk factors for severe disease (for older participants). A high proportion of deaths appear to be associated with infectious disease symptoms; disease prevention programs need to include focus on informal settlements, where residents have traditionally been neglected in health promotion efforts.

488

HEALTH EFFECTS AND COPING STRATEGIES TO FLOODS IN KUMI DISTRICT, EASTERN UGANDA

Peter Kirabira, Christopher G. Orach, David Mukanga

Makerere University College of Health Sciences, School of Public Health, Kampala, Uganda

Globally, floods account for over 40% of natural disasters and contribute to 37% of all mortality. During 2007, Uganda experienced prolonged floods that affected the eastern and northern districts including Kumi. We assessed the health effects and coping mechanisms to the 2007 floods in Kumi district.

Methods We conducted a cross-sectional study during May 2008. Data was collected from all the 26 health facilities in Kumi district using record reviews and 17 key informant interviews were conducted. Quantitative data were analyzed using SPSS version 13.0 while qualitative data were analyzed using Manifest-Content Analysis technique. The leading causes of morbidity were malaria (OPD:45.44%; IPD:53.13%), respiratory infections (OPD:14.14%; IPD:9.42%) and injuries (OPD:3.40%; IPD:3.71%), while malaria (27.36%), respiratory infections (9.92%) and injuries (4.62%) were the leading causes of mortality. Diarrheal diseases (4.42%), injuries (3.09%) and respiratory infections (1.57%) had highest case fatality rates. Under-fives were most affected (OR=1.06, 95%CI 1.01-1.11), females were more likely to be admitted during the floods than before or after (OR=1.05, 95%CI 1.00-1.10). There was higher under-five mortality during the floods (OR=1.72, 95%CI 1.22-2.69) and after (OR=1.76, 95%CI 1.15-2.61) compared to before. 15/26 health facilities had disruption of routine services; there was no rapid response team prior to the floods. Main challenges were lack of district disaster management plan, low budget, understaffing and sustainability of clean safe water. In conclusion, the main causes of morbidity and mortality were communicable diseases (malaria, respiratory infections) and injuries. Under-fives and women were most vulnerable. There is need for the district to develop a disaster management plan and budget, focusing on communicable disease control, injuries, and on vulnerable under fives and women during disaster response and recovery period. The district should set up early warning systems to improve disaster management.

489

SEROPREVALENCE OF ZOONOSES IN MONGOLIA: SURVEILLANCE AND RISK FACTOR ASSESSMENT

Roman Wölfel[1], Damdin Altantuul[2], Ilona Mossbrugger[1], Lhagvajav Zorig[2], Biraasuren Enkhtuvshin[3], Rendoo Davaadorj[4]

[1]Bundeswehr Institute of Microbiology, Munich, Germany, [2]Central Clinical Hospital of the Mongolian Armed Forces, Ulaanbaatar, Mongolia, [3]General Authority for Border Protection of Mongolia, Ulaanbaatar, Mongolia, [4]Mongolian Armed Forces General Staff, Ulaanbaatar, Mongolia

Public health surveillance in Mongolia currently relies, primarily, upon passive disease reporting from health care providers. It is suspected that there is presently a substantial underreporting especially of various zoonotic infections. The true prevalence of infections such as plague, tularaemia, brucellosis, tick-borne encephalitis (TBE), Q-fever, Congo-Crimean Hemorrhagic Fever (CCHF) and hanta virus infections within Mongolia is unknown. Therefore, we performed a seroprevalence study in five different regions of Mongolia in order to determine the prevalence of exposure to the disease agents of interest and to assess the demographics associated with prior exposure to these infections. During the study period blood samples were obtained from 765 consenting members of the Mongolian Armed Forces (670 male, 95 female). All participants were asked to complete a questionnaire identifying their demographics, medical history and possible risk factors for exposure to these infections. Samples were tested for disease-specific IgG antibodies by ELISA and/or immunofluorescence assays. Results indicate no or infrequent exposure to the CCHF virus (2.9%) and *Francisella tularensis* (1.7%). In contrast

to this, high seroprevalence rates for *Yersinia pestis* (22.6%), *Brucella sp*. (14.9%), *Coxiella burnetii* (13.9%) and hanta viruses (10%), suggest that human infections with these zoonotic bacteria are frequent and largely unrecognized in Mongolia. Exposure to *Y. pestis* (range 7.3-38.8%) and TBE virus (range 0.6-29.8%) appeared to be significantly different between certain rural regions of Mongolia. Demographic features of seropositive persons did suggest distinct epidemiology, ecology and risks for brucellosis, TBE and plague, whereas specific associations between the other diseases and certain risk factors could not be demonstrated. The results of this first nationwide study allow an estimation of the baseline disease prevalence for the above mentioned infections among the Armed Forces in different regions of Mongolia. Further investigations are necessary to develop a better understanding of risk factors important for the reduction of exposure of these zoonotic diseases in Mongolia.

490

TEN-YEAR CANCER TREND AND AVAILABLE INSTITUTIONAL CAPACITIES FOR RESPONSE IN UGANDA, 2010

Moses Tetui, Suzanne Kiwanuka, Danstan Bagenda

Makerere University College of Health Sciences, School of Public Health, Kampala, Uganda

Globally, cancer is the biggest non communicable disease (NCD); estimated to have accounted for about 13% of all deaths in 2007. The burden of NCDs for developing and newly industrialized countries is expected to rise over 60% by 2020 compared to a rise of 10% in developed countries. This study established the cancer trends (1999 to 2008) from the pathology department of Mulago National Referral Hospital, describes the demographic distribution and assessed institutional capacities for response in Uganda. This was a descriptive retrospective study, which employed quantitative and qualitative methods. Records were reviewed to establish cancer trends. We held key informant interviews with leaders at various departments that offer cancer related services to assess institutional capacities. Quantitative data was analysed using STATA Version 10 and qualitative data analysed using thematic manifest techniques. The most commonly diagnosed cancers between 1999 and 2008 were; Kaposi's sarcoma, cervical cancer and cancer of the eye. The three commonest cancers in men were Kaposi's sarcoma, prostate and cancer of the eye, while in women it was cancers of the cervix, breast and ovary. Majority of the cancers are on the increase in Uganda and females contribute to 58% of all the cancers diagnosed. The median age for cancer diagnosis was 40 years (IQR 27, 55). The proportion of persons affected by breast cancer has significantly increased between 1999 and 2008 (82 cases vs 142, $p<0.001$). HIV related cancers are still prevalent although they are declining. Critical service gaps identified were: inadequate access to diagnostic and treatment services and shortage of human resources. In conclusion, there is a steady increase of cancer in Uganda with females being more affected. The existing institutional capacities are insufficient to match the increasing trend. There is an urgent need for government and partners to increase human resources and accessibility to diagnostic and treatment services for cancers in order to improve cancer outcomes.

491

THE EFFECTS OF MATERNAL HELMINTH INFECTION AND CO-INFECTION WITH MALARIA ON BIRTHWEIGHT AND SUBSEQUENT GROWTH IN OFFSPRING IN A POPULATION ON THE COAST OF KENYA

Jessica K. Fairley[1], Indu Malhotra[1], Peter Mungai[2], Eric Muchiri[2], Christopher L. King[1], Charles H, King[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Division of Vector Borne Diseases, Nairobi, Kenya

While there is abundant data on the effects of maternal malaria infection during pregnancy on the infant, data on the effects of maternal helminth infection and malaria-helminth co-infection on the offspring are lacking. We seek to better define the associations between maternal infection and early childhood growth in an endemic area. A cohort of pregnant woman and their infants from the Kwale district in Kenya were followed prospectively. The women were tested for malaria and helminth infection at delivery and birthweight was documented. Subsequent height, weight, hemoglobin, and malaria infection status were measured on the offspring every 6-12 months up to age 60 months. There were 696 live births of full-term infants and 2072 follow-up data points. 42.7% of the mothers were infected with *Plasmodium falciparum*, 30.6% with Schistosoma haematobium, 36.2% with filariasis, 31.5% with hookworm, and 5.9% with Trichuris trichiura. 29.5% were infected with *P. falciparum* and helminthes, 13.2% with *P. falciparum* alone, 41.1% with one or more helminthes, and 16.2% with none of the parasites. 8.3% of the infants had low birthweights with a z-score of -2 SD or below. There were no significant differences in mean birthweights between those with no infection and the other three infection groups. In the follow-up data, the percentage of underweight ranged from 13.6%-19.2%. For height, the data showed a range of prevalence of stunting of 40.2-50.7%. There were no significant differences in height z-scores on univariate analysis between the four infection groups described above, however, in most age groups, the "no infection" group tended to have a significantly worse mean weight z-score than the other groups. This data analysis does not show a significant effect of maternal infection on infant growth, however, the relatively small percentage of mothers without infection may make it insufficiently powered to detect a true difference. Multivariate analysis controlling for presence of malaria infection and anemia in the children may also prove to unmask differences.

492

A SYSTEMATIC REVIEW OF SAFETY DATA REPORTING FROM MALARIA, TUBERCULOSIS, AND HIV VACCINE TRIALS: THE NEED FOR INTERNATIONAL GUIDELINES

Cindy Tamminga[1], Santina Maioletesi[1], Charlotte Fedders[1], Jan Bonhoeffer[2], Uli Heininger[2], Vasee Moorthy[3], Thomas Richie[1], Judith Epstein[1]

[1]United States Military Malaria Vaccine Program, Naval Medical Research Center, Silver Spring, MD, United States, [2]Division of Infectious Diseases and Vaccines, University Children's Hospital, Basel, Switzerland, [3]Initiative for Vaccine Research, World Health Organization, Geneva, Switzerland

Malaria, tuberculosis and HIV combine to kill approximately 5 million people each year with the poor of the world most severely affected. The development and testing of promising candidate vaccines for each of these three poverty related diseases (PRD) is of critical importance. The speed and efficiency of developing safe and effective vaccines would be facilitated if researchers could objectively compare the clinical safety results of trials conducted in various settings. As a first step to creating a standardized reporting format, we assessed the current reporting practices of safety data from clinical trials of candidate vaccines for these PRD. A systematic literature review was performed of articles published in the English language during the time period January 2000 to June 2009. Approximately 150 articles met inclusion criteria and were reviewed. Differences in the methods of collecting, analyzing, and reporting adverse events were evaluated. Particular attention was paid to unique challenges related to conducting clinical trials in resource poor countries. The results of this review, demonstrating the heterologous nature of safety data reporting, will be presented. The Brighton Collaboration, an international voluntary collaboration to facilitate the development, evaluation, and dissemination of high quality information about the safety of human vaccines, will utilize the results of our extensive review to develop guidelines aimed at improving the detail, accuracy, completeness, and comparability of vaccine safety data from clinical trials conducted on vaccines for PRD.

493

COMPUTER-BASED MODELING IN SUPPORT OF GLOBAL ERADICATION OF INFECTIOUS DISEASES

Phillip Eckhoff

Intellectual Ventures, Bellevue, WA, United States

Malaria and polio are current targets of Global Eradication campaigns, and success of these campaigns and any future disease eradication campaigns will provide lasting benefits to humanity. Modern computing and modeling can assist rational planning of disease eradication campaigns to maximize the probability of success in the face of significant challenges and obstacles. We present a new computational framework which is designed to answer questions posed by Eradication campaigns with specific focus on malaria as an example. New detailed models for single malaria infections and mosquito population dynamics are developed and integrated into a large spatial-scale dynamic simulation. The single malaria infection model includes detailed descriptions of parasite intrahost development and human immunology which combine to provide mechanistic explanations of phenomena such as infection duration and adapted response to re-infection. The model for mosquito population dynamics captures the effects of multiple simultaneous vector control interventions upon the mosquito population and the resulting change in parasite population dynamics. The integration of these detailed micro-models into a large-scale spatial simulation with individual resolution allows study of many possible combined-intervention malaria eradication campaigns. Overall probability of campaign success for different combined approaches in the presence of systemic, campaign, and model uncertainty is studied and conclusions for locally-tailored approaches are discussed.

494

ENVIRONMENTAL PRESSURE ON THE ANTIBODY RESPONSE TO A CHILDHOOD VACCINE IN NORTHERN SENEGAL

Lobna Gaayeb Juliàn[1], Emmanuel Hermann[2], Jean-Baptiste Hanon[3], Jean Biram Sarr[1], Mamadou Ousmane Ndiath[4], Anne-Marie Schacht[1], Franck Remoué[5], Gilles Riveau[3]

[1]Laboratoires de Recherche Médicale - Espoir Pour la Santé, Saint-Louis, Senegal, [2]Université Lille Nord de France, Lille, France, [3]Centre of Infection and Immunity of Lille - Unité 1019 Institut National de la Santé et de la Recherche Médicale, Lille, France, [4]Unité Mixte de Recherche 198 - Institut de Recherche pour le Développement, Dakar, Senegal, [5]Unité de Recherche 016 - Institut pour la Recherche et le Développement, Cotonou, Benin

Environmental factors play a role in vaccine induced immunity. Season-dependent elements have particularly been involved in the modulation of immune responses in developing countries. In Senegal, a sub-Saharan country with two distinct seasons, a dry and a wet one, we conducted a study to investigate whether there is a modulation of the immune response to a childhood vaccine according to seasonal factors. Whooping cough is a vaccine-preventable respiratory disease caused by *Bordetella pertussis* infection, against which Senegalese children are immunized with the Diphteria-Tetanus-whole Pertussis vaccine (DTwP). To assess the level of immunization against whooping cough, we conducted a cross sectional and longitudinal study (1.5 year) in which serum samples were collected from 410 children aged 1 to 10 from 5 villages in Northern Senegal. We tested these sera for antibodies (Ab) against two major antigens of *B. pertussis*: filamentous hemagglutinin (FHA) and pertussis toxin (PT). Although most children were immunized with DTwP, FHA-specific IgG response was significantly different according to age. Until the age of 5, response to FHA was low, and got higher in the older group. Assessment of anti-PT IgG response suggested evidence of recent exposures to the pathogen. Moreover, IgGs to another antigen included in the DTwP vaccine, the tetanus toxoid (TT), was quantified. A high specific Ab response, which decreased with age, was observed. This suggests that the detected low levels of FHA-specific Ab, especially in the younger group of children, were not due to a failure in vaccination. Noteworthily, significant differences in the specific Ab responses to FHA, PT and TT were observed between villages in the same studied area. Besides, when the results from sera collected every three months were compared, a strong effect of seasonal factors on the Ab response to DTwP antigens was detected. The results of this work should be critical in the scope of a better understanding of the role of environmental factors on the establishment and maintenance of immunity to vaccines.

495

MALARIA INCIDENCE AND PREVALENCE AMONG CHILDREN LIVING IN A PERI-URBAN AREA ON THE COAST OF BENIN, WEST AFRICA: A LONGITUDINAL STUDY

Alain M. Nahum[1], Annette Erhart[2], Ambroisine Mayé[3], Daniel Ahounou[1], Chantal van Overmeir[2], Joris Menten[2], Harry van Loen[2], Harry van Loen[2], Martin Akogbeto[1], Marc Coosemans[2], Achille Massougbodji[4], Umberto D'Alessandro[2]

[1]Centre de Recherche Entomologique de Cotonou, Cotonou, Benin, [2]Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium, [3]Hôpital de Zone, Abomey-Calavi, Benin, [4]Laboratoire de Parasitologie, Faculté des Sciences de la Santé, Cotonou, Benin

Clinical malaria incidence was determined over 18 months in a cohort of 553 children living in a peri-urban area near Cotonou. Three cross-sectional surveys were also carried out. Malaria incidence showed a marked seasonal distribution with 2 peaks, the first corresponding to the long rainy season and the second to the overflowing of Lake Nokoue. The overall *Plasmodium falciparum* incidence rate was estimated at 84/1,000 person-months, its prevalence at over 40% in the two first surveys and 68.9% in the third. Multivariate analysis showed that girls and people living in closed houses had a lower risk of clinical malaria. Bed net use was associated with a lower risk of malaria infection. Conversely, children of families owing a pirogue were at higher risk of clinical malaria. Considering the high pyrethroids resistance, indoor residual spraying with either a carbamate or an organophospate insecticide may have a major impact on the malaria burden.

496

COMBINED UTILITY OF TOURNIQUET TEST AND WHITE BLOOD CELL COUNT AS TRIAGE CRITERIA FOR DENGUE IN THE AMERICAS

Christopher Gregory[1], Luisa Alvarado-Domenech[2], Lissy Colon[3], Ramon Cruz-Rivera[3], Liv Cuyar-Bermudez[3], Ivonne Galarza[2], Carlos Garcia-Gubern[3], Olga Lorenzi[1], Fernando Ortiz-Baez[3], Luis Santiago[1], Kay Tomashek[1]

[1]Centers for Disease Control and Prevention, San Juan, Puerto Rico, [2]Department of Pediatrics, San Lucas Hospital/Ponce School of Medicine, Ponce, Puerto Rico, [3]Department of Emergency Medicine, San Lucas Hospital/Ponce School of Medicine, Ponce, Puerto Rico

As the clinical presentation of dengue can be non-specific, and rapid diagnostic tests are not readily available, finding easily obtainable markers that can distinguish dengue from other acute febrile illnesses is a priority. Data from Thailand suggests that the combination of a positive tourniquet test (TT) and leukopenia can distinguish dengue from other febrile illnesses in children; little data exists on the utility of these tests in adults or in the Americas. We evaluated the utility of the TT and leukopenia (white blood cell count <4000/mm^3) for identifying dengue as part of a febrile illness surveillance study conducted in the Emergency Department of the Hospital San Lucas in Ponce, Puerto Rico. From September to December 2009, 284 patients presenting to the ED with fever for 2-7 days and no identified source of infection were enrolled. Participants were tested for influenza, dengue, leptospirosis, and enteroviruses. Thirty-one (10.9%) of patients were confirmed as having dengue; a definitive etiology was determined for 142 others (136 influenza, 2 leptospirosis, 3 enterovirus) and 111 patients had no infectious etiology identified. Fifty-two percent of laboratory-positive dengue cases had a positive TT versus 18% of

patients without dengue (p <.0001), and 71% of dengue cases compared to 22% of non-dengue cases had leukopenia (p<0.001). The combination of a positive TT and leukopenia had a sensitivity of 39%, specificity of 96%, positive predictive value (PPV) of 52% and negative predictive value (NPV) of 93%. Having either a positive TT or leucopenia was 84% sensitive, 71% specific, and had a PPV of 27% and NPV of 97%. The tourniquet test was more sensitive in dengue patients with a platelet count of >100,000 than in patients with a count <100,000, but this was not significant (78% vs. 41%, p=0.11). Our study supports the combined use of the tourniquet test and leukopenia as useful markers of dengue infection in adults and children in Puerto Rico. Few patients with early dengue infection would be missed using these tests as triage criteria.

497

COMMUNITY-BASED STUDY OF CHAGAS DISEASE PREVALENCE IN LOS ANGELES COUNTY

Mahmoud I. Traina[1], Salvador Hernandez[1], Louis V. Kirchhoff[2], Aiman M. Smer[1], Haneen Khamag[1], Eva Padilla Garcia[1], Sheba K. Meymandi[1]

[1]Olive View-University of California at Los Angeles Medical Center, Sylmar, CA, United States, [2]University of Iowa, Iowa City, IA, 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 million persons are infected. Chronic CD results in symptomatic cardiac and/or gastrointestinal disease in 10-30% of infected persons, and each year roughly 20,000 deaths are attributed to the illness in the endemic countries. Approximately 17 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. Considerable information regarding CD prevalence among U.S. blood donors has been published, but community-based perspectives on CD prevalence are largely lacking. The Chagas Community Screening Project was initiated in Los Angeles County in April 2008 by the Center of Excellence for Chagas Disease at Olive View-UCLA Medical Center. Randomly-selected adults 18 to 60 years old residing in Los Angeles County who had lived in a Chagas-endemic country for 1 year or more were enrolled in the study, mostly through church groups. Blood samples were screened serologically in a prototype ELISA in which chimeric recombinant proteins FP3, FP6, and FP10 were used as target antigens. Samples positive in the ELISA then underwent confirmatory testing in the Chagas RIPA. To date 985 subjects have been tested. The median age was 45. The endemic countries in which the subjects had spent 1 year or more were as follows: Mexico 719 (72.8%), El Salvador 141 (14.3%), Guatemala 77 (7.8%), Peru 17 (1.7%), Columbia 11 (1.1%), Honduras 7 (0.7%), Nicaragua 6 (0.6%), Costa Rica 3 (0.3%), Ecuador 2 (0.2%), Argentina 1 (0.1%), and Venezuela 1 (0.1%). A total of 10 subjects were RIPA-positive (1.0%). The median age of the positive subjects was 48. 5 patients were from Mexico (0.7%), 4 from El Salvador (2.8%), 1 from Honduras (14.3%), and none from the other countries. Our study demonstrates a substantial prevalence of CD in Latin American immigrants in Los Angeles County and suggests that similar numbers of persons with CD are present in other communities in which immigrants from Chagas-endemic countries have settled. Serologic screening of immigrants at geographic risk for CD should be performed so that appropriate monitoring and treatment can be carried out.

498

KNOWLEDGE, ATTITUDES, AND PRACTICES OF PRACTITIONERS WHO PROVIDE PRE-TRAVEL CONSULTATIONS

Laura Kogelman[1], Elizabeth D. Barnett[2], Emad Yanni[3], Michael R. Winter[4], Christine E. Chaisson[4], Lin H. Chen[5], Mary E. Wilson[6], Adolf W. Karchmer[7], Winnie W. Ooi[8], Erika Gleva[5], Gary Brunette[3], Davidson H. Hamer[9]

[1]Tufts Medical Center, Boston, MA, United States, [2]Boston Medical Center, Boston, MA, United States, [3]Division of Global Migration and Quarantine-Traveler's Health Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Data Coordinating Center, Boston University School of Public Health, Boston, MA, United States,[5]Mt. Auburn Hospital, Cambridge, MA, United States, [6]Harvard School of Public Health, Boston, MA, United States, [7]Beth Israel Deaconess Medical Center, Boston, MA, United States, [8]Lahey Medical Center, Burlington, MA, United States, [9]Center for Global Health and Development, Boston, MA, United States

International travel by US residents is increasing. Many health professionals are asked to provide pre-travel advice but may not be prepared to provide these specialized services. We designed an anonymous internet survey to assess knowledge, attitudes and practices of primary-care providers (PCP) and specialists who provide pre-travel consultations. The survey was sent to ~20,000 randomly selected PCPs in the Pri-Med Institute (now pmiCME) database and >3,000 US-based travel medicine specialists, identified from ISTM, ASTMH, and CDC yellow fever vaccine provider mailing lists. Of 14,932 e-mails sent to valid e-mail addresses, 902 yielded complete or partially completed surveys (6.0%). Respondents included 51% MDs, 25% NPs, 9.6% PAs, 8.5% RNs and 5.1% DOs. Identified specialties included Internal Medicine (30%), 32% Family Practice, 18% Primary Care, 9% Pediatrics, and 28% other. 87% were interested in attending a travel medicine course and 80% personally provided pre-travel consultations. 47% of the 625 respondents who provided pre-travel consultation saw fewer than 50 travelers/year, 15% saw 51-100, 26% 101-500, and only 12% >500/y. Familiarity with travel-specific vaccines (e.g. yellow fever, Japanese encephalitis) and provision of written educational materials increased as annual volume of travelers increased. More respondents who saw <30 travelers/y than those who see >500/y would prescribe chloroquine for malaria chemoprophylaxis for travel to sub-Saharan Africa (an inappropriate drug for this destination) (11% vs. 5.2%, p = 0.14). Those who saw <30/y were less likely to prescribe chloroquine (first-line drug) to travelers to malaria-endemic areas of Central America (36% vs. 81% who saw >500/y, p <0.001). When asked about *Campylobacter* antibiotic resistance in SE Asia, 32% of providers who saw <30/y incorrectly selected azithromycin resistance as a problem and 10% incorrectly thought antibiotic resistance was not an issue. Fewer providers who saw <30/y knew fluoroquinolone resistance was a problem (47% vs. 75% who saw >500/y, p <0.001). Many survey respondents provided pre-travel advice, but most saw very few travelers. Travel medicine knowledge and use of supplementary educational materials increased with volume of patients seen. Specific knowledge and practice deficits among study participants demonstrated a need for additional travel medicine education especially for those seeing fewer travelers.

499

CORRELATION OF BRAIN MR IMAGING AND CLINICAL MANIFESTATIONS OF EOSINOPHILIC MENINGITIS CAUSED BY *ANGIOSTRONGYLUS CANTONENSIS* IN SOUTHERN TAIWAN

Hung-Chin Tsai, Yu-Ting Tseng, Susan Shin-Jung Lee, Yao-Shen Chen

Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan

The most common cause of eosinophilic meningitis is the rat lungworm *Angiostrongylus cantonensis*, a parasite that is endemic in the southeast Asia and Pacific regions. Correlation of Brain Magnetic Resonance (MR)

imaging findings and clinical features are rarely reported in the literature. This study is aimed to analysis the Brain imaging features and clinical manifestations in patients with eosinophilic meningitis in southern Taiwan. This retrospective cohort study consisted of all of the patients diagnosed with eosinophilic meningitis at Kaohsiung Veterans General Hospital from December 1991 through Septemper 2009. The associations between laboratory parameters and brain MR imaging findings were analyzed by Mann-Whitney U test. Thirty-seven patients were diagnosed to have eosinophilic meningitis during a period of 18 years. Age ranged from 2 to 80 years. Most of the patients (35/37, 95%) were adults. The median incubation period was 10.5 days (range 3-80). Thirty percent of the patients complained of hyperesthesia. Patients who had hyperesthesia tended to have longer incubation period, low serum IgE levels and longer duration between onset of symptoms and spinal taps. Three patients presented with lymphocytic meningitis initially. Brain MR imaging was performed in 26 patients. Leptomeningeal enhancement was noted in 17 patients. Increased signal intensity at the subcortical white matter of bilateral cerebral or cerebellar hemisphere on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images was seen in 10 patients. Those patients who had leptomeningeal enhancement and increased signal intensity on T2-weighted and FLAIR imagings tended to have younger age and short incubation period. (Mann-Whitney U test, $p<0.05$). The presence of brain MR imaging abnormalities were not associated with timing between onset of symptoms and spinal tapping. In conclusion, hyperesthesia, rarely found in patients with bacterial and aseptic meningitis, is relative common in our patients. Patients who had younger age and short incubation period were more likely had leptomeningeal enhancement and increased signal intensity in Brain MR imagings. The possibility of eosinophilic meningitis can not be totally excluded despite absence of eosinophilia initially in the CSF. Detailed food intake history and laboratory tests are important to obtain the correct diagnosis.

500

TREATMENT OF STUBBORN SCALP, SKIN AND NAILS INFECTION OF FUNGAL AND BACTERIAL ORIGINS

Praise I. Oronsaye

University of Benin, Benin City, Nigeria

The purpose of this study, was to determine the efficacy of this new lotion in treating fungal and bacterial infection of scalp, skin and nails infection that have defiled remedies by known and commonly prescribed anti-fungal and anti-bacterial drugs by Physicians. These infections are common health problems of people in tropical and developing countries of the world. Two hundred and fifty patients with various scalp, skin and nails infections of fungi and bacterial organisms attending University of Benin Teaching hospital from January to December 2008 were randomly recruited into the study. They comprised of 150 females and 100 males whose ages range from children, adult and the elderly. The infections includes eczema, ringworm, scabies, witlow, sores and bruises. The composition of the lotion is as follows: salicylic 20% wt/vol, absolute ethanol 100 mls, glycerin 3% vol/vol. The salicylic was dissolved in the ethanol and glycerin was added, mixed thoroughly and allowed to stand for not less than 3 hours before application. Application was made using cotton wool bud on the affected sites only. Satisfactory clinical response was achieved within 3-7 days depending on severity of the infection. We present in this study, the dramatic effect of this lotion 'magic bullet' in treating scalp, skin and nail infection of fungal and bacteria origins.

501

DETERMINANTS OF ANTIBIOTICS PRESCRIPTION FOR SCHOOLCHILDREN AT ALLADA, SOUTH BENIN

Ghislain K. Koura[1], André Garcia, T. Beheton[1], Philippe Deloron[1], Michel Cot[2], Jean-François Faucher[3]

[1]Mère et enfant face aux infections tropicales, Paris, France, [2]Faculté de Pharmacie, Université Paris Descartes, Paris, France, [3]Hôpital Saint-Jacques, Service des maladies infectieuses et tropicales, Besançon, France

The objective of this study was to study the determinants of antibiotics prescriptions for schoolchildren by nurses. Data were collected during a prospective study on treatment of parasitologically-confirmed cases of malaria in four schools (Allomé, Centre, Dankoli, Dogoudo) of the district of Allada in the Republic of Benin. One thousand six hundred thirty children were included from February till June 2008. For each patient, sociodemographic characteristics, reasons for consultation, final diagnosis and therapeutic prescriptions were collected. A malaria rapid diagnosis test was used to screen for malaria diagnosis. Data were entered and validated with Epidata® software, and analyzed with STATA 10® software. Fever was the first reason for consultation (57 %), followed by digestive (27%) and respiratory (24%) symptoms and skin lesions (17%). A malaria diagnosis was confirmed in 61% of the children attending for fever. Antibiotic was prescribed for 40% of children (21% with confirmed malaria diagnosis and 57% with a non-malarial-fever). We found a significant association between an antibiotic prescription and a respiratory infection diagnosis (OR [IC 95 %]: 41.09 [24.34-69.33]), and to a lesser extent between an antibiotic prescription and a cutaneous infection diagnosis (OR [IC 95 %]: 5.78 [4.20-7.97]). In conclusion, the rational use of the antibiotics is a major challenge in poor resource countries. A better knowledge of the determinants of antibiotics prescription is critical in order to establish rules of this rational use of antibiotics. We found that, by far, the diagnosis of respiratory infection is the main factor associated with an antibiotic prescription. Was this finding firmly established, further clinical research studies would be needed in order to find the most appropriate ways of restricting antibiotics prescriptions for children who complain with respiratory symptoms.

502

IS THE *IN SITU* INFLAMMATORY PROFILE CORRELATED WITH THE CLINICAL PRESENTATION OF HUMAN SPOROTRICHOSIS?

Fernanda N. Morgado[1], Armando O. Schubach[2], Monica B. Barros[2], Marta A. Santiago[3], Marcela Costa-Santos[1], **Fatima Conceiçao-Silva**[1]

[1]Oswaldo Cruz Institute - Fiocruz, Rio de Janeiro, Brazil, [2]Evandro Chagas Institute - Fiocruz, Rio de Janeiro, Brazil, [3]BioManguinhos - Fiocruz, Rio de Janeiro, Brazil

Sporotrichosis, caused by the fungus *Spotothrix schenckii,* is a granulomatous skin disease whose most common clinical presentations are the lymphocutaneous (LC) and fixed cutaneous (F) forms. Although these forms are clinically well characterized, little is known about the immunopathological processes that determine the difference between clinical presentations. The study was developed in order to evaluate the composition of the in situ inflammatory reaction and to correlate it with clinical aspects. The composition of the *in situ* inflammatory process was analyzed by immunohistochemistry and clinical data were collected from two groups of patients with sporotrichosis: LC (n = 19) and F (n = 11). LC patients presented a larger number of lesions ($p = 0.001$), longer disease duration ($p = 0.026$) and longer duration of treatment ($p = 0.049$) when compared to F group. A greater fungal burden was also observed in LC lesions (LC: 0 to 6.5 and F: 0 to 1.5; $p = 0.021$). The percentage of neutrophils was significantly higher in LC lesions (median: 24.7%) than in F lesions (median: 6.7%) ($p = 0.002$), as well as the percentage of CD4+ cells (LC median: 40.9% and F median: 30.0%, $p = 0.001$) and CD22+ cells (LC median: 15.3% and F median: 2.9%, $p = 0.048$), and the intensity of NOS2 expression ($p = 0.009$). In conclusion, LC patients

presented more lesions, that were clinically more severe and presented a longer duration. The more marked inflammatory character of LC lesions was related to the larger number of neutrophils and CD4+ cells and to higher NOS2 expression, as well as the larger fungal load. The host-mediated immune response in sporotrichosis shows some peculiar characteristics of cellularity and inflammatory activity that might be determinant for progression of the different clinical forms.

503

BIBLIOMETRIC REVIEW FOR MALARIA IN PREGNANCY

Anna M. van Eijk, Jenny Hill, Sue Povall, Alison Reynolds, Helen Woods, Feiko Ter Kuile

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

The Malaria in Pregnancy (MiP) Library (http://www.update-software.com/Publications/Malaria/) is a bibliographic database of literature relating to malaria in pregnancy. The MiP Library was created in 2005 and is a product of the MiP Consortium. We conducted a bibliometric review of published and unpublished reports to obtain a better understanding of the available number and sources of information for malaria in pregnancy and changes over time. Every four months, the literature is screened online for new information using a standardized search protocol. The published literature includes journal articles, books, reports, academic theses, and policy guidelines. The unpublished literature includes registered studies (including trials), unpublished theses, meeting reports and other unconventional literature. There is no language restriction. By May 2009, the MiP Library contained 4373 entries, consisting of 3317 journal articles (75.9%), 348 reports (8.0%), 135 academic theses (3.1%), 97 books or book chapters (2.2%), and 355 conference proceedings (8.1%), 74 registered studies (1.7%) and 47 'other' (0.9%). Most of the sources were in English (87.2%), followed by French (7.2%) and Spanish (1.7%). About a third of the source material was publicly available on the internet (36.4%), and the remaining accessible with restricted access (37.5%) or not available (26.1%). The number of journal articles increased from 40 publications in the 1960s, to 699 in the 1990s, and to 1932 between 2000 and 2009; articles were sourced from 884 different journals. Among the journal articles published since 1959, the top 3 sources were the Am J Trop Med Hyg (194), Trans R Soc Trop Med Hyg (132), and the Malaria Journal (104), followed closely by J infect Dis (93), Lancet (74), and Trop Med Int Health (68). In conclusion, the last decade has seen a dramatic increase in publications related to malaria in pregnancy; an increasing proportion is now publically available through online sources. The MiP Library is an excellent scholarly source for literature and systematic reviews related to malaria in pregnancy.

504

LABORATORY SURVEILLANCE FOR THE POSSIBLE INFECTIOUS CAUSES OF UNDIFFERENTIATED FEBRILE ILLNESSES IN THE COUNTRY OF GEORGIA

Tinatin Kuchuloria[1], Danielle V. Clark[2], Tamar Akhvlediani[1], Alexander Nanuashvili[3], Manana Makhviladze[4], Marine Kanashvili[4], Tengiz Tsertsvadze[5], Marina Endeladze[5], Maiko Chokheli[6], Tamar Chikviladze[6], Marine Chubinidze[6], Brent House[7], Margaret Farrell[7], Mohamed Abdel Maksoud[7], Matthew J. Hepburn[8], Guillermo Pimentel[7]

[1]I. Javakhishvili Tbilisi State University; Technology Management Company, Tbilisi, Georgia, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Service of Antimicrobial Chemotherapy, Tbilisi, Georgia, [4]V. Bochorishvili Anti-Sepsis Center, Tbilisi, Georgia, [5]Infectious Diseases, AIDS and Clinical Immunology Research Center, Tbilisi, Georgia, [6]National Center for Disease Control and Public Health, Tbilisi, Georgia, [7]Global Disease Detection and Response Program, United States Naval Medical Research Unit No. 3, Cairo, Egypt, [8]United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, United States

Information on the relevant infectious causes of undifferentiated febrile illness (UFI) in a region is essential for effective treatment and prevention. Presumptive treatment with antibiotics is common in the Caucasus, where laboratory diagnostics are not systematically used for zoonotic and vector-borne infections. Laboratory based UFI surveillance was conducted at 5 hospitals in Georgia, starting in May, 2008. Patients ≥4 years of age with fever ≥38°C for ≥48 hours were considered for inclusion. Blood culture and serologic testing (ELISA) were conducted for leptospirosis, brucellosis, West Nile virus (WNV) infection, Crimean-Congo hemorrhagic fever (CCHF), Q fever, tick-borne encephalitis (TBE), hantavirus infection, typhoid fever, and rickettsial infections. To date, 309 subjects have been enrolled in the study. Enrolled subjects represent all but 2 regions of Georgia. Fever of unknown origin (FUO) was the preliminary diagnosis in 86% of patients. The median duration of fever was 15 days, with the maximum duration of 1000 days; gradual onset of fever was noted on 75% of the cases. The majority of patients reported antibiotic use prior to enrollment (71%) and 40% of patients reported self-medicating. Several *Streptococcus* and *Staphylococcus* species were isolated. Samples were positive by serology for brucellosis (7%), hantavirus infection (6.8%), leptospirosis (5.5%), Q fever (4.5%), typhus group rickettsial infection (4%), and TBE (1.2%). Currently 177 samples have been tested with CCHF and WNV IgM ELISA; only 1 CCHF-positive sample was recorded. In conclusion, clinical awareness and laboratory capacity are essential to diagnose infectious etiologies of febrile illnesses. As a result of this study, physicians and public health authorities will be informed of the relative frequency of the studied pathogens. Information from this ongoing study will be utilized to enhance clinical suspicion and focus efforts to develop diagnostic capacity and treatment options for these infections in the Caucasus.

505

FACS ANALYSIS OF KEY INNATE IMMUNE CELLS IN PERIPHERAL BLOOD OF DENGUE PATIENTS

Jih-Jin Tsai[1], Yen-Hua Jen[1], Jung-San Chang[1], Hui-Mien Hsiao[2], Guey Chuen Perng[2]

[1]Tropical Medicine Center, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, [2]Department of Pathology and Laboratory Medicine, Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA, United States

The importance of innate immune system in defense of host against pathogens has been well documented. As the first line of host defense system, timing becomes a critical step in order to have an efficient effect on the engagement with and controlling pathogens. Dengue is a timing acute disease and frequently dengue patients do not see for helps until 2-3 days of prodrome occurs. Thus, the role of innate immune system in dengue patients remains largely unexplored. Comprehensive FACS

profiling of key innate immune cells in peripheral blood of dengue patients varying in onset of fever was performed. Four FACS panels were used to evaluate NK cells, platelet-leukocyte aggregates, inflammatory monocytes, and plasmacytoid and myeloid dendritic cells, respectively. Serological results revealed that majority of dengue patients were primary dengue. FACS results showed the followings: i)NK cells, CD56+CD16+ or CD56+CD16-, were significantly dropped on the 5th day after onset of fever and were gradually resumed to normal within two weeks of illness. ii)Biphasic platelet-leukocyte aggregates was observed; reached to maximum levels on the 6th-8th days and on the 11th-16th days after onset of fever. The platelet-monocyte aggregates were the most frequent event. iii)Inflammatory monocytes, CD14+CD16+HLADR+, were consistent lower on 5th-8th days after onset of fever, and were gradually returned to normal level in the second week of illness. iv)Plasmacytoid dendritic cells, CD3-CD20-CD14-CD123+CD11c-, reached to the maximum on the 5th day after onset of fever, and were gradually declined to the baseline level after one week of illness. In contrast, myeloid dendritic cells, CD3-CD20-CD14-CD11c+CD123-, were somewhat fluctuated during the first week of illness, and thereafter returned to baseline level in the second week of illness. These results were the first phenotypically documented the key innate immune elements in peripheral blood of dengue patients. The most interest findings in current investigation was the biphasic platelet-leukocyte aggregates, in particular the platelet-monocyte aggregates. Perhaps these innate immunological parameters may be a crucial factor, which could dictate in understanding the complicated pathogenesis of dengue disease.

506

PREVALENCE OF ROTAVIRUS AMONG PATIENTS WITH ACUTE DIARRHEA IN DIFFERENT REGIONS OF UZBEKISTAN

Gulnara Ibadova[1], Adkham Mamatkulov[2], Gulnara Abdukhalilova[3], Ruslan Madyarov[3], Aybek Khodiev[2], Carl Mason[4], Ladaporn Bidhidatta[4]

[1]Tashkent Institute of Postgraduate Medical Education, Tashkent, Uzbekistan, [2]Technology Management Company, Tashkent, Uzbekistan, [3]Research Institute of Epidemiology, Microbiology and Infectious Diseases, Tashkent, Uzbekistan, [4]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

The rotavirus is one of the major causes of diarrhea. The purpose of study was to reveal prevalence and pattern of rotavirus diarrhea in different regions of Uzbekistan. 2450 stool samples from patients with acute diarrhea (AD) were collected from different regions of Uzbekistan. All samples were tested for bacterial diarrheagenic pathogens with culture on standard selective media and biochemical tests followed by serotyping. The rotavirus was detected with commercial ELISA following the instruction of commercial kit (IDEIA, DakoCytomation, UK). Rotavirus identified in 40.04% of samples (981 out of 2450 samples). The prevalence of rotavirus detection differed by regions. The high prevalence of rotavirus reveled in south regions of Uzbekistan (Kashkadarya and Surkhandarya regions and Tashkent city) - 43.1%, 44.69% and 43.13% accordingly. In Karakalpak republic and Khorezm region rotavirus identified in 37.88 and 33.71% accordingly. The difference of rotavirus identification revealed between inpatient and outpatient hospitals - 39.0% and 46.0% accordingly. Rotavirus was only identified pathogen in 25.35% of patients, the association of rotavirus and other diarrheagenic pathogens identified in 14.61% of patients. The most frequent association of rotavirus identified with the following pathogens (out of all associations): E. coli - 68 samples (19.0%), Citrobacter spp. - 65 samples (18.16%), Enterobacter spp. - 62 samples (17.32%), Salmonella typhimurim - 43 samples (12.01%) and Shigella spp. - 32 samples (8.94%). The significant seasonal prevalence of rotavirus diarrhea was not observed. In conclusion, 1) Rotavirus may be the cause of diarrhea in 40.0% of patients admitted with AD and has no seasonal prevalence; 2) rotavirus prevails in south regions of Uzbekistan; 3) rotavirus diarrhea more frequently found in outpatient oral rehydration facilities then in inpatients departments; 4) rotavirus as the only pathogen identified in 25.0% of AD cases and in 15.0% in association with other diarrheagenic pathogens.

507

EVALUATION OF FEVER IN AN INTERNATIONAL TRAVELER

Adetunji Adejumo[1], Abhijeet Nadkarni[2], Adenike Adeyinka[1], Shefiu Shittu[1], Olutayo Olabige[1], Cyrus Badshah[1]

[1]Harlem Hospital/Columbia University, New York, NY, United States, [2]Springs Memorial Hospital, Lancaster, SC, United States

The differential diagnosis of fever in an international traveler is broad and often times presents a diagnosis dilema for clinicians. Travel medicine has become an important part of medical practice in a world that is now a global village. The patient was a 57yrs old Caucasian man who returned 5days prior to presentation from a 1 week trip to New London, South Africa. He complained of fever-102ºF, severe headache, fatigue, lower extremity itching with erythematous rash and right groin swelling that started 1day before he left South Africa. He reported camping activity abroad and noticed ticks on his body. He denied mosquito bites or animal contacts and no sexual exposure. He had no diarhoea or dyusria. He had no improvement after course of keflex used upon arrival. On examination, he was febrile to 102ºF and stable. The right lower extremity skin had multiple erythematous patches measuring 1-1.5cm in diameter on the medial aspect of popliteal surface resembling eschars. There was right inguinal lymphadenopathy and no penile ulceration, discharge or scrotal swelling. Routine laboratory studies were normal. Based on the history and physical findings, a working diagnosis of African tick bite fever by *Rickettsia africae* was made. Rickettsia serology was sent to an outside laboratory for confirmation. He was commenced on doxycycline 100mg bid. At 1 week follow up, his fever has resolved and the lower extremity eschar was clearing. He completed 2weeks of therapy with complete resolution of the skin lesions and symptoms. An important emerging infectious disease, the incidence of Rickettsia infections worldwide is estimated at 5.6-11% in groups of travelers returning from Sub-Sahara Africa who developed acute febrile illness after returning from Africa. The causative agent of African tick-bite fever, is transmitted by *Amblyomma hebraeum* and *A. variegatum* ticks. These ticks are common in western, central, and southern Africa. Adults rarely feed on humans, although nymphs attach more. In conclusion, a high index of suspicion for African tick-bite fever is needed in persons who seek treatment with a history of tick bites and clinical signs of fever, headache, and multiple eschars after traveling to an endemic area.

508

ANTIBODIES IN ACTION: ROLE OF OPSONINS IN CLEARING *SALMONELLA* TYPHI IN HUMANS

Janet C. Lindow, Kelly A. Fimlaid, Janice Y. Bunn, Beth D. Kirkpatrick

University of Vermont, Burlington, VT, United States

Typhoid fever causes ~21 million illnesses and up to 600,000 deaths annually. The assessment of new typhoid vaccine efficacy is difficult, because no correlate of protection has been identified. We have developed several assays to test the function of *S.* Typhi-specific antibodies generated following vaccination with a next-generation typhoid vaccine M01ZH09 (*S.* Typhi Ty2 Δ*aroC* Δ*ssaV*) by Emergent Biosolutions. Using an opsonophagocytosis assay, we found that post-vaccination sera increases the uptake of wild type *S.* Typhi Ty2 by human macrophage-like cells (THP-1) up to 2.3-fold relative to pre-vaccination (day 0) or placebo serum samples (p=0.017). Addition of purified immunoglobulins from post-vaccination serum recapitulates opsonophagocytosis results demonstrating that antibodies are largely responsible for the phenotypes observed. Using microscopy, we verified that more bacteria are internalized by macrophages when opsonized with post-vaccination sera than with day 0 or placebos (2-9.5-fold more bacteria/phagocytosing macrophage). Most

importantly, we discovered that the survival of wild type *S.* Typhi, which generally replicates within human macrophages, is reduced up to 50% when opsonized with post-vaccination sera relative to day 0 or placebo serum samples (p=0.049). We also show that antibodies are generated which can be recognized by complement factors and be used to kill wt *S.* Typhi using a bactericidal assay. We show post-vaccination sera has significantly higher bactericidal antibody titers at day 7 (mean = 2972; p=0.031) and day 14 (mean = 5194, p=0.031) relative to day 0 (mean = 886) or placebo controls (mean = 625 all days). These assays are the first to assess the functionality of post-vaccination antibodies in the protection from typhoid infection. This work may lead to the identification of correlates of protection for typhoid fever and may help identify individuals most at risk of acquiring the disease.

509

EVALUATION OF THE TRADITIONAL AND REVISED WHO CLASSIFICATIONS OF DENGUE DISEASE SEVERITY

Federico Narvaez[1], Douglas Elizondo[2], Gamaliel Gutierrez[2], Maria Angeles Pérez[1], Katherine Standish[2], Andrea Nuñez[3], Angel Balmaseda[3], **Eva Harris**[4]

[1]Hospital Infantil Manuel Jesús de Rivera, Managua, Nicaragua, [2]Sustainable Sciences Institute, Managua, Nicaragua, [3]Departamento 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, a mosquito-borne viral illness, is a major public health problem worldwide and continues to increase in incidence. Infection with one of the four dengue virus serotypes leads to a range of outcomes, including subclinical infection, undifferentiated febrile illness, dengue fever (DF), life-threatening syndromes associated with fluid loss and hypotensive shock, or other severe manifestations such as bleeding and organ failure. The long-standing World Health Organization (WHO) dengue classification and management scheme has recently been revised, replacing the schema of DF, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) with dengue without warning signs, dengue with warning signs (intense abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, or hemoconcentration ≥20%) and severe dengue (SD; dengue with plasma leakage leading to respiratory distress, severe hemorrhaging, hypotensive shock or organ failure). We interpreted SD to include compensated shock based on the dengue case management algorithm in the 2009 WHO Guidelines. We evaluated the old and new classification schemes against clinical intervention levels to determine how each captures disease severity using data collected over five years (2005-2010) of a hospital-based study of pediatric dengue in Managua, Nicaragua. Laboratory-confirmed dengue cases (n=557) were categorized using both of the classification schemes, and by level of care (1-3). Level 1 was out-patient care, level 2 was in-patient care that did not meet criteria for level 3, which included intensive care defined by admission to ICU, hypotensive shock, ventilation, administration of inotropic drugs, or organ failure. We therefore tested the sensitivity and specificity of the new and old classifications for severe dengue to identify level 3 care. Sensitivity and specificity for DSS were 45.9% and 76.7%, respectively; sensitivity and specificity for SD were 99.1% and 67.3%, respectively. We are currently extending the analysis to include dengue-negative febrile illnesses, as the DHF/DSS classification is reported to be specific even without laboratory confirmation of dengue. Among dengue-confirmed cases, the new WHO classification for severe dengue appears to have higher sensitivity and specificity to identify cases in need of heightened care, although it is no longer specific for a particular pathogenic entity.

510

HIGH GENOMIC STABILITY OF CHIMERIC YELLOW FEVER/ DENGUE VACCINE STRAINS PRODUCED *IN VITRO*

Yves Girerd-Chambaz, Nathalie Mantel, Patrice Riou, Jean Lang, **Veronique Barban**

Sanofi pasteur, Marcy l'Etoile, France

Most RNA viruses exist as quasi-species in nature and are known to evolve mainly by accumulation of mutations in their genome. The 17D-204 YF attenuated strain is an exception among these viruses and exhibits a remarkable genomic stability in vitro and in vivo, probably due to the low error-rate of the viral RNA polymerase. This enzyme is also the enzyme that ensures viral replication of chimeric Yellow Fever/Dengue (CYD) vaccine viruses, and thus a high genomic stability of these viruses was expected.

The full genome sequence of the 4 CYD viruses was established at various passage levels, from laboratory lots, to GMP vaccine lots (phase 1, phase 2 and phase 3 lots). Sequencing was performed at each step of the manufacturing process from premaster seed lot (PMSL) to master seed lot (MSL), working seed lots (WSL), bulks lots (BL), and at a late step (BL10). Compared to PMSL, no nucleotide substitution was observed, for any serotype, in BL produced for phase 1, 2 and 3 studies despite a cell substrate change at phase 2 level (use of serum-free adapted Vero cells) and the production scale up. No nucleotide substitution was observed at BL10 of CYD1 and CYD2 phase 1 and 2 lots, compared to the corresponding sequence of the PMSL. Regarding CYD3, 3 nucleotide substitutions were detected. A silent mutation at NS5-73 (C>T) was observed in BL10 of phase 2 lots, and 2 amino-acid substitutions, one at NS4B-177 (L>F) and one at E302 (N>N/D) were detected in BL10 of phase I and 2 viruses, respectively The NS4B mutation was also observed at late non-GMP passages of CYD3, during clone selection of CYD2 candidate and at BL10 of phase 1 CYD4 virus, suggesting that it could be the result of adaptation to cell substrate. No mutation was detected at BL10 of phase 2 CYD4. Suckling mice neurovirulence assay was performed for non-conservative mutations, and no increase in neurovirulence was observed.

511

THROMBOCYTOPENIA IN DENGUE PATIENTS: THE EFFECTS OF BODY TEMPERATURE AND CIRCULATING CD41$^+$CD61$^+$ CELLS

Sansanee Noisakran[1], Nattawat Onlamoon[2], Kovit Pattanapanyasat[2], Hui-Mien Hsiao[1], Kulkanya Chokephaibulkit[3], Guey Chuen Perng[1]

[1]Emory University School of Medicine, Atlanta, GA, United States, [2]Office for Research and Development, Siriraj Hospital, Mahidol University, Bangkok, Thailand, [3]Department of Pediatrics, Siriraj Hospital, Mahidol University, Bangkok, Thailand

Dengue has been recognized as one of the most important vector-borne emerging infectious diseases. Severe form of dengue hemorrhagic fever and/or dengue shock syndrome may occur and if left untreated the death rate can be as high as 50%. Currently, there is no preventive vaccine or anti-viral treatment available. Thrombocytopenia and viremia are prominent clinical features in dengue patients. Prolonged fever is a unique feature in dengue patients and yet its association with circulating immune complexes found on the surface of platelets has not been systematically documented in dengue patients, thus factors leading to these clinical features remain elusive. Systematic investigations on the subject with hospitalized dengue patients were initiated. Daily body temperature (BT) and blood samples were measured and collected, respectively, from 36 dengue patients for 9 days. CBC, dengue virus antibodies, viral load (VL), blood smear, and PBMC were obtained. Morphology of and viral antigen in cells were evaluated on blood smears. High levels of VL and BT were observed at the early time points of specimen collection and

declined over time. Their kinetic patterns correlated well with decreasing numbers of platelets. The nadir of platelets count was noticed at the time that VL was undetectable and BT reached normal. Morphological studies revealed that cells with $CD41^{+}CD61^{+}$ surface marker were likely positive for viral antigen. Further investigation suggested that these cells possessed the characteristics of megakaryocytes. Our results imply that a) body temperature associated with circulating-immune complex may be responsible for the low platelets counts, and b)$CD41^{+}CD61^{+}$ cells with megakaryocytic characteristic feature may directly link to dengue viremia. In conclusion, perfect storm of synergy factors interacting with one another including direct infection of megakaryocytes by dengue virus and clearance of dengue virus containing platelets by immune-complex may account for the observed clinical features in dengue patients.

512

PHYLOGENETIC ANALYSIS OF AN ISOLATE OF DENGUE VIRUS TYPE 2 FROM GUATEMALA, 2009

German Añez[1], Caren J. Chancey[1], Andriyan Grinev[1], Maria E. Morales-Betoulle[2], Maria Rios[1]

[1]United States Food and Drug Administration, Bethesda, MD, United States, [2]Universidad del Valle de Guatemala, Guatemala City, Guatemala

Dengue virus (DENV) (genus Flavivirus, family Flaviviridae) is the most prevalent mosquito-borne virus worldwide. The four DENV serotypes (DENV-1 to -4) infect 50-100 million persons annually mostly in Southeast Asia, the Pacific and the Americas. DENV is asymptomatic in approximately 70% of infections, and its spectrum of disease can range from a mild flu-like fever to severe dengue characterized by hemorrhagic manifestations that can lead to shock and death. Dengue has become a public health problem not only in endemic areas but also in non-endemic countries including the US due to importation. Because of the lack of specific treatment or predictive biomarkers for progression to the severe form of the disease, in which survival depends on supportive care, molecular diagnostic techniques are of capital importance for early detection of DENV infections. Here we report the molecular analysis of an isolate obtained from a serologically confirmed case of uncomplicated dengue from Guatemala, collected during the 2009 dengue epidemic. Blood specimens from the febrile phase were made available to us for further investigation. The sample was subjected to quantitative real-time RT-PCR (qRT-PCR) and viral isolation in mosquito C6/36 cells. qRT-PCR results were positive for DENV-2 and the characteristic cytopathic effect of the virus was observed in infected C6/36 cells. Cell culture supernatant from C6/36 cells also tested positive for DENV-2 by qRT-PCR within the first week of culture. The whole genome of the isolate termed Gua09 was sequenced and identified by phylogenetic analysis as closely related to DENV-2 strains from Nicaragua and to belong to the American/Asian genotype of the virus, which has been circulating in Central America for several years now. To our knowledge this is the first report of a fully characterized DENV human isolate from Guatemala. Genetic characterization of DENV isolates are of relevance for the development of molecular diagnostic tools for early identification of infection and proper care of critical patients.

513

IDENTIFICATION OF NEUTRALIZING AND ENHANCING EPITOPES ON DENGUE VIRUS ENVELOPE PROTEIN

Kristen M. Kahle, Kimberly-Anne Mattia, Benjamin J. Doranz

Integral Molecular, Inc., Philadelphia, PA, United States

Characterizing the binding sites of monoclonal antibodies (MAbs) on target antigens, their 'epitopes', can aid in the discovery and development of new vaccines, therapeutics, and diagnostics. Integral Molecular has generated high-resolution epitope maps for human antibodies against the immunodominant envelope protein (prM/E) of Dengue virus (DENV). MAbs used in this study were derived from different immunogens (natural infections and vaccinations), different disease states (Dengue Fever and Dengue Hemorrhagic Fever), and different exposure times (primary and secondary infections). To obtain detailed MAb epitope maps at the resolution of individual amino acids, we developed a novel technology, Shotgun Mutagenesis Mapping. This approach offers the capability of mapping both linear and conformational epitopes, even for structurally complex proteins such as oligomeric Env proteins. A comprehensive mutation library for DENV serotype 3 prM/E protein was created in which *every* residue of the Env protein was individually mutated, expressed in human cells, and analyzed for its effect on antibody immunoreactivity. For each MAb tested, Shotgun Mutagenesis Mapping identified a comprehensive set of amino acids on DENV3 prM/E that are critical for antibody binding. These residues comprise an epitope map that can be visualized on the prM/E three dimensional protein structure. Our goal is to map epitopes on all four DENV Env serotype proteins, and to determine whether the epitopes are shared by different DENV serotypes, if they contribute to antibody-dependent enhancement of infection, and how they relate to the residues that are required for Env function. We expect that this approach will help define the full range of immunodominant structures on Dengue virus and identify novel enhancing and neutralizing antibody epitopes.

514

EVIDENCE FOR *IN VIVO* SUPPRESSION OF TLR-INDUCED INFLAMMATORY BUT NOT OF B-CELL ACTIVATION DURING DENGUE VIRUS INFECTION IN NON-HUMAN PRIMATES

Carlos A. Sariol[1], Yesseinia Anglero[2], Francheska Rivera[3], Petraleigh Pantoja[3], Idia V. Rodriguez[4], Luis Giavedoni[5], Vida Hodara[5], Jorge L. Munoz-Jordan[6], Teresa Arana[3], Laura J. White[7], Melween Martinez[3], Kristina Abel[8], Edmundo N. Kraiselburd[3]

[1]University of Puerto Rico, UMC-CPRC, Departments of Microbiology and Internal Medicine, San Juan, PR, United States, [2]University of Puerto Rico, San Juan, PR, United States, [3]University of Puerto Rico, UMC-CPRC, San Juan, PR, United States, [4]University of Puerto Rico, UMC, San Juan, PR, United States, [5]Department of Virology and Immunology, Southwest National Primate Research Center, Southwest Foundation for Biomedical Research, San Juan, TX, United States, [6]Molecular Virology and Surveillance Laboratory; Centers for Disease Control and Prevention, Dengue Branch, Puerto Rico, San Juan, PR, United States, [7]The Carolina Vaccine Institute, University of North Carolina, Chapel Hill, NC, United States, [8]Department of Microbiology and Immunology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Dengue is the second most important arthropod-borne disease after malaria, with 50-100 million cases of DF) and 500,000 cases of DHF/DSS each year. In recent years, key roles in determining the outcome of DENV infection have been assigned to the innate immune response, particularly to the PRR such as TLR 7/8 (TLRs) [6,7,8]; the RIG-I/MDA5 [9,10] and the IFN signaling pathways [9-11]. The role of the innate immune response, particularly of DC and of NK cells at early stages after the infection has become a focus of high interest and debate [16,17,18]. However, little is known about the effect of TLRs in the early innate immunological mechanisms that drive adaptative immune responses to dengue virus *in vivo*. On this work we sought to determine the role of such innate signals on the quality of the anti-DENV immune response *in vivo* in NHP. For this purpose animals were infected or mock infected with DENV-1 (WP-74 strain). One infected cohort was also stimulated with TLR 3 and 7/8 agonists and one cohort received TLR agonists only. TLR agonists abrogate the viremia peak at day 4 after the infection and induce a higher frequency of double positive CD40/CD86 mDC. Coincident we the activation of the mDC we found significant higher serum levels of CXCL-10 in the group receiving only the TLR agonists. On the other hand DENV was able to suppress or counteract the TLR-induced inflammatory effect by inhibiting the activation of mDC and controlling the scrum levels of CXCL-10 and IL-1Ra. Of note, the quantity of total specific anti-DENV antibodies was also significantly higher one month after infection in animals receiving TLR agonists. Finally the stimulation with TLR agonists modifies the quality of the anti-DENV B cells response by inhibiting the

IgG1 class switching and increasing the IgG1/IgG2 ratio. For first time we are showing, in NHP model, a quantitative and qualitative modification of the adaptative immune response by TLRs stimulation and activation of the innate immune mechanisms in the setting of an acute viral infection, particularly after DENV infection.

515

COEVOLUTIONARY IMPLICATIONS OF MOSQUITO CGMP-DEPENDENT KINASE AND MOSQUITO-BORNE FLAVIVIRUSES

Julie A. Keating, Dipankar Bhattacharya, Robert Striker

University of Wisconsin-Madison, Madison, WI, United States

Mosquitoes are efficient transmitters of flaviviruses but the molecular mechanisms required to adapt viruses to mosquito transmission are unknown. We have found that mammalian protein kinase G (PKG), a cGMP-dependent protein kinase, directly interacts with and phosphorylates multiple sites in mosquito-borne flaviviral NS5 proteins. PKG also alters insect behavior in Drosophila, honeybees, and ants by mediating phototactic behavior. PKG specifically phosphorylates mosquito-borne flaviviral NS5 at Thr449, but both tick-borne and mammalian-associated flaviviruses lack a phosphoacceptor at this site. Additional PKG phosphorylations occur in the N-terminal methyltransferase of NS5 from mosquito-borne flaviviruses. Interestingly, while PKG phosphorylates both *Aedes* and *Culex*-transmitted flaviviruses, the primary sequence of mosquito PKGs (especially in the regulatory domains) and viral phosphoacceptors differ between mosquito genera. The overall level of phosphorylation of the methyltransferase domain is higher in mosquito-borne than tick-borne flaviviruses. These phosphorylation differences may be linked to the evolution of flaviviruses in adapting to particular insect vectors. While phosphorylation of flaviviral NS5 by mammalian PKG has been identified, we have isolated *Ae. aegypti* PKG and are investigating if and where mosquito PKG phosphorylates NS5. Since PKG upregulation is associated with increased foraging in non-vector insects, we compared the flight activity of mosquitoes whose PKG was pharmacologically activated with control mosquitoes. We found a 3-fold increase in flight activity while maintaining a diurnal pattern, highlighting the importance of PKG in flaviviral insect vectors. Further studies examining PKG's role in flaviviral vector behavior are ongoing. Overall, PKG phosphorylates multiple sites in mosquito-borne flavivirus NS5. The role of PKG in cellular replication of flaviviruses and insect spread of mosquito-borne flaviviruses will be discussed.

516

CHOLESTEROL IS A RISK FACTOR FOR SEVERE DENGUE

Aubree Gordon[1], Crisanta Rocha[2], Federico Narvaez[2], Douglas Elizondo[3], Andrea Nuñez[4], Maria Angeles Pérez[2], Sheyla Silva[2], Angel Balmaseda[4], Eva Harris[1]

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

Dengue is a mosquito-borne illness that is a major public health worldwide. Clinical manifestations range from dengue fever (DF) to severe forms of the disease associated with hemorrhagic manifestations, vascular leak, and hypovolemic shock. To investigate the association between serum cholesterol levels and development of severe dengue in laboratory-confirmed dengue patients, we performed a prospective hospital-based study of children in Managua, Nicaragua. All children who presented to the National Pediatric Reference Hospital between August 2005 and February 2010 with suspected dengue were eligible to participate in the study. Demographic data and clinical history were collected at enrollment. Data on signs, symptoms, treatments, and laboratory results were collected systematically. Children were considered dengue-positive if dengue virus was detected by RT-PCR or virus isolation or if acute and convalescent sera demonstrated seroconversion by IgM ELISA or a $\geq$4-fold increase in dengue-specific antibodies. Dengue-positive patients were classified as DF, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) following 1997 WHO guidelines. Fasting blood samples for cholesterol measurements were collected each morning from all hospitalized participants. A total of 774 children participated, of which 557 (72%) were hospitalized. Of these, 184 children were dengue-negative, 235 were classified as DF, 93 as DHF, and 45 as DSS. Mean cholesterol levels on the first day of hospitalization were the highest in dengue-negative patients (101.5 mg/dL) and decreased as severity of illness increased; among dengue-positive patients, mean cholesterol levels were 95.7, 84.6, and 70.4 mg/dL, respectively for DF, DHF, and DSS patients. Significant differences ($p<0.001$) in serum cholesterol level were observed between all dengue disease severity categories as well as when comparing dengue-negative patients with all dengue-positive patients. Similar results were observed when comparing HDL cholesterol levels. In multivariable models, the odds of severe disease among dengue patients was 1.3 times higher per 10-unit drop in cholesterol (95% CI 1.2-1.4) when adjusted for day of presentation, age, and sex. Multivariable longitudinal analysis is currently underway to characterize lipid profile changes over the course of disease progression as well as to investigate the prognostic value of cholesterol testing.

517

PENTOXYFILLINE USE TO MODULATE TUMOR NECROSIS FACTOR IN CHILDREN WITH DENGUE HAEMORRHAGIC FEVER

Doris M. Salgado, Tatiana Zabaleta, Martha Rocio Vega, Carlos F. Narvaez, Jairo A. Rodriguez

Universidad Surcolombiana, Neiva, Colombia

Dengue is the main arthropod-borne viral infection in the world, severe forms become a challenge for the clinician because at present no specific treatment is available and there is an increase in the number cases, complications and mortality. The objective of this study was to establish the efficacy of pentoxyfilline for the modulation of the immune response in pediatric patients with dengue hemorrhagic fever. A prospective randomized double-blind study was developed from April to August 2009. A total of 55 patients with symptoms of dengue according to WHO criteria and serologic confirmation of dengue, were included and assigned into 2 groups who were treated with usual treatment, but in the first group Pentoxyfilline was included, whereas in the second was placebo. Complete clinical monitoring and TNFα serum levels were determined during 3 consecutive days. A statistically significant decrease of TNFα levels in dengue patients treated with pentoxyfilline was found ($p=0.02$), this result was more significant in patients classified clinically as having dengue III ($p = 0.003$). In conclusion, taking into account the role of TNFα in the pathophysiology of dengue, pentoxyfilline is suggested as a cost-effective therapeutic measure during the acute phase of severe dengue leading to reduction of complications and death.

518

VIRAL INTERFERENCE BETWEEN DENGUE-2 AND YELLOW FEVER VIRUSES IN VERO CELLS

Flávia A. Saiki, Emiliana P. Costa, **Benedito A. Fonseca**

School of Medicine of Ribeirão Preto, Ribeirão Preto, S.P., Brazil

Dengue is the most important disease caused by an arbovirus worldwide, especially in tropical and subtropical countries, while yellow fever is an important disease to parts of Africa and South America. Although a safe vaccine against yellow fever has been available for over 60 years, there has been an increase in the number of infected people since the beginning of the 1980s in several South American countries, including Brazil. Even though both viruses can be transmitted by the same vector (*Aedes aegypti*) and share some epidemiological features, such as prevalence in

areas where the vector population is abundant, the urban transmission of yellow fever virus hasn't been detected. The causes for this phenomenon are unclear and several hypotheses have been raised to explain this finding. The present study presents a new hypothesis and investigated a phenomenon known as viral interference, a situation where the infection by a particular virus prevents the infection of the same cells by a different virus. We studied the dynamics of both virus replication when dengue-2 virus (DENV-2) and yellow fever virus vaccine strain 17D (YFV-17D) infected the same culture of mammalian cells (Vero cells). We have investigated this phenomenon in *Aedes albopictus* cells (C6/36 cells) using DENV-2 and YFV-17D, and in that case, it was observed a decrease in YFV-17D replication in C6/36 cells previously infected with DENV-2. In this study, we investigated whether or not the same findings were observed in mammalian cells since the interaction between these two important arboviruses in different cell systems can contribute for the understanding of the different aspects related to biology and epidemiology of these two *Flaviviruses.* Our results show that Vero cells chronically infected with YFV-17D (evidenced after 3 days post-infection by RT-PCR) and infected with DENV-2 (YFV-17D-DENV-2) sustained an intense viral interference on the DENV-2. The same interference on the YFV-17D replication was observed when the cells were first infected with DENV-2 (DENV-2-YFV-17D). For both experiments, the MOI was 0.1. These results show the presence of viral interference between these two different *Flaviviruses* in eukaryotic cells and should help to understand the dengue and yellow fever pathophysiology during co-infections in human beings.

519

SEVERE LIVER INFECTION IN AN ADULT PATIENT WITH DENGUE HEMORRHAGIC FEVER: EVIDENCE FOR A BETTER USE OF THE NEW WHO DENGUE CASE CLASSIFICATION

Patrícia Rolim Mendonça, Rômulo Rebouças Lobo, **Benedito A. Fonseca**

School of Medicine of Ribeirão Preto, Ribeirão Preto, S.P., Brazil

Dengue clinical manifestations range from an acute febrile illness (dengue fever) to severe disease [dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)]. However, other distinct clinical manifestations have been described, such as central nervous system and liver involvement. Based on this plethora of clinical manifestations, the World Health Organization is suggesting the use of a new dengue case classification. To compare the two disease classification, we describe an unusual clinical course of a patient with DHF and presenting with severe liver dysfunction. A 56-year-old male patient was admitted to a university teaching hospital with a 5-day history of fever, headache, myalgia and malaise. He had no past medical history of note. One day before admission he reported epigastric pain, hematemesis, melena and bleeding gums. On admission, at physical examination he was afebrile, oriented, showed normal vital signs, and presented with a slight maculopapular rash, mild mucosal bleeding, and abdominal tenderness. Initial laboratory tests showed leukocytosis, thrombocytopenia, elevated liver enzymes (AST=6,234 U/L; ALT=5,127 U/L) and bilirubin levels, renal failure (creatinine: 3.9mg/dL) and decreased prothrombin activity. In the following days, the patient developed oliguria, drowsiness and episodes of gastrointestinal bleeding, requiring transfusion of blood components due to anemia and a bleeding disorder. A marked increase in liver enzymes developed with peak values of 16,950 U/L and 8740 U/L for AST and ALT, respectively. CPK was normal (132 units/L). An abdominal computed tomography showed mild ascites and splenomegaly. NS1 detection, IgG and IgM antibodies against dengue virus were positive. Improvement in liver function tests occurred gradually in the following days, but superimposed bacterial infection developed and the patient needed orotracheal intubation and vasoactive drugs. Hepatic involvement is a well known feature of dengue but it is usually mild, and liver dysfunction is only observed in the most severe cases. The mechanism of hepatic damage in dengue is poorly understood but viral infection of hepatocytes, induction of apoptosis and immune mediated hepatocyte injury are all possible answers. We present here a dengue case with a severe hepatitis and renal failure, which would complicate the previous WHO classification but it easily classified as severe dengue in the new classification.

520

PEDIATRIC DENGUE SURVEILLANCE IN COLOMBO, SRI LANKA: ANNUAL SERO-CONVERSION

Hasitha A. Tissera[1], Aravinda M. de Silva[2], A. Dharshan De Silva[3], Nihal Abeysinghe[4], Paba Palihawadana[5], Clarence Tam[1], Sunethra Gunasena[6], Ananda Amarasinghe[7], G. William Letson[7], Harold Margolis[7]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]University of North Carolina School of Medicine, Chapel Hill, NC, United States, [3]Genetech Research Institute, Colombo, Sri Lanka, [4]Ministry of Health, Colombo, Sri Lanka, [5]Epidemiology Unit Ministry of Health, Colombo, Sri Lanka, [6]Medical Research Institute, Colombo, Sri Lanka, [7]Pediatric Dengue Vaccine Initiative, Seoul, Republic of Korea

Dengue virus (DENV) infections have been reported in Sri Lanka for nearly 50 years. Cyclical dengue fever/ dengue hemorrhagic fever (DF/ DHF) epidemics have been a regular phenomenon after 1989. Recent epidemics were reportedly more severe. The worst ever epidemic was reported in 2009 with over 35,000 cases. We established a community-based, enhanced passive fever surveillance with a household enrolment model in Colombo, Sri Lanka to estimate burden of dengue infection in the pediatric population. The study is based in Colombo Municipal area which has a high reported annual caseload. A population census of the study area was conducted at the beginning. An age stratified sample of 800 children $\leq$ 12 years of age were selected proportional to the population of each census block to be followed up over a minimum of one year. During the initial recruitment, finger-prick blood samples were collected onto filter paper discs to determine baseline flavivirus sero-status. A repeat finger-prick sample was collected at 12 months to determine the annual sero-conversion rate. An in-house IgG assay was done in order to measure IgG levels in all baseline and one year follow-up samples. Baseline results indicate an overall flavivirus seroprevalence of 52%. Age stratified seroprevalence range from 14% among infants to 72% in children aged 12 years. A preliminary analysis of the first year results shows that 12% of children in the cohort were infected during the follow-up period. For every clinically apparent DENV infection there appears to be two asymptomatic infections. This is the first community based follow-up study in Sri Lanka to estimate burden of dengue infections among children. These results demonstrate the disease endemicity and intense transmission of DENV infection among children in this study area. The study results would be useful in strengthening prevention and control activities of dengue, including dengue vaccine introduction in future.

521

HUMAN DC-SIGN TRANSGENIC MICE AS A MODEL TO STUDY DENV-INDUCED HEMORRHAGE

Betty Wu-Hsieh, Yu-Ting Yen, Ta-Chun Lin, Feng-Chiang Wu, Shin- Yi Jou, Chia-Lin Lai

National Taiwan University College of Medicine, Taipei, Taiwan

In this study, we generated hDC-SIGN transgenic mice in C57BL/6 background. The hDC-SIGN gene was constructed under the polymerase II promoter, followed by a poly(A) tail and two insulators. By RT-qPCR, we showed that the transgenic mice expressed the hDC-SIGN gene in most of the tissues. Flow cytometric analysis showed that around 30-50% of monocytes and peritoneal macrophages expressed hDC-SIGN. Intradermal inoculation of dengue virus (DENV) strain 16681 induced hemorrhage development in hDC-SIGN transgenic mice and the transgenic mice were more susceptible to DENV-induced hemorrhage. To investigate the mechanism of how hDC-SIGN transgenic mice are more susceptible to DENV-induced hemorrhage, we found that macrophages from the transgenic mice produced significantly higher levels of TNF-α

after stimulation by DENV than macrophages from the wild type mice. Furthermore, treatment with neutralizing antibody against TNF-α significantly reduced the incidence and the severity of hemorrhage in transgenic mice. These results together indicate that greater susceptibility of hDC-SIGN transgenic mice to DENV-induced hemorrhage is due to higher TNF-α production by the macrophages in the transgenic mice. The hDC-SIGN transgenic mice will be very useful for testing new drugs and vaccines against DENV.

522

SURVEILLANCE OF DENGUE VIRUS IN FIELD-CAUGHT *AEDES AEGYPTI* FROM THE FLORIDA KEYS BY REAL-TIME RT-PCR

Catherine A. Pruszynski

Florida Keys Mosquito Control, Key West, FL, United States

Dengue Fever and Dengue Hemorrhagic Fever infect as many as 100 million people yearly, and is a significant cause of illness and death in the tropics and subtropics. Though dengue is endemic to South and Central America as well as the Caribbean islands, it rarely occurs in the United States. However, in Fall of 2009, the Florida Department of Health confirmed 22 cases of locally-acquired dengue in Key West, Florida. The prevention and control of dengue relies on the surveillance of circulating virus in the human population and its vector *Aedes aegypti*. Due to the asymptomatic nature of the disease and the high number of tourists traveling throughout the Florida Keys, human serological data are difficult to obtain. A more consistent method for surveillance is the detection of virus RNA in field-caught *Aedes aegypti* mosquito pools. From June through October 2010, mosquito pools were collected from BG Sentinel traps throughout the Florida Keys and assayed by real-time reverse transcriptase polymerase chain reaction for dengue viral RNA. Positive pools were confirmed by the Florida Department of Health Laboratory in Tampa, FL. Results indicate that Key West has the highest prevalence of circulating dengue in *Ae. aegypti* mosquitoes compared to those trapped in Marathon and Key Largo. Vector surveillance through real-time RT-PCR remains a reliable method for dengue detection, and is necessary in order to implement immediate and effective control strategies.

523

COMPARISON OF NEUTRALIZING AND ENHANCING TITERS OF PATIENT AND VACCINEE SERA USING A HIGH-THROUGHPUT DENGUE REPORTER VIRUS DETECTION SYSTEM

Kimberly-Anne Mattia, Elizabeth Christian, Bridget A. Puffer, Benjamin J. Doranz

Integral Molecular, Inc., Philadelphia, PA, United States

The lack of reliable, high-throughput tools for characterizing anti-Dengue virus (DENV) antibodies in large numbers of serum samples has been an obstacle in understanding the impact of neutralizing and enhancing antibodies on disease progression. DENV reporter virus particles (RVPs) have been developed to facilitate the genetic manipulation and biological characterization of DENV virions. RVPs are produced by combining a subgenomic replicon encoding an optical reporter with structural components from each of the four defined serotypes of DENV. RVP infection is monitored by expression of the reporter gene using standard optical detection platforms. RVPs are antigenically equivalent to wild-type virions but lack the viral machinery required for a productive infection, making them a safe reagent to rapidly assess humoral immune responses to all four serotypes. In this study, we demonstrate the diagnostic utility of DENV RVPs for characterizing antibodies in human serum samples. RVPs expressing either GFP or luciferase reporters were tested for optimal detection of infection, serotype-specific neutralization, and enhancement of infection. We assessed the suitability of RVPs for long-term, large-scale studies by optimizing storage conditions, and testing the reproducibility of infection in different cell lines. Neutralization titers obtained using RVPs were statistically identical to those derived using the plaque reduction neutralization test (PRNT). Finally, RVPs were used to identify and score sera from individuals vaccinated with live attenuated DENV and from patients naturally exposed to DENV. Comparison of sera neutralization and enhancement allows for a more complete antibody profile, documenting the potentially protective and pathogenic humoral immune response against each serotype of DENV within each patient. These experiments validate DENV RVPs as a high-throughput reagent for measuring neutralizing and enhancing antibody responses, and present a novel tool for understanding the effects of natural infection and vaccination on large patient populations.

524

LONG-TERM DOMINANCE OF DENGUE VIRUS TYPE II PUERTO RICAN LINEAGES THROUGHOUT MAJOR EPIDEMIOLOGICAL CHANGES

Gilberto A. Santiago[1], Kate L. McElroy[1], Niall J. Lennon[2], Bruce W. Birren[2], Matthew R. Henn[2], Jorge L. Muñoz-Jordán[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]Broad Institute of MIT and Harvard, Cambridge, MA, United States

Previous studies of dengue virus serotype 2 (DENV-2) Asian/American genotype in Puerto Rico had documented positive selection, rare introductions and a clade replacement prior to its drastic decline in the early 2000's. The virus re-emerged in 2003 and has become the only of the four DENV serotypes to have been uninterruptedly transmitted on the island since the early 1980's. We re-examine the evolution of the autochthonous, Puerto Rican DENV-2 over a period of 22 years by conducting full genome sequencing of an expanded sampling of 160 genomes, including 53 from the 2002-2007 period covering the decline and re-emergence of DENV-2. Although our phylogenetic analysis confirms lineage turn-over events, we show that DENV-2 evolves through a strong, negative, purifying selection, and document new findings of epidemiological importance. Puerto Rican DENV-2 lineages are defined by temporal and geographical associations and the virus persisted in a restricted circumscription through 3 years of extreme paucity, to then re-emerge and disperse across the island. We also re-examine the role of re-introductions in the epidemiology of DENV-2 in Puerto Rico and find that foreign strains are frequently but transiently transmitted across periods of high DENV-2 dominance; but rarely displace the autochthonous virus. All together, our analyses indicate superior fitness of the Puerto Rican DENV-2 lineages, which may explain its long term endurance despite great epidemiological changes.

525

IMPLEMENTING A SEXUAL ASSAULT TRAINING FOR NURSES AT JFK MEMORIAL CENTER IN MONROVIA, LIBERIA

Abiola A. Fasina

Mount Sinai School of Medicine, New York, NY, United States

Liberia's first civil war lasted from 1989 till 1997. Violence erupted again in the second civil war from 1999 till 2003. Qualitative data from these periods indicates high rates of sexual assault, coercion and rape. The United Nations Mission now maintains peace in Liberia but women are still experiencing the impact of gender based violence (GBV) from during and after the war and there are few resources to care for survivors. At present, Medecins Sans Frontieres (MSF) is providing services but is planning to leave Liberia in June, 2010. The purpose of this project was to train health care providers at JFK Memorial Center (JFK-MC) in the capital city of Monrovia to provide care for survivors of GBV when MSF leaves. Adapting a training tool developed by the International Rescue Committee (IRC), a curriculum was developed for nurses who would be caring for survivors of GBV. The purpose was to familiarize the nurses with international standards and appropriate referrals for survivors. This 3 day training consisted of case based scenarios, review of clinical management, role play and a DVD reviewing the various components of the exam with actors demonstrating key points. A post training survey was administered

to participants. Nurses were selected by senior nursing management from the departments of obstetrics, emergency medicine, internal medicine and the trauma service. The project was reviewed and approved by the hospital's general administrator who functions as the chief managing officer. 15 nurses participated in the training. The proportion of nurses who were 'very comfortable' providing medical care for survivors of sexual assault increased from 14% to 93% as a result of the training. Paired T-test (SPSS 17) indicated that this change was significant ($p < 0.01$). The proportion that felt very comfortable with counseling and referral increased from 29% to 71%. This change was also significant by paired T-test (SPSS 17, $p < 0.05$). 93% of participants rated the training as very good or satisfactory. In conclusion, training programs can improve knowledge and ability to care for survivors of GBV.

526

DIARRHEA MORTALITY IN CHILDREN AGED 5 TO 14 YEARS IN INDIA

Shaun K. Morris[1], Diego G. Bassani[2], Shally Awasthi[3], Rajesh Kumar[4], Prabhat Jha[2], for the MDS Collaborators[5]

[1]The Hospital For Sick Children, Toronto, ON, Canada, [2]Centre for Global Health Research, Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, ON, Canada, [3]Department of Pediatrics, King George's Medical University, Lucknow, India, [4]School of Public Health, Postgraduate Institute of Medical Education and Research, Chandigarh, India, [5]India

Diarrheal diseases are a leading cause of death in children under the age of 5. However, very little is known about diarrhea mortality in children over the age of 5. Our objective is to provide the first nationally representative estimate of diarrheal mortality in Indian children aged 5 to 14 years and to understand the distribution of these deaths based on geographic region, age, and gender. This study uses data from the verbal autopsy based Million Death Study (MDS). The MDS surveyed 6.3 million people in 1.1 million nationally representative Indian households for vital status between 2001 and 2003. Diarrheal deaths were defined ICD-10 codes A00 and A02-A09. Estimates of number of deaths were obtained by applying the proportion of deaths by gender, age, and state caused by diarrhea from the MDS to the corresponding UN Population division derived death envelopes for India in 2005. We estimate there are approximately 45,000 annual deaths due to diarrhea in children aged 5 to 14 years in India. The mortality rate is approximately 35% higher for girls than in boys for both age groups 5 to 9 years and 10 to 14 years. There were significant differences by region; the diarrhea mortality rate in children older than 5 ranged from a high of 39.3 per 100,000 in the Northeast to a low of 3.6 per 100,000 in the South. In conclusion, there are no existing estimates for the burden of diarrhea mortality in older Indian children, however, our estimate of 45,000 annual deaths is significantly larger than the Global Burden of Disease estimate of approximately 1,000 deaths in children aged 5 to 14 years in all of South Asia. Additional research is needed to better understand the etiologic causes of these illnesses as well as underlying risk factors for death. The large gender and geographic differences in mortality rates suggest the potential for significant numbers of lives saved though strengthening health education and access to health services.

527

HEALTH PROBLEMS AMONG JAPANESE EMBASSY PERSONNEL IN HANOI, VIETNAM

Yasutaka Mizuno[1], Thi Bach Duong Hoan[2]

[1]National Center for Global Health and Medicine, Tokyo, Japan, [2]Embassy of Japan in Vietnam, Hanoi, Vietnam

The number of Japanese expatriates has increased in recent years through internationalization. According to data from the Ministry of Foreign Affairs of Japan, there were 106 million Japanese expatriates in the world in 2006. Although many studies about health problems among short-term travelers have been conducted, relatively few reports exist on expatriates who reside for extended periods of time in developing countries. The objective of this study is to evaluate health problems among Japanese expatriates in Vietnam, and to clarify whether there were differences in the illnesses reported by Japanese expatriates and local people who were examined at the medical division of the Japanese embassy in Vietnam. Data on embassy personnel, their dependents, and local employees with any health problems, who visited the medical division in the embassy from May 2007 to April 2008, were retrospectively collected. The patients were examined by a medical attache in order to make a clinical diagnosis. According to these reported diagnoses, we evaluated the difference of health problems between Japanese and Vietnamese personnel. A total of 696 patients visited the medical division for the purpose of medical consultation, laboratory examination, and vaccinations and because of health problems. Of these, 421 (60.5%) were Japanese. The mean age was 33.95 ± 12.11 years. The remaining 275 (39.5%) patients were Vietnamese. The mean age was 39.56 ± 7.98 years. The most frequent purpose of visit was due to respiratory problems (n=188), followed by vaccinations (n=111), and due to gastro-intestinal (G-I) problems (n=96). G-I and eye problems were more frequently seen in Japanese, whereas genital, orthopedic, and skin problems were more frequently seen in Vietnamese. In conclusion, although health problems among Japanese expatriates seem similar to short term travelers, some illnesses are possibly related to the local environmental situation. Additionally, there were slight differences of illnesses between Japanese expatriates and Vietnamese local residents. These results indicate that differences in health problems might have been due to culture and lifestyle differences. Thus, in order to provide appropriate expatriate health care, travel health practitioners should consider not only disease prevalence but also the environmental and cultural situation of a country.

528

WHAT DOES IT COST TO ASSESS TRACHOMA PREVALENCE?

Chaoqun Chen[1], Elizabeth A. Cromwell[2], Paul M. Emerson[2], Jonathan D. King[2]

[1]Georgia Institute of Technology, Atlanta, GA, United States, [2]The Carter Center, Atlanta, GA, United States

Trachoma prevalence surveys are used to map the disease, for planning trachoma control program implementation, and to assess program impact. Many national programs have not conducted trachoma prevalence surveys because of the perception that they are too expensive. We analyzed the cost and the factors that influence cost the most (cost drivers) for 193 surveys from seven national programs.

Actual field costs were collected from the national programs and the country offices of the implementing agencies for trachoma prevalence surveys conducted from 2006 to 2010. Surveys included in this analysis used a cluster random sampling design with15-24 clusters surveyed per domain. Data were reviewed for accuracy and checked against financial reporting records. Costs were converted to USD using the three year average exchange rate. The median cost per district (domain) surveyed was $4,738 (Inter Quartile Range [IQR]: $3,315-$5,898) and the median cost per cluster was $291 (IQR: $110-$356). The main cost drivers were: field work (per diem and accommodation for the survey team) which accounted for 41.3% of all costs; transport (driver per diem, fuel and associated costs of running a program vehicle, and vehicle rentals), 18.9%; and data entry, 10.1%. These data can be used to project future prevalence survey costs in areas not yet mapped for trachoma.

529

IMMUNE SYSTEM DEVELOPMENT DURING EARLY CHILDHOOD IN TROPICAL LATIN AMERICA: EVIDENCE FOR THE AGE-DEPENDENT DOWN REGULATION OF THE INNATE IMMUNE RESPONSE

Philip Cooper[1], Rommy Teran[1], Maritza Vaca[1], Silvia Erazo[1], Gisela Oviedo[1], Martha Chico[1], Marc Huebner[2], Quentin Bickle[3], Laura C. Rodrigues[3], Edward Mitre[2]

[1]Laboratorio de Investigaciones FEPIS, Quito, Ecuador, [2]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [3]London School of Hygiene and Tropical Medicine, London, United Kingdom

There are important differences in the prevalence of inflammatory diseases such as asthma between populations living in urban and rural areas of tropical Latin America (LA). The immune response that develops in early childhood is considered to underlie the development of such diseases but there are few data. The present study investigated the effects of age and environment (urban vs. rural) on the development of immunity during the first 5 years of life in tropical LA. A cross-sectional study was conducted of Afro-Ecuadorian children aged 6-9 months, 22-26 months, and 48-60 months living in urban and rural Esmeraldas Province in Ecuador. Data was collected by parental questionnaire and blood samples were collected to measure innate and adaptive immunity. Data obtained clearly demonstrated that the immune system is actively developing throughout the first five years of life as frequencies of naïve CD4+ T cells declined with age while those of memory CD4+ and CD8+ T cells increased. Infants aged 6-9 months had evidence of hyper-reactive innate immune responses to TLR agonists compared to older children. Regulatory responses including T-cell production of IL-10 and frequencies of FoxP3+ T-regulatory cells decreased with age. No substantial effects of environment were observed on these innate and adaptive immune responses. These results suggest that innate immune responses decline with age during early childhood in tropical LA in parallel with declines in regulatory responses. Enhanced innate immunity in early life may be important for host defense against pathogens but may increase the risk of immunopathology.

530

ONE HEALTH INITIATIVE: A FOCUS FOR CROSS-SPECIES MEDICINE

Jack Woodall, Thomas P. Monath, Laura H. Kahn, Bruce Kaplan

One Health Initiative, Sarasota, FL, United States

The One Health Initiative is an internet forum promoting co-equal, all inclusive collaboration between physicians, veterinarians and professionals in health related disciplines, endorsed by major medical organizations and health agencies, including the American Medical Association, American Veterinary Medical Association, American Society of Tropical Medicine and Hygiene, U.S. Centers for Disease Control and Prevention (CDC), Association of American Medical Colleges, Association of American Veterinary Medical Colleges, Association of Schools of Public Health, American Society for Microbiology and many others. More than 500 prominent scientists, physicians and veterinarians worldwide have endorsed the initiative. The One Health concept is a worldwide strategy for expanding interdisciplinary collaboration and communication in all aspects of health care for humans and animals. The synergism achieved will advance health care for the 21st century and beyond by accelerating biomedical research discoveries, enhancing public health efficacy, expeditiously expanding the scientific knowledge base, and improving medical education and clinical care. When implemented it will protect and save untold millions of lives in our present and future generations. Recognizing that human and animal health are inextricably linked, One Health seeks to promote, improve, and protect the health and well-being of all species by enhancing cooperation and collaboration between physicians, veterinarians and other scientific health professionals, and by promoting strong leadership and management to achieve these goals. The vision of the One Health Initiative is to serve the medical, veterinary medical, nursing, public health and environmental health communities by providing a forum for the exchange of One Health research findings and innovative ideas in education, clinical care and public health. The ultimate goal is to improve the health of all species including humans, domestic animals, wildlife and plants. The authors are independent of any other entity or organization; we support and augment efforts of other organizations to recognize, promote and implement One Health. This autonomous endeavor is sustained *pro bono* due to our firm conviction regarding the enormous value of the "One Health/One Medicine" concept.

531

MEASURING CHILD PSYCHOSOCIAL STIMULATION BY CAREGIVERS IN THE DOMINICAN REPUBLIC

Jessica E. Sjoblom, John D. McLennan

University of Calgary, Calgary, AB, Canada

Child psychosocial stimulation by caregivers during the early years has an important role in the development of children. However, there are few reports on these practices from low-and middle-income countries (LMICs) and few feasible instruments to measure these practices. This study (i) describes caregiver psychosocial stimulation practices in a peri-urban community in the Dominican Republic, (ii) examines socio-demographic predictors of these practices, and (iii) determines the test-retest reliability of the Family Care Indicators (FCI), a tool to measure psychosocial stimulation. Caregivers of young children (n=220) living in a peri-urban district on the outskirts of Santo Domingo, Dominican Republic participated in a structured interview. The interview included items from the FCI and additional questions regarding play materials and caregiver engagement with children in play activities. Socio-demographic predictors were examined using regression analysis. Test-retest reliability of FCI items between baseline and follow-up interviews was determined. Typical play materials of children were store bought and both play materials and activities of children usually involved pretend play and movement. Socio-demographic predictors of practices included child and caregiver age, number of children of the caregiver, and caregiver's level of educational attainment. Test-retest reliability of FCI items ranged from moderate to good. In conclusion, in order to increase the reliability of the FCI, some items may need to be reworded to reduce ambiguity. Inclusion of items on pretend play may improve the scope of the instrument to better capture a more diverse range of important psychosocial stimulation practices. More research is necessary to determine the utility of the FCI, or a modified FCI, in quantifying stimulation practices cross culturally.

532

IN SEARCH OF COMPREHENSIVE HEALTH CARE: BIOMEDICINE AND BEYOND

Catherine M. Sebuguzi[1], Florence Nankya[1], Joaniter Nankabirwa[1], James Kizito[1], Christine Nabirye[1], Clare Chandler[2], Sarah G. Staedke[2]

[1]Infectious Disease Research Collaboraton, Kampala, Uganda, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom

Formal health facilities, focusing on delivery of biomedical treatment, are one of many sources of care for febrile patients in sub-Saharan Africa. To increase prompt access to appropriate biomedical treatment, rationales behind current treatment-seeking must be understood, and any gaps in the services offered by the formal sector must be considered as areas for improvement. We carried out a study of community perceptions, preferences and experiences in treatment seeking in Tororo district, Eastern Uganda. We carried out 10 focus group discussions with community members, including primary care givers (n=5) and household heads (n=5). We triangulated the findings with in-depth interviews with 100 community medicine distributors who acted as key informants with insight into treatment-seeking patterns.

In this area, first-line treatment for most conditions was with a biomedical drug. Sources of these drugs included government-run health centers, private clinics and drug shops. Nearly all community members had visited their local health centre, but dissatisfaction with experiences there was high. Herbal medicine was frequently used and interestingly, community members also relied heavily on shrines, churches and prayers for treatment. Choice of health care was influenced by the following factors: (1) initial perceptions and beliefs about etiology and severity of the illness that would, from experience, require a particular source of treatment. Often, experience showed health centers to be a poorer source of care than other providers for common illnesses; (2) accessibility of the preferred treatment, which relied on distance to the provider as well as opening hours, spousal support in meeting costs, opportunity costs of leaving the home and travelling to the provider, ability to negotiate the logistical and social rules of the provider's institution, and availability of treatment at that provider; and (3) trial and error in moving between treatment sources.

Our results suggest that care from health facilities frequently does not meet patients' expectations. Biomedical drugs were valued as a first port of call, but the wider process of care at health centers was unsatisfactory, leading patients to seek care from alternative, non-medical sources. Interventions designed to improve health care delivery need to attend to the wider needs of patients beyond the biomedical paradigm of pharmaceutical treatment.

533

MAPPING THE GLOBAL DISTRIBUTION OF TRACHOMA: AN UPDATED ATLAS

Jennifer Smith[1], Simon Brooker[1], Danny Haddad[2], Sarah Polack[1], Pamela Hooper[2], Emma Harding-Esch[1], Robin Bailey[1], David Mabey[1]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]International Trachoma Initiative, Task Force for Global Health, Atlanta, GA, United States

With the aim of eliminating trachoma as a cause of blindness by the year 2020, it is increasingly important to finely target and coordinate strategies to control this focal disease. A geographically targeted approach relies on the availability of reliable and updated maps of trachoma prevalence. Although the number of surveys conducted has increased in recent year, the data are rarely consolidated or presented in a form easily accessible to policy makers, programme managers and potential donors. A previous trachoma atlas developed in 2005 by the London School of Hygiene and Tropical Medicine collated information at a district level, and there is now a need to update this atlas and allow for changing administrative boundaries. We describe the search strategy and assembly methods for development of an updated, open-access, *Global Atlas of Trachoma*. Estimates of active trachoma and trichiasis were disaggregated to the village level to enable detailed mapping of disease distribution and a flexible approach in calculating prevalence estimates. Details of survey population, diagnostic methods, sample size and numbers examined and infected were abstracted into a single database, and all surveyed locations assigned to a specific longitude and latitude using standardized geolocation procedures. Population coverage of different SAFE interventions was included in a linked database. Based on information assembled in the updated atlas, we quantify the current geographical distribution of trachoma and evaluate progress in control. Information sources date from 1985 and include surveys conducted by ministries of health and NGO partners, as well as academic research studies. Currently, 46 countries have district-level data included and follow-up is ongoing for disaggregated data. Three countries have nationwide surveys and an additional 13 have substantial geographic coverage of endemic areas. The majority of data are from population based surveys (70%), but rapid assessment (20%) and other sampling methodologies are also represented. It is envisioned that these maps will have important applications in quantifying the global burden of trachoma and targeting of future control efforts.

534

PRICE MARK-UPS AND PRICING DETERMINANTS OF ARTEMISININ-COMBINATION THERAPY (ACT) IN THE PRIVATE COMMERCIAL SECTOR DISTRIBUTION CHAIN FOR ANTIMALARIAL DRUGS IN CAMBODIA

Edith Patouillard[1], Benjamin Palafox[1], Sarah Tougher[1], Sochea Phok[2], Duong Socheat[3], Kate OConnell[4], Immo Kleinschmidt[1], Kara Hanson[1], Catherine Goodman[1]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]Population Services International, Phnom Penh, Cambodia, [3]National Centre of Entomology, Parasitological and Malaria Control, Phnom Penh, Cambodia, [4]Population Services International, Nairobi, Kenya

In many developing countries, the private commercial sector is an important provider of malaria treatment. To design effective interventions for improved malaria control, there is a need to understand retailers' behaviour and identify the factors influencing their stocking and pricing decisions. Retailers are the last link in a chain of wholesalers and retail outcomes are likely to be influenced by what happens further up the chain. However, little is known about the retail sector distribution chain and its influence on the availability and prices of antimalarials that consumers can access. This study is part of ACTwatch, a multi-country project that aims to provide evidence to policymakers on the use, availability and prices of quality malaria treatment. In Cambodia, nationally representative samples of retailers and wholesalers selling antimalarials were surveyed: 1127 structured interviews and 27 in-depth interviews with providers operating at different levels of the distribution chain collected data on providers' characteristics, and stocking and pricing behaviour for antimalarials. Data collection took place during the malaria transmission risk season between June and November 2009. Median percentage mark-ups on ACT ranged at retail level between 33.5% in village shops and 50.0% in drug shops, and at wholesale level between 39.9% at the level supplying retailers and 39% at that supplying higher levels of the distribution chain. In absolute terms, median mark-ups on one adult equivalent treatment dose amounted to US$0.50 in village shops, US$0.68 in drug shops, US$0.18 at the level supplying retailers and US$0.14 at the level supplying higher levels. Whilst there was no evidence of a difference in percentage mark-ups for ACT between retail and wholesale levels ($p= 0.882$), there was strong evidence that absolute mark-ups at retail level exceeded mark-ups at wholesale levels ($p<0.0001$). Findings will also be presented on the influence on ACT pricing of structural aspects of the distribution chain and relationships between providers.

535

A GLOBAL MAP OF RESEARCH AND DEVELOPMENT FOR MALARIA - WHO IS DOING THE WORK?

Mary Moran, Javier Guzman, Alina McDonald, Klara Henderson

The George Institute for International Health, Sydney NSW, Australia

Over $US 1 billion was spent on research and development (R&D) of new malaria products in 2007 and 2008, but there is limited information on how funds were invested. We have therefore analysed these malaria funding recipients by organisation type, location and type of research. Unpublished data from the G-FINDER survey has allowed us to create a map of the malaria R&D workforce. Seventy percent (US$760M) of 2007 and 2008 malaria R&D funding was disbursed to over 250 recipients including public researchers, small biotechnology companies (SMEs) and multinational pharmaceutical companies (MNCs) in more than 30 countries, half being developing countries. Twenty percent (US$250M) was disbursed to Product Development Partnerships (PDPs) and other intermediaries. Over two thirds was received by organisations in the USA (32%), UK (21%) and Switzerland (15%). If funding to PDPs and intermediaries is excluded - since this is mostly disbursed on to third organisations - organisations based in the USA and UK still received nearly half of all funding (22% and 20%). Basic research received almost 25%

of funds, and product development around 40%. Public institutions represented 75% of basic research funding; discovery and preclinical research were conducted by MNCs (36%), public institutions (33%), PDPs (12%) and SMEs (9%); while PDPs and MNCs led clinical development (around 40% each). Public groups were previously primarily responsible for discovering new malaria product leads, taking these through to Phase II. Government incentives then sought (largely unsuccessfully) to encourage industry to take leads through clinical development to registration and large-scale manufacture. Today, while public groups continue to generate vital basic research, it is companies and PDPs who are primarily responsible for discovering leads and taking these through early clinical trials, while PDPs also manage 40% of clinical development investment. It is not clear that policy settings have kept up with this shift in activity.

536

ENCOURAGING PRIVATE SECTOR INVOLVEMENT THROUGH ELIMINATION OF IMPORT TARIFFS ON ANTI-MALARIA MEDICINES

Halima Mwenesi, **A. Elisabeth Sommerfelt**, Kenyetta Lovings

Academy for Educational Development, Washington, DC, United States

The United Nations (UN) has emphasized the need for multi-sectoral involvement for successful control, elimination, and eventual eradication of malaria. In addition to contributions from international donors and public sector engagement, this requires large-scale involvement of various actors in the private sector for sustained coverage with effective interventions. When import tariffs are eliminated, the likelihood of private sector participation increases, keeps prices low and universal access high. International fora (including the UN General Assembly, World Health Assembly and various regional commitments) have called for the elimination/reduction of taxes and tariffs on anti-malaria tools/interventions (including treated bednets, medicines used with rapid diagnostic tests, and indoor residual spraying). Based on our extensive analysis of data on tariffs reported by countries, ~1/3 of countries identified by Roll Back Malaria as having a substantial malaria problem still impose tariffs on all these interventions. For information on the private sector as a source of care for under-five children with recent fever, we analyzed nationally representative survey data from the Demographic and Health Surveys Program (DHS). Among countries that impose tariffs on all these interventions, we selected those with recent DHS surveys (from 2007) and a substantial malaria problem (D.R. Congo, Indonesia, Philippines, Sierra Leone) (sample sizes of children ranged from ~5000 to ~16000), analyzing sources of care reported by mother. Illustrative findings from the Indonesia 2007 DHS show that care was sought for 9 of 10 children with fever; ~20% received care from a public facility and almost 60% from a private facility/provider. There were differentials according to poverty-wealth quintile for care received from public (~25% of poorest vs. slightly above 10% of richest) and private (almost 50% of poorest vs. almost 75% of richest) sources. These findings demonstrate that the private sector is an important source of care for fever (and malaria) even for the poorest subgroups.

537

ACCEPTABILITY OF TRANSPORT COST PAYMENT FOR MOTHERS SEEKING ANTENATAL AND DELIVERY CARE SERVICES: A SURVEY OF TRANSPORTERS' ATTITUDES - EASTERN UGANDA, 2009

Moses Tetui, Elizabeth K. Ekirapa, John Bua, Aloysius Mutebi, Raymond Tweheyo, George W. Pariyo

Makerere University College of Health Sciences, School of Public Health, Kampala, Uganda

Geographical accessibility is a major limitation to utilisation of maternal health services in Uganda. This study explored; transport availability, perceptions of subsidising transportation costs for antenatal (ANC) and delivery care services in four districts of Eastern Uganda. This was a cross-sectional study using qualitative approaches, carried out in Tororo, Pallisa, Soroti and Kamuli districts of Eastern Uganda in October 2009. Key informant interviews and focus group discussions were held with bicycle and motorcycle riders, and their transport leaders. Thematic content analysis was used. Findings revealed that mothers had great difficulties in accessing ANC and delivery services partly because of lack of transport. Most mothers who sought the services walked to health facilities, although public bicycles, motorcycles and vehicle transport means were available for their use. Service users were often unable to afford the transport charges. The project to help the mothers was generally acceptable in all the four study districts. Majority of the transport providers preferred weekly cash payments to be made either to them or their leaders rather than through bank accounts. The transporters acknowledged that project implementation could encounter potential challenges such as; insecurity at night, conflicts with non-beneficiaries (those who are not pregnant), conflicts with husbands, lack of night duty midwives, delays at the health centres, poor communication, lack of ownership of transport means, delayed payments, determining payments during day and night. In conclusion, findings suggest that subsidizing transportation for ANC and delivery care for pregnant women was acceptable, however, with potential social and institutional challenges. The study recommends accommodation of stakeholder suggestions in the project planning cycle, involvement of local authorities and community sensitization through various media.

538

NORTH-SOUTH COLLABORATIONS IN NON-COMMERCIAL CLINICAL RESEARCH: OPEN CHALLENGES

Raffaella M. Ravinetto[1], Ambrose Talisuna[2], Halidou Tinto[3], Umberto D'Alessandro[1], 4 Artemisinin-Based Combinations Study Group[4]

[1]Institute Tropical Medicine Prince Leopold, Antwerp, Belgium, [2]Uganda Malaria Surveillance Project, Kampala, Uganda, [3]Clinical Research Unit, Centre Muraz, Nanoro, Burkina Faso, [4]Multicentre, Antwerp, Belgium

North-South collaborations in clinical research have a double goal: addressing public health needs and building capacity and co-ownership. The 4-ABC trial, Evaluation of 4 artemisinin-based combinations for treating uncomplicated malaria in African children (ClinTrialGov NCT00393679) was funded by EDCTP, sponsored by the Antwerp Institute of Tropical Medicine (ITM) and conducted in Burkina Faso, Gabon, Mozambique, Nigeria, Rwanda, Uganda and Zambia. Protocol and amendments were sequentially submitted to the ITM Institutional Review Board, Ethics Committee (EC) of Antwerp University Hospital and EC and Competent Authorities in host countries. 4114 patients were recruited in 10 sites; data were recorded via an electronic CRF, cleaned at ITM and analyzed in Liverpool with the participation of an African statistician. PCR were read at ITM with the participation of an African biologist. The study group, co-chaired by an ITM and an African malaria expert, faced context-related, budgetary and structural constraints. In externally-funded trials, budgets tend to be inadequate to fully comply with GCP formal requirements and promptly react to unexpected situations. In addition, North-South non-commercial consortia have limited structural resources for clinical research tasks (data management, pharmacovigilance, regulatory affairs). Specific difficulties were linked to multiple ethical reviews; harmonization of QC procedures; samples' shipment; staff retraining; safety reporting; rationalization of monitoring expenses; cultural adaptation of informed consent in various urban and rural contexts; shelf life extension/discontinuation of two study drugs; set up of off-line remote data entry system; lack of insurers in host countries to cover study-specific risks. The capacity building effort brings empowerment, networking and capacity transfer to the South. However, the transfer of capacity to lead and sponsor trials is delayed by persisting obstacles, including the lack of secured funds for local structural costs and the need to translate universal GCP principles in contextualized procedures.

539

ASPIRATIONS FOR QUALITY HEALTH CARE: HOW DO WE GET THERE?

Clare I. Chandler[1], James Kizito[2], Deborah DiLiberto[1], Sarah G. Staedke[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Uganda Malaria Surveillance Project, Infectious Disease Research Collaboration, Kampala, Uganda

Access to 'prompt, effective treatment' is the dominant discourse in policies and programmes to reduce the burden of diseases such as malaria. However, decades of efforts to achieve this through public, private and community-based initiatives have barely dented morbidity and mortality levels in many settings, particularly in rural Africa. We carried out an assessment of perceptions of quality health care and barriers to achieving this in Tororo, Eastern Uganda. We carried out 69 in-depth interviews and 6 focus group discussions with health workers, 100 in-depth interviews with community medicine distributors and 10 focus group discussions with community members across 5 sub-counties in Tororo district. We found that aspirations for good quality care were similar amongst the three groups, and identified the most frequently discussed values: good clinical care and treatment; good interpersonal interactions between health workers and patients; well-managed health centres; providing advice and explanations to patients; welcoming and guiding the patient through health centres; professionalism amongst health workers; provision of free treatment at convenient times; and being seen quickly on arrival at the health centre. At health centres, immediate barriers to quality care included drug stock-outs and lack of equipment; high patient to staff ratio; use of volunteer health workers; language barrier between health workers and patients and discriminatory treatment of patients. Underlying these barriers were poor motivation of staff; poor management of the health centre; lack of patient-centred culture and poor relationship between health workers and communities. We traced these factors to district level determinants, including prioritisation of funds and politicking by district officials, and to wider systemic issues and cultural values. We argue that in order to attract patients to health centres and improve health outcomes, interventions need to build on the values and aspirations of health workers and community members rather than focus on narrow biomedical goals. We will present strategies designed to tackle these wider issues, to be evaluated through a 2-year cluster randomised controlled trial.

540

EFFECT OF TEMPERATURE AND PROINFLAMMATORY CYTOKINES ON PHOSPHATIDYLSERINE EXPRESSION ON *PLASMODIUM FALCIPARUM* MALARIA-INFECTED RED BLOOD CELLS DURING PARASITE MATURATION

Kovit Pattanapanyasat[1], Panudda Sratongno[1], Pattamawan Chimma[1], Supapart Chitjamnongchai[1], Korakot Polsrila[1], Kesinee Chotivanich[2]

[1]Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, [2]Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Malaria caused by *Plasmodium falciparum* remains one of the world's largest burdens of disease. Cerebral malaria is a life-threatening complication believed to be associated with parasitized red bloods (pRBCs) sequestration within the microvasculature of vital organs. Intra-RBC maturation of the malaria parasite corresponds with profound changes in the asymmetry of phospholipids in the lipid bilayer of the pRBCs. These changes may contribute to adherence of pRBCs to endothelial cells. The present study investigates the effect of febrile temperature and pro-inflammatory cytokines usually encountered during symptomatic human malaria infection on phosphatidylserine (PS) expression on the surface membrane of pRBCs during parasite maturation. The expression of PS on the pRBCs was determined by flow cytometry using fluorescence-labeled annexin V, which specifically binds to PS and a vital nucleic acid fluorochrome for parasite staining. The results showed that PS expression on the surface of pRBCs increased in association with parasite maturation, particularly at the late parasite stage. Exposure to febrile temperature led to significant increases in the expression of PS on the surface of pRBCs, especially at the late parasite stage associated with the virulence strain of the parasite. In contrast, pro-inflammatory cytokines had no detectable effect on PS expression on pRBCs. Interestingly, the growth of parasites also accelerated senescence of the uninfected RBCs in parasite cultures if cultured under febrile temperature. These data imply that febrile temperature in association with parasitemia, parasite strain and virulence but not pro-inflammatory cytokines induces more expression of PS molecules on pRBCs. These findings contribute to our understanding of the possible factors that are involved in malaria pathogenesis.

541

THE ANGIOPOIETIN-TIE-2 SYSTEM IS ASSOCIATED WITH RETINOPATHY AND MORTALITY IN MALAWIAN CHILDREN WITH SEVERE MALARIA

Andrea L. Conroy[1], Michael Hawkes[1], Simon Glover[2], Karl B. Seydel[3], Terrie Taylor[3], Malcolm Molyneux[4], Kevin C. Kain[1]

[1]University of Toronto, Toronto, ON, Canada, [2]University of Malawi College of Medicine, Blantyre, Malawi, [3]Michigan State University, Lansing, MI, United States, [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Severe malaria is associated with microvascular endothelial activation. The angiopoietins (Ang-1 and Ang-2) and their receptor Tie-2 are important regulators of endothelial quiescence. Previous reports have demonstrated increased Ang-2 and decreased Ang-1 in patients with cerebral malaria (CM). We tested plasma samples from febrile Malawian children who had been prospectively recruited with clinically defined CM (n=155) or moderately severe malaria (n=50) for Ang-1, Ang-2 and the soluble form of Tie-2 (sTie-2). Children's pupils were dilated and the fundi were examined with direct and indirect ophthalmoscopy. Malarial retinopathy was defined by the presence of haemorrhage, whitening, or vessel changes and retinal changes were clinically graded by an experienced ophthalmologist. Of the 205 children enrolled in the study, there were 78 children that had none of the signs of retinopathy, and 117 had one or more. There was a significantly lower median Ang-1 level ($p=0.048$) and significantly higher median Ang-2 ($p<0.0001$) and sTie-2 levels ($p<0.0001$) in children with retinopathy compared to those without. Correlations between retinal grading and the angiopoietins demonstrated an inverse correlation between Ang-1 and retinal whitening (Spearman's rho: -0.153, $p<0.05$), whereas Ang-2 and sTie-2 were positively correlated with retinal whitening and vessel changes (Ang-2:0.421, $p<0.01$; sTie-2: 0.411, $p<0.01$). None of the markers was associated with papilloedema. Finally, median Ang-2 and sTie-2 were higher in those with a fatal outcome (n=61) compared to survivors (n=141), $p<0.001$ for both. Receiver operator characteristic (ROC) curves were generated to assess the prognostic accuracy of the markers and Ang-2 and sTie-2 had areas under the ROC (AUROC) curve of 0.73 (95% CI: 0.66-0.81) and 0.71 (0.64-0.79) respectively. The AUROC of venous lactate, a known prognostic marker in malaria, was 0.66 (0.57-0.75), $p<0.001$. These data suggest that the angiopoietin-sTie-2 system could have diagnostic and prognostic value in children with severe malaria.

542

IMPLICATING COMPLEMENT C5 ACTIVATION IN MALARIA-INDUCED FETAL GROWTH RESTRICTION

Andrea L. Conroy[1], Karlee L. Silver[1], Kathleen Zhong[1], Joseph Fletcher[1], Stephen Rogerson[2], Kevin C. Kain[1]

[1]University of Toronto, Toronto, ON, Canada, [2]University of Melbourne, Melbourne, Australia

Placental malaria (PM) is a leading cause of poor fetal outcomes including spontaneous abortion, preterm delivery and low birth weight. Complement component C5a has been shown to interact with *Plasmodium falciparum* bioactive products (glycosylphosphatidylinositol), to induce synergistic release of inflammatory cytokines and chemokines. Using a murine model of experimental PM (BALB/c mice and *Plasmodium berghei* ANKA), we show that malaria-infected mice had elevated serum C5a at 3 days post infection ($p<0.05$) and elevated levels of placental mRNA encoding the C5a receptor (C5aR) at 6 days post-infection ($p=0.003$). To test the hypothesis that C5a was causally implicated in poor fetal outcomes, we compared pregnancy outcomes of malaria-infected C5aR deficient mice versus wild type (WT). C5aR deficiency resulted in increased fetal viability ($p=0.021$) compared to WT mice and viable fetuses from C5aR-deficient mice experienced less growth restriction ($p<0.001$). Further, C5aR deficiency was associated with improved regulation of angiogenesis. These observations were extended to a population of malaria-exposed women in Malawi. Plasma samples were collected in a case-control design from pregnant women at delivery (n=495). In a univariate analysis, median levels of placental C5a were elevated in women with PM (n=146) compared to women without PM (n=349), $p<0.0001$. There were also changes in angiogenic factors, with decreased angiopoietin-1 (Ang-1, $p<0.0001$), and increased Ang-2 ($p=0.001$) and sFlt-1 ($p=0.002$). In order to extrapolate causal relationships from these data, structural equation modeling was performed using AMOS v17.0 for SPSS. Our model suggests that parasites in the placenta lead to mononuclear cell infiltration, which in turn drives C5a production, dysregulated placental angiogenesis and culminates in fetal growth restriction. Overall, the model was a good fit with an rmsea 0f 0.033 (95% CI: 0.022-0.043). Together, these data suggest that C5a may be an early mediator of placental malaria pathogenesis and may contribute to angiogenic dysregulation and placental insufficiency.

543

PLASMA URIC ACID LEVELS CORRELATE WITH PARASITE DENSITY, INFLAMMATORY CYTOKINE LEVELS, AND DISEASE SEVERITY IN MALIAN CHILDREN WITH MALARIA

Tatiana M. Lopera-Mesa

National Institutes of Health, Rockville, MD, United States

Several *Plasmodium falciparum* factors have been proposed to elicit excessive host inflammatory responses involved in malaria pathogenesis. One of these is uric acid (UA) which can be generated from hypoxanthine and xanthine accumulated by *P. falciparum*-infected red blood cells (RBCs) during parasite maturation. These purines are released at schizont rupture and are converted to UA in plasma by the human enzyme xanthine oxidase. *In vitro*, UA generated in this manner potently activates PBMCs to produce inflammatory cytokines associated with severe malaria. To explore whether parasite-generated UA drives inflammation *in vivo*, we hypothesized that UA levels (i) are elevated during malaria episodes, (ii) positively correlate with parasite density, and (iii) positively correlate with levels of cytokines and chemokines. To test these hypotheses, we measured the plasma levels of UA and inflammatory mediators (IL-1, IL-6, IL-8, TNF, MCP-1, IFNg, IL-10, IP-10, sTNFR II) in 266 Malian children (aged 6 months to 5 years) presenting with their first episode during the 2008 transmission season. Out of 266 children, 248 had mild malaria and 18 had severe malaria (9 with impaired consciousness). UA levels were significantly elevated in children with malaria compared to 18 healthy children without parasitemia ($P=0.001$). Log transformed UA levels correlated with log parasite density ($r=0.26$, $P<0.0001$) and mean values significantly increased with disease severity (mean ± SEM; 4.7 ± 0.09 mg/dl for mild malaria vs. 5.6 ± 0.28 mg/dl for severe malaria, $P=0.002$). Inflammatory mediators also increased with disease severity. Log UA levels positively correlated with log-transformed levels of TNF ($r=0.27$, $P<0.0001$), IL-6 ($r=0.4$, $P<0.0001$), IL-1 ($r=0.22$, $P=0.006$), IL-10 ($r=0.35$, $P<0.0001$), IL-8 ($r=0.29$, $P<0.0001$), MCP-1 ($r=0.27$, $P<0.0001$), IP-10 ($r=0.21$, $P=0.001$), and sTNFR II ($r=0.3$, $P<0.0001$). These data suggest that parasite-derived UA contributes to the pathogenesis of uncomplicated and severe malaria.

544

RELATIVE ANGIOPOIETIN LEVELS ALTER SUSCEPTIBILITY TO EXPERIMENTAL CEREBRAL MALARIA

Karlee L. Silver[1], Sarah Higgins[2], John G. Hay[3], Susan E. Quaggin[4], W. Conrad Liles[5], Kevin C. Kain[5]

[1]McLaughlin-Rotman Centre for Global Health; University of Toronto, Toronto, ON, Canada, [2]McLaughlin-Rotman Centre for Global Health; Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada, [3]Division of Pulmonary and Critical Care Medicine, New York University School of Medicine, New York, NY, United States, [4]Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, Toronto, ON, Canada, [5]McLaughlin-Rotman Centre for Global Health; Department of Medicine, University of Toronto, Toronto, ON, Canada

Cerebral malaria is characterized by endothelial activation. The angiopoietins are critical regulators of the endothelium. Constitutive interaction of angiopoietin (Ang)-1 with endothelial-expressed Tie-2 maintains the integrity and quiescent nature of mature vascular endothelium. Ang-2 can displace Ang-1 from Tie-2 to activate the endothelium to a pro-inflammatory state primed to respond to cytokines such as TNF. Therefore, the relative level of Ang-2 and Ang-1 is believed to play a pivotal role in the regulation of endothelial activation and integrity. Systemic Ang-2 and Ang-1 levels have been shown to be informative biomarkers of malarial disease severity. Due to events indicative of endothelial activation and loss of blood-brain barrier associated with severe malaria, we hypothesized that malaria-induced alteration of Ang-2 and Ang-1 levels plays an important role in the pathogenesis of cerebral malaria. We examined serum and brain Ang levels of inbred mouse strains infected with *Plasmodium berghei* ANKA (PbA): Serum Ang-1 levels of strains susceptible to experimental cerebral malaria (129Sv/J, C57BL/6J and B10.D2/nSnJ) dropped prior to onset of neurological symptoms, and earlier after PbA infection than in resistant strains (B10.D2/oSnJ, AKRJ). Whole brain Ang-2 and the Ang-2/Ang-1 ratio were elevated in infected susceptible B10.D2/nSnJ mice as compared to congenic resistant B10.D2/oSnJ at day 6 post PbA infection. Increased brain Ang-2 was also observed by immunohistochemical staining of brain sections from infected versus uninfected control mice. To show that relative Ang-2/Ang-1 levels were able to determine susceptibility to experimental cerebral malaria, we used an adenovirus gene-delivery system to over-express Ang-1 in susceptible (C57BL/6J) mice and a conditional knockout system to delete Ang-1 in resistant (BALB/c) mice. Reversing the strain susceptibility to experimental cerebral malaria following PbA infection by altering the relative Ang-2/Ang-1 levels supports a pathophysiologic role for these proteins.

545

CYCLOOXYGENASE (COX)-2 PROMOTER HAPLOTYPES ARE ASSOCIATED WITH PROTECTION AGAINST PEDIATRIC SEVERE MALARIAL ANEMIA

Samuel B. Anyona[1], Evans Raballah[1], Prakasha Kempaiah[2], Collins O. Ouma[1], Tom Were[1], Gregory C. Davenport[2], John M. Vulule[3], John M. Vulule[3], James B. Hittner[4], Charity W. Gichuki[5], John M. Ong'echa[1], Douglas J. Perkins[2]

[1]University of New Mexico/KEMRI, Kisumu, Kenya, [2]University of New Mexico, Albuquerque, NM, United States, [3]KEMRI, Kisumu, Kenya, [4]College of Charleston, Charleston, SC, United States, [5]Kenyatta University, Nairobi, Kenya

Inducible cyclooxygenase (COX; prostaglandin-endoperoxide H synthase)-2 is up-regulated by pro-inflammatory mediators and generates the production of elevated levels of prostaglandins (PGs) as part of the host-immune response to infections. We have previously shown that COX-2 transcripts and protein in peripheral blood mononuclear cells, and circulating PGs, are suppressed in children with severe malaria. Although previous studies demonstrated that variation in COX-2 conditions the clinical outcomes in several autoimmune and inflammatory diseases, no studies to date have reported the association between COX-2 genetic variation and susceptibility to severe malarial anemia (SMA). As such, the association between COX-2 -608A/G and -765G/C promoter variants and susceptibility to SMA was investigated among parasitemic children (age: 3-36 months; n=551) with acute malaria presenting at Siaya District Hospital, western Kenya. Demographic, clinical and laboratory measures were determined and children stratified, based on hemoglobin (Hb), into non-SMA (Hb>6.0g/dL; n=316] and SMA (Hb<6.0g/dL; n=235). Genotyping was performed using TaqMan 5′ allele discrimination and PCR-RFLP methods. Proportions of -608 and -765 genotypes were comparable between non-SMA and SMA groups (*P*=0.791 and *P*=0.624, respectively). Similarly, frequencies of haplotype constructs failed to show differences between the groups [-608A/-765C (AC; *P*=0.987), AG (*P*=0.143), and GG (*P*=0.846), respectively]. However, prevalence of the GC haplotype was significantly lower in children with SMA relative to the non-SMA group (*P*=0.016). Multivariate logistic regression analyses, controlling for co-variates, revealed that carriers of GC haplotype had a 78% reduced risk of developing SMA (OR=0.22, 95% CI-0.060-0.782; *P*=0.020). These results suggest that variation at -608 and -765 in the COX-2 promoter may play an important role in conditioning susceptibility to SMA in children resident in *Plasmodium falciparum* endemic areas.

546

ASSOCIATION OF FUNCTIONAL RANTES PROMOTER AND INTRONIC HAPLOTYPES WITH SEVERE MALARIAL ANAEMIA AND MORTALITY IN KENYAN CHILDREN WITH *PLASMODIUM FALCIPARUM* MALARIA

Tom Were[1], Gregory C. Davenport[2], Collins O. Ouma[1], Samuel B. Anyona[1], Prakasha Kempaiah[2], Evans O. Anyona[1], James B. Hittner[3], John M. Vulule[4], John M. Ong'echa[1], Douglas J. Perkins[2]

[1]University of New Mexico/KEMRI, Kisumu, Kenya, [2]University of New Mexico, Albuquerque, NM, United States, [3]College of Charleston, Charleston, SC, United States, [4]KEMRI, Kisumu, Kenya

Regulated on activation normal T-cell expressed and secreted (RANTES, CCL-5) is an important immunoregulatory chemokine that forms part of the intercellular networks that regulate hematopoiesis. We previously showed that suppression of RANTES is associated with severe malarial anemia (SMA, Hb<6.0g/dL) and suppression of erythropoiesis in African children with malaria. Previous studies also demonstrated that genetic variation in RANTES regulates outcomes of inflammatory, auto-immune and infectious diseases, and plasma RANTES levels. However, the role of RANTES gene variation in conditioning SMA, appropriate erythropoiesis (reticulocyte production index, RPI>3.0), mortality and RANTES production in children with malaria is unknown. Associations of RANTES intronic (A+307G, rs2280789) and promoter (G-403A, rs2107538 and A-4120T, rs16971624) haplotypes with SMA, erythropoiesis, three-month post-enrolment mortality, and circulating RANTES were therefore investigated in children (*n*=535) with malaria from western Kenya. Prevalence of the AGT haplotype was 7.1% in the SMA and 13.2% in the non-SMA (Hb>6.0g/dL) groups (*P*=0.024). Multivariate regression modeling controling for covariates showed that haplotype AGT was associated with reduced risk of SMA (OR, 0.501; 95%CI, 0.269-0.934; P=0.030) and appropriate erythropoiesis (OR, 2.247; 95%CI, 1.100-4.591; P=0.026), while haplotype AAA predicted reduced risk of three-month post-enrolment mortality (OR, 0.319; 95%CI, 0.101-1.003; P=0.051). Functional analyses illustrated higher circulating RANTES levels [ng/mL, median (IQR)] in AGT [18.3 (68.3) vs. 11.4 (36.3); P=0.047] and AAA [21.5 (93.1) vs. 11.3 (36.8); P=0.038] haplotype carriers. These results suggest elevated RANTES production, conditioned by genetic variation, is associated with enhanced erythropoiesis, protection against SMA, and reduced malaria-associated mortality.

547

EVALUATION OF PUTATIVE IMMUNOGENIC PROTEINS FROM *VIVAX* MALARIA BLOOD STAGE BY HIGH-THROUGHPUT SCREENING ASSAYS

Eun-Taek Han[1], Jun-Hu Chen[1], Jae-Wan Jung[2], Yue Wang[1], Kwon-Soo Ha[2], Takafumi Tsuboi[3]

[1]Department of Parasitology, School of Medicine Kangwon National University, Chuncheon, Gangwon-do, Republic of Korea, [2]Department of Molecular and Cellular Biochemistry, School of Medicine Kangwon National University, Chuncheon, Gangwon-do, Republic of Korea, [3]Cell-free Science Technology and Research Center and Venture Business Laboratory, Ehime University, Matsuyama, Ehime, Japan

Completed genome sequences and stage-specific transcriptome of the intraerythrocytic developmental cycle of *Plasmodium vivax* offers chances for discovery of new malaria vaccine candidates using innovative screening approaches. Herein, a panel of putative immunogenic antigens from *P. vivax* blood stage was selected using data mining by comparative genomics. Total of 94 ORFs were high-throughput cloned to wheat germ cell-free expression vector (pEU) from 99 PCR products (94.9%) by In-Fusion cloning method. Ninety-five percent (95%, 89/94) genes of *P. vivax* were expressed by wheat germ cell-free system. The putative immunogenic proteins screened with *P. vivax* infected patient sera by protein arrays, a total of 18 (19.1%, 18/94) highly immunoreactive proteins were identified, including 3 of well-characterized vivax vaccine candidates (AMA1, MSP1-42 and MSP1-19), 2 GPI-anchored proteins (MSP8 and MSP10) and MSP3β. Other 12 ORFs have not been previously described as immunologically reactive. These novel immunogenic proteins of vivax malaria blood stage will be further studied as potential vaccine candidates. The results indicates that the In-Fusion cloning method combined with wheat germ cell-free system and protein arrays technology can be used to perform high-throughput screening assays to determine immunogenicity of candidate antigens from the *P. vivax* genome.

548

THE ROLE OF *PLASMODIUM* PARASITES DERIVED MIFS DURING MALARIA INFECTION

Dingding Shao[1], Cong Han[1], Yahui Lin[1], Guangliang Shan[1], Zaixing Zhang[2], Xiaodong Sun[2], Zhensheng Wang[1], Chunyan Wei[1], Yan Deng[2], Lianhui Zhang[1], Lingyi Bu[1], Heng Wang[1]

[1]Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and School of Basic Medicine, Peking Union Medical College, Beijing, China, [2]Yunnan Institute of Parasitic Diseases, Puer, Yunnan Province, China

It has been hypothesized that parasites can modulate the host immune response to benefit their survival by their own molecules,

such as macrophage migration inhibitory factor (MIF). Recently, several *Plasmodium* parasites derived MIFs (*P*MIFs) have been identified to function on host immune cells *in vitro* and furthermore, our group had reported the crystal structure and function comparison between *P*MIF and its host MIF, and confirmed that, 1) as a tautomerase, *P*MIF had a distinct substrate binding pattern and obviously lower enzymatic activity than the host MIF; and 2) both the parasite and host derived MIFs showed identical activities on host cell respectively, however, the combination activities of *P*MIF and host MIF on immune cells was complex. These results suggest the potential regulatory effect of *P*MIF during malaria infection. In this report, by using the monoclonal antibodies specifically against *P. falciparum* MIF (PfMIF) or *P. vivax* MIF (PvMIF), we investigated the correlation of these two *P*MIFs with the course of malaria infection. Data from the epidemiologic studies of the two *P*MIFs shown that the concentrations of the two molecules in the peripheral blood of malaria patients were positively correlated with the level of parasitemia, TNF-α, IL-10 and MCP-1, but not correlated with TGF-β1 and IL-12. Moreover, multiple stepwise regression analysis also showed that parasitemia, IL-10,and HuMIF expression are significant predictors of *P*MIFs production. In addition, by tracing these two *P*MIFs levels during anti-malaria drug treatment, we found decrease of *P*MIFs level following the decrease of parasitemia in most of the patient samples. Our data for the first time shows that the circulating level of *P*MIF is a reflection with *in vivo* malaria parasite density and disease severity and is helpful for further understanding the role of *P*MIF during malaria infection.

549

PLASMODIUM FALCIPARUM ERYTHROCYTE MEMBRANE PROTEIN 1 EXPRESSION IN ISOLATES FROM CHILDREN WITH SEVERE MALARIA

Pamela A. Magistrado[1], Anja T. Jensen[1], Louise Turner[1], Thomas Lavstsen[1], Louise Joergensen[1], Davis John[2], John P. Lusingu[3], Martha Lemnge[3], Raimos Olomi[2], Thor G. Theander[1]

[1]Institute of International Health, Immunology and Microbiology, Copenhagen, Denmark, [2]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania, [3]National Institute for Medical Research, Tanga, United Republic of Tanzania

Plasmodium falciparum malaria remains one of the world's leading causes of human suffering and poverty. Most deaths are caused by specific severe malaria syndromes such as cerebral malaria, pregnancy-associated malaria, and severe normocytic *P. falciparum*-related anaemia. In areas of stable transmission of *P. falciparum* parasites, mortality and severe morbidity from malaria is restricted to the first 5-10 years of life, as protective immunity is gradually acquired. It is well-established that protective immunity acquired in response to repeated infections is mediated by IgG, and that a principal target of this IgG is parasite-encoded, clonally variant surface antigens (VSA), exposed on the surface of infected erythrocytes. The best-characterized VSA are the family of high-molecular weight (200-400 kDa) proteins called *P. falciparum* erythrocyte membrane protein 1 (PfEMP1). PfEMP1 based vaccines are attractive because these molecules probably are the targets of natural acquired immunity. They are problematic because they are large and diverse. Establishing the parts of the PfEMP1 molecule which are targets for protective antibodies is a key to vaccine development. In another study, PfEMP1 transcription in isolates from Tanzanian children with malaria was analyzed. It was found that severe malaria syndromes such as cerebral malaria, severe anaemia and hyperparasitemia are associated with certain PfEMP1 variants such as VAR4, VAR5, VAR6 and VAR8 while asymptomatic infections are not. In the current study, specific antibodies targeting such PfEMP1 variants were produced and tested in flow cytometry for reactivity with *P. falciparum* isolates taken directly from Tanzanian children with severe malaria. To date, 32% of the 28 isolates tested reacted with VAR4 antibodies. Flow cytometry analyses using PfEMP1 variant specific antibodies and fresh clinical isolates provide clues on PfEMP1 expression in the field. This knowledge aids in the development of a morbidity-reducing malaria vaccine for children in Africa.

550

PLASMODIUM FALCIPARUM GAMETOCYTE CARRIAGE IS ASSOCIATED WITH SUBSEQUENT *P. VIVAX* RELAPSE AFTER TREATMENT

Jessica T. Lin[1], Delia B. Bethell[1], Stuart D. Tyner[1], David L. Saunders[1], Phisit Khemawoot[1], Sabaithip Sriwichai[1], Kurt Schaecher[1], Duong Socheat[2], Steven R. Meshnick[3], Youry Se[1], Chanthap Lon[1], Mark M. Fukuda[1]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [3]Gillings School of Public Health, University of North Carolina, Chapel Hill, NC, United States

Up to one-third of patients in Southeast Asia develop *Plasmodium vivax* relapse shortly after treatment of what appears to be a *P. falciparum* (Pf) mono-infection. These patients are thought to harbor cryptic vivax infection not apparent at presentation. A previous retrospective analysis of an artesunate monotherapy trial completed in western Cambodia in 2006-7 showed for the first time that those with *P. falciparum* gametocytes on admission were more likely to develop relapse with *P. vivax* upon follow up. Now results from a larger follow on trial conducted in 2008-9 at the same site further confirms this association. In total, over the two trials, 244 patients with uncomplicated *P. falciparum* malaria received short-acting antimalarials in the form of artesunate monotherapy or quinine/tetracycline for 7 days. 18% of these (44/244) had Pf gametocytes on a peripheral blood smear at presentation. Of those, 55% went on to develop *P. vivax* infection during the 28 or 42 day follow up period, as opposed to 17% of those who were not gametocytemic at presentation. Thus, the presence of Pf gametocytes on an initial blood smear was associated with a 3 fold greater risk of *P. vivax* relapse (RR=3.2, 95% CI 2.1-4.8, $p<0.0001$). This difference could not be explained by duration of illness prior to presentation, initial asexual parasitemia, or history of previous malaria episodes. Patients with a history of malaria in the previous month were excluded. PCR confirmed that a very low proportion of subjects had Pv detectable at baseline, indicating that *P. vivax* parasites resided in the liver at the time of presentation in the majority of patients who relapsed. These data indicate that in areas with substantial rates of mixed *P. falciparum*/*P. vivax* infection, gametocytes seen at presentation may be a potential marker for liver-stage P. *vivax* infection. We hypothesize that the presence of a second competing malaria species may boost *falciparum* gametocytogenesis. If this is true, patients who harbor mixed infection may contribute disproportionately to ongoing malaria transmission.

551

THE GENETIC COMPOSITION OF MULTIPLE-CLONE MALARIA INFECTIONS

Standwell Nkhoma, Shalini Nair, Tim J. Anderson

Southwest Foundation for Biomedical Research, San Antonio, TX, United States

*Plasmodium falciparu*m infections containing multiple clones are common in endemic regions, but the genetic composition of such infections is poorly understood. If these result from superinfection (i.e. sporozoite innoculation from multiple mosquitoes), we would expect component clones to be predominantly unrelated. However, if clones come from a single mosquito inoculation we would expect parasites to be related. To test these predictions, we isolated individual clones from Malawian mixed infections by limiting dilution and genotyped these using 384 SNPs distributed across the genome. We found up to 8 clones per host. There were examples of both related and unrelated parasites within infections, suggesting that both processes occur in nature. The most striking feature of these data was the discovery of parasite clones that are extremely closely related (sharing identity at >90% SNPs genotyped). The differences observed were found in blocks suggesting that the divergent genome regions result from a recombinational process. We suggest two

processes that may be responsible for these observations. First, if multiple clone infections are serially transmitted from one patient to another, then repeated inbreeding will progressively diminish variation among clones, as occurs during generation of recombinant inbred lines in laboratory model organisms such as mice.Alternatively, these divergent blocks could be generated by a novel genetic mechanism such as mitotic recombination, although this is not currently suspected to occur in *Plasmodium*. In conclusion, these data suggest that a simple superinfection model cannot explain the complex relatedness structure observed within multiple clone infections. These results (1) identify a valuable new resource for genetic mapping, (2) refine our understanding of *Plasmodium* population structure and genetics, and (3) have important implications for our understanding of malaria traits such as virulence and sex ratio where kinship is critical.

552

GENETIC DIVERSITY OF THE MEROZOITE SURFACE PROTEINS 8 (MSP-8) AND 10 (MSP-10) IN *PLASMODIUM* SPP

M. Andreina Pacheco, Alamelu D. Elango, Abir A. Rahman, Ananias A. Escalante

Center for Evolutionary Medicine and Informatics, Arizona State University, Tempe, AZ, United States

Assessing how natural selection, negative or positive, operates on genes with low polymorphism is challenging. In *Plasmodium*, the merozoite surface proteins (MSP-1 to MSP-10) are expressed on the merozoite surface. Given their role in the invasion of the red blood cell (RBC), several of them are considered promising vaccine candidates. Among all MSPs identified to date, MSP-1, MSP-8 and MSP-10 have two epidermal growth factor-like domains (EGF) at the C-terminal, which have been proposed to act as ligands during the invasion of RBCs. Those domains are highly antigenic, making them immunogenic and functionally conserved among the different *Plasmodium* species. We investigated the genetic diversity of orthologous genes encoding the Merozoite Surface Protein 8 (MSP-8) and 10 (MSP-10). We applied evolutionary genetic methods to study the polymorphism in MSP-8 and MSP-10 from *Plasmodium falciparum* and *P. vivax*, the two parasites responsible for most human malaria morbidity and mortality. In addition, we studied MSP-8 and MSP-10 orthologous from closely related malarial species found in non-human primates. Overall, genes encoding MSP-8 and MSP-10 are highly conserved in all the *Plasmodium* spp. included in this investigation. Both, MSP-8 and MSP-10, have low polymorphism in *P. falciparum* and *P. vivax* in comparison with the orthologs from other *Plasmodium* species. We found that observed polymorphism in MSP-8 and MSP-10 in *P. vivax* and MSP-8 *P. falciparum* appears to be neutral. There is limited evidence suggesting that MSP-10 in *P. falciparum* could be under positive selection. Yet, we found evidence that the orthologous genes in non-human primate parasites (*P. cynomolgi*, *P. inui*, and *P. knowlesi*) are under purifying (negative) selection. We discuss how selective pressures may differ among orthologous genes in closely related malarial parasites species. It is important to consider the effect of negative selection while studying genes encoding proteins with low polymorphism using comparative approaches.

553

MALARIA MICROSCOPY COMPETENCY IN LIBERIA

Luis Benavente[1], Henry Kohar[2], Joel Jones[2], Hannan K. Bestman[3], Emmanuel Ouma Yamo[4], Kassahun Belay[5], Sean Fennell[1]

[1]Medical Care Development Inc., Silver Spring, MD, United States, [2]National Malaria Control Program, Monrovia, Liberia, [3]Medical Care Development Inc., Monrovia, Liberia, [4]African Medical and Research Foundation, Nairobi, Kenya, [5]United States Agency for International Development, Monrovia, Liberia

Background: In many malaria endemic countries, malaria microscopy is the most common method of malaria diagnosis. However, there are many constraints to achieving competence in malaria microscopy. In addition, in Liberia the war has disrupted the educational system and created a major human resource gap. Until recently, NMCP's training emphasis was in strengthening competence using malaria rapid diagnostic tests (RDTs). Although RDTs are sensitive and specific, they are more costly than microscopy and cannot inform about species or parasite density. Therefore a program of refresher training in malaria microscopy was instituted in Liberia. Methodology: In 2009 and 2010, the National Malaria Control Program (NMCP), the National Public Health Reference Laboratory (NPHRL) and the Improving Malaria Diagnostics (IMaD) project conducted two malaria microscopy refresher training courses for a total of 45 laboratory technicians. The courses combined assessment with training and practice in slide reading. Competency was assessed at the beginning and end of each course using slide sets of known composition. Results of slide reading were graded at the end of every day so participants had the opportunity to review failed slides on the following day.Results: Both theoretical knowledge of malaria diagnosis and performance (sensitivity, specificity, species identification and parasite counting) were very low prior to refresher training. After training overall sensitivity was 84%, overall specificity 87%, species identification 26%, and parasite counting 26%. The percentage of technicians attaining a "pass" level was 54% for sensitivity, 63% for specificity, 0% for species identification and 17% for parasite counting. Out of six microscopists attending both refresher training courses, only two dropped in performance level against the standard although they remained within the 95% confidence interval. The distribution of participants in both training courses combined by quintile (Pf ID) was bimodal both in pre-and post training, suggesting that participants were a heterogeneous mix of high and low performers. Conclusions: There is a need to strengthen skills in malaria microscopy in Liberia using regular training courses that combine assessment with training and practice, especially for national and regional supervisors.

554

NEW LAB-ON-A-CHIP FOR FAST DIAGNOSIS OF HUMAN MALARIA SPECIES AND DETECTION OF DRUG RESISTANCE

Joseph Mugasa, Lisa Ranford-Cartwright

Glasgow University, Glasgow, United Kingdom

Malaria continues to be a major cause of morbidity and mortality worldwide. This has been partly attributed to the resistance of *Plasmodium falciparum* to commonly used antimalarials. Rapid and accurate diagnosis of *Plasmodium* spp is essential for the rational treatment of malaria. Microscopy remains the gold standard tool for malaria diagnosis, but needs skilled manpower, and is tedious and time- consuming. Currently, there are no tools for diagnosis of drug resistance in patients other than relying on reports of failure of curative treatments. Available molecular methods are laborious and time-consuming preventing timely and appropriate decisions on clinical management. Here we describe a collaborative project for the development of a new Lab-on-a-Chip (LoC) based platform (In-check™) for molecular diagnosis of malaria species and drug resistant variants. The In-Check™ Platform is an integrated system combining a fast PCR and microarray based diagnostic test using a single Lab-on-a-Chip. Detection of the five human malaria-causing *Plasmodium* species (*P. falciparum*, *P. vivax*, P. ovale, *P. knowlesi* and P. malariae) is performed using PCR based on 18S rRNA gene followed hybridisation with species-specific probe on the microarray. For detection of drug resistant parasites, polymorphic regions of the genes PfCRT, PfDHFR, PfDHPS and PfCytb that confer resistance to Chloroquine, Sulphadoxine -Pyrimethamine and Malarone are analysed by PCR and hybridisation to specific probes on the microarray. The sensitivity and specificity of the In-Check™ Platform has been assessed using parasite samples at different parasitaemia and samples for which the species present has been determined by other methods. The performance of the In-Check™ Platform relative to slide diagnosis and standard species-specific PCR will be presented. The LoC offers simultaneous diagnosis of the infecting malaria species, together with a prediction of the likely response to commonly used antimalarials for *P. falciparum*. The process takes less than an hour, a considerably shorter period than the current molecular diagnostic tests for malaria.

555

COMBINED RNA AND DNA RT-QPCR ASSAY FOR USE IN MALARIA DIAGNOSIS AND INTERVENTION TRIALS

Edwin Kamau[1], Nancy Nyakoe[2], Linda Muringo[2], Christian F. Ockenhouse[1], John Waitumbi[2]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Walter Reed Project, Kenya Medical Research Institute, Kisumu, Kenya

Microscopy is the gold standard for detection and quantification of malaria asexual parasitemia. Unfortunately, a number of factors mitigate utility of malaria microscopy i.e; the method is poorly reproducible and even then, requires considerable expertise for correct diagnosis and quantification. As such, nucleic acids and antigen based assays are increasingly being used to resolve problems of malaria diagnosis. In a series of studies, we have developed a quantitative real time reverse transcription PCR (RT-qPCR) that is based on amplification of total nucleic acids (RNA and DNA) from 18s rRNA genes for genus *Plasmodium* and the four species of malaria: *Plasmodium falciparum, ovale, malariae and vivax*. For *P. falciparum*, total nucleic acid was extracted using Qiagen Kit from whole-blood samples spiked with cultured, washed, ring-stage-infected red blood cells (iRBCs). The assay has a dynamic range of 0.125-750 iRBCs/µL with Ct values of 37.6 and 21.02 for the lowest and highest parasitemia respectively. By diluting patient total nucleic acid to provide Ct measurements within the dynamic range, *P. falciparum* parasitemia of > 106/µL can be quantified, thus allowing identification of parasite burden within a very broad range. Importantly, the combined nucleic acids RT-qPCR have more than log fold sensitivity over DNA only. We conclude that the combined nucleic acids RT-qPCR is a suitable adjunct to microscopy and could benefit malaria diagnosis and intervention trials.

556

TRENDS IN MALARIA DIAGNOSIS: COMBINING THE USE OF TELEDIAGNOSIS, MICROSCOPY AND PCR IN THE IDENTIFICATION OF *PLASMODIUM* SPP

Blaine A. Mathison, Stephanie Johnston, Maniphet Xayavong, Paul Arguin, Alexandre J. da Silva

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

Microscopic examination of blood smears continues to be the gold standard for laboratory diagnosis of malaria. PCR can also be used for confirmatory testing, especially in cases where an accurate identification cannot be made by microscopy, e.g. low parasitemia, poor preparation or staining, or when a dual infection is suspected. Since 1998, the CDC's DPDx Project has implemented telediagnosis as an effective means of parasite identification, including malaria. Telediagnosis is inexpensive and allows diagnosis to be made in minutes to hours; a good diagnostic alternative in laboratories with adequate infrastructure. Here, we evaluate the results of four years of telediagnosis submissions for malaria, from October 1, 2005 to September 30, 2009 (CDC Fiscal Years 2006-2009). During this time period, the DPDx Team received 1,192 telediagnosis inquiries, 423 of which (35.5%) were for malaria diagnosis. Of the 423 cases, 298 (70.4%) were confidently identified to the species level, identified as *Babesia*, or reported as negative by telediagnosis. However, in 125 (29.6%) of these cases, a definitive identification could not be made by images alone and follow-up material was requested. Requested follow-up material (slides and/or EDTA blood) was received for 79 (63.2%) of the cases. In 36 of the 79 (45.6%) cases where follow-up material was received, the species-level identification was made by microscopy, and in 5 of these 36 (13.9%), PCR further confirmed the microscopy. In 8 of the 79 (10.1%) cases where follow-up material was received, only EDTA blood was received and only PCR was performed. In 33 of these 79 (41.8%) cases, the follow-up examination of smears could not further identify the *Plasmodium* sp. present. PCR was successfully used in 23 of these 33 (69.7%) cases where EDTA blood was also received. Two of the 79 (2.5%) cases where follow-up material was received turned out to be positive for *Babesia* sp. Our data show that telediagnosis is an effective tool for rapid diagnosis of malaria, or to screen clinical specimens for further testing, to better improve patient management.

557

DEVELOPMENT OF A GOLD NANOPARTICLE BASED DIAGNOSTIC ASSAY FOR *PLASMODIUM FALCIPARUM*

Gloria E. Oramasionwu[1], Michael S. Cordray[2], Rebecca R. Richards-Kortum[2]

[1]Baylor College of Medicine, Rice University, Houston, TX, United States, [2]Rice University, Houston, TX, United States

Current malaria diagnosis requires experienced microscopists, expensive equipment, or may be insensitive at low parasitemia, thus a sensitive diagnostic test is needed for limited-resource settings where malaria is prevalent. A recently developed, gold nanoparticle aggregation assay has been shown to specifically detect 107 copies of bacterial DNA. The simplicity of this assay, when coupled with amplification of target DNA, could yield specific malaria diagnosis in low resource settings. Loop mediated isothermal amplification (LAMP) amplifies target DNA up to 109 fold with detection of product by the naked eye, though simple visual turbidity assessment has low specificity. Our goal is to integrate gold nanoparticle mediated aggregation and isothermal amplification to develop a simple, sensitive malaria diagnostic assay that improves upon the low specificity encountered with LAMP. We have employed LAMP to amplify the *Plasmodium* 18s rRNA gene in a region unique to *P. falciparum*. Oligonucleotide-functionalized gold nanoparticle probes were designed to specifically hybridize to adjacent sequences on this amplified portion of the *P. falciparum* 18s rRNA gene in order to generate aggregated complexes of gold nanoparticles in the presence of the target DNA. Target DNA and the gold nanoparticle probes were heated and allowed to hybridize. Upon specific hybridization to the target DNA sequence, the color of the sample changes from pink to purple, the light scattered by the gold changes from green to orange and the shift in scattering spectra is measured by a simple total internal reflection (TIR) spectroscopy device. With LAMP, we are able to amplify and detect as few as 102 copies of the 18s rRNA gene in under 1 hour. Without prior amplification, we are able to specifically detect 333 picomolar or 108 copies/µL of ssDNA, equivalent to 108 parasites using serial dilutions of *P. falciparum* oligonucleotide target and TIR imaging. In conclusion, we have developed an assay that specifically detects the equivalent of 108 *P. falciparum* parasites/µL. Optimization of the isothermal amplification process should improve the limit of detection of the integrated assay diagnostic platform by 107 fold, such that it is comparable to the sensitivity of conventional microscopy, yielding a simple, sensitive alternative for malaria diagnosis in resource-limited environments.

558

PERFORMANCE OF THE TWO RAPID DIAGNOSTIC TESTS FOR MALARIA IN PARA STATE, BRAZILIAN AMAZON

Danielle Regina Barbosa[1], Giselle Maria Viana[1], Nathália Nogueira Chamma[1], Roberto A. Franco[2], Marinete M. Póvoa[1]

[1]Instituto Evandro Chagas, Ananindeua, Brazil, [2]Laboratório Central de Saúde Pública do Estado do Pará, Belém, Brazil

Rapid diagnostic tests (RDTs) for malaria can increase the availability of diagnostic methods, especially in remote areas and in epidemic situations. These tests use immunochromatographic methods to detect *Plasmodium*-specific antigens in blood samples and, differently from microscopy, results can be readily available with minimal equipment and technical expertise. Thus, RDT use tends to increase in the coming years, but they usually present limitations to detect malaria infection, especially in low parasitemic infections. Our objectives were to determine sensitivity, specificity and accuracy of SDBioline® and OptiMAL-IT® in detecting *Plasmodium spp.* in an endemic area of Para State. We used light microscopy as the gold standard. Blood samples were collected from individuals with clinical

signals suggestive of malaria in the Tucurui municipality. Thick smears and RDTs were performed. Out of the 90 samples analyzed, 15.5% (14/90) were positive (range of parasitemia: 0.001%-2%) and 84.4% (76/90) negative by microscopy. OptiMAL-IT® detected 12.2% (11/90) positives and 87.8% (79/90) negatives, and SDBioline® 8.9% (08/90) and 91.1% (82/90), respectively. The statistical parameters obtained by OptiMAL-IT® in relation to microscopy were sensitivity of 78.6%, specificity of 100%, and accuracy of 96.67%; by SDBioline® we found sensitivity, specificity, and accuracy of 50.0%, 96.7% and 91.1%, respectively. Kappa agreement index was 86.1% for the OptiMAL-IT® (almost perfect agreement) and 59.0% for SDBioline® (moderate agreement). These results suggest that OptiMAL-IT® can be used with caution in remote areas of the Brazilian Amazon Region and its sensitivity decline in parasitemias below 500 parasites/µL. For SDBioline®, it is advisable other studies to better assess its performance.

559

DEVELOPMENT OF FIELD-USABLE DRY FORMAT ASSAY FOR THE QUALITATIVE DETECTION OF G6PD ENZYME ACTIVITY

Hyeonsuk Kim, Seung Hee Han, Tae-Hee Koo, Jaean Jung, Young Ho Choi

Access Bio Inc., Monmouth Junction, NJ, United States

Glucose-6-Phosphate Dehydrogenase deficiency is the most common human enzyme deficiency affecting 400 million people. It is an X-linked recessive hereditary disease characterized by abnormally low levels of glucose-6-phosphate (G6PD) and is manifested as anemia, with RBCs being prematurely destroyed by oxidative stress. G6PD deficiency is highly prevalent in malaria endemic areas where 300-500 million people are infected annually with malaria. It implies that many patients that are treated for malaria with oxidative anti-malarial drug will suffer from the treatment because of oxidative stress in their G6PD deficient erythrocytes. To prevent this, it is important to screen G6PD deficiency before treatment with oxidant drugs. Most malaria prevalent areas are economically underdeveloped, and have limited healthcare facilities and limited trained medical care staffs. To meet the need in field situations, we have developed a rapid one step dry format assay for the qualitative detection of G6PD enzyme activity. The assay is based on a formazan method using tetrazolium compound which the color turns yellow to purple under reducing condition. Test strip use capillary power to develop visual signal in the window of device. By using two differential strips with two windows in the device, this test kit allows to distinguish the severity of deficiency. Accelerated stability studies showed that test strips were stable for 2 months at 45oC and 3 days at 60oC. The test procedure is very simple. Just add 2 µl of whole blood to the sample well in the device and followed by adding 2 drops of assay buffer in the assay buffer well. There is no need for the pre-lysis of red blood cells with lysing buffer, a step required by most conventional assays. Since the sample volume needed is only 2 µl, it is possible to use capillary blood as well as venous blood. The assay is rapid (<10 min), easy to operate, inexpensive, portable, and has no special storage requirements.

560

EVALUATION OF TWO RAPID DIAGNOSTIC TESTS FOR MALARIA (OPTIMAL-IT® AND PALUTOP+4®) IN AN ENDEMIC AREA OF PARA STATE, BRAZILIAN AMAZON REGION

Giselle Maria Viana, Nathália Nogueira Chamma, Danielle Regina Barbosa, Ediclei Lima do Carmo, José Maria Nascimento, José Mário Peres, Marinete M. Póvoa

Instituto Evandro Chagas, Ananindeua, Brazil

Rapid diagnostic tests (RDTs) are of simple and fast implementation and some tests may differentiate between *Plasmodium falciparum* and non *P. falciparum*. They are especially useful in situations where conventional microscopy is difficult. This study aimed to evaluate the accuracy of OptiMAL-IT® and PALUTOP+4® in detecting human malaria in Para State, Brazil and to analyze their performance against conventional microscopy and nested-polymerase chain reaction (PCR). Blood samples were collected from individuals with clinical signals suggestive of malaria in the Tucurui municipality. Thick smears, RDTs, and PCR tests were performed. Out of 178 samples, 64.6% (115/178) were positive (range of parasitemia: 0.001%-2%) and 35.4% (63/178) negative by microscopy; OptiMAL-IT® detected 47.8% (85/178) positives and 52.3% (93/178) negatives, and PALUTOP+4® 75.8% (135/178) and 24.2% (43/178) respectively. Nested-PCR detected 66.9% (119/178) positive and 33.2% (59/178) negative samples. The statistical parameters obtained by OptiMAL-IT® in relation to microscopy were sensitivity of 73.9%, specificity of 100%, and accuracy of 83.2%; sensitivity, specificity, and accuracy by PALUTOP+4® were 85.2%, 53.8% and 72.3%, respectively. Kappa agreement index for OptiMAL-IT® was 66.7% when compared to microscopy and 62.4% when compared to nested-PCR (substantial agreement); the same index for PALUTOP+4® was 40.5% (moderate agreement) and 32.0% (fair agreement) compared to microscopy and nested-PCR, respectively. Thus, these results suggest that OptiMAL-IT® can be used with caution in areas of difficult access of the Amazon region and PALUTOP+4® needs further investigation in different transmission settings.

561

EPIDEMIOLOGY OF MALARIA DIAGNOSTICS WITH THE INTRODUCTION OF RAPID DIAGNOSTIC TESTS IN AFRICAN REFUGEE CAMPS, 2007-2008

David A. Townes[1], Christopher Blanton[1], Christopher Haskew[2], S. Patrick Kachur[1], Holly Williams[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]United Nations High Commissioner for Refugees, Geneva, Switzerland

In Africa approximately 1.1 million refugees are under the protection of the United Nations High Commissioner for Refugees (UNHCR), with the majority living in malaria-endemic areas. Historically, malaria has been diagnosed clinically or by microscopy. Introduced in African refugee camps in 2008, malaria rapid diagnostic tests (RDTs) offer a portable, rapid, easy to use and potentially cost effective addition to malaria diagnostics. We describe changes in malaria testing and case confirmation in the year following the introduction of RDTs in refugee camps in six African countries. In the UNHCR health information system (HIS), health indicators, including malaria diagnostics, are recorded on a paper form at camp level and electronically at country level for subsequent analysis. Malaria confirmation is by microcopy or RDT. Malaria diagnostics data from Chad, Ethiopia, Kenya, Sudan, Tanzania, and Uganda (2007-2008) were analyzed with SAS version 9.2 and Microsoft Office Excel 2003. In their first year of introduction, over 105,000 RDTs were performed. Malaria testing increased in five of the six countries (median 352%, *range 20-1543%*) with RDTs accounting for the majority of the increase in Chad, Ethiopia and Tanzania. Malaria testing decreased in Kenya. The percentage of malaria cases that were confirmed increased in Chad, Ethiopia and Kenya (median 19%, *range 10-28%*), decreased in Sudan (5%) and Tanzania (14%) and was unchanged in Uganda. During their first year of introduction, the use and impact of RDTs varied widely. The observed differences among countries are likely due, in part, to inconsistent integration of RDTs into existing guidelines for use of diagnostic tests in suspected malaria, disparities in training of staff on the use of RDTs, and inconsistent availability of both RDTs and microscopy supplies. These results indicate a willingness to use RDTs to supplement existing diagnostics but highlight the need for specific guidelines and training for their integration in these settings to meet 2010 WHO Guidelines for Diagnosis and Treatment of Malaria.

562

A NEWER WAY TO CATCH THE AGE-OLD BUG

Shujaat A. Khan, Deborah Asnis, Elisa Bravo

Flushing Hospital Medical Center, Flushing, NY, United States

Worldwide, an estimated 350-500 million clinical cases and approximately 1 million deaths caused by malaria occur annually, primarily among children aged <5 years living in sub-Saharan Africa (*1*). The majority of the malaria cases diagnosed in US are imported from malaria-endemic regions. A Giemsa-stained blood film is usually the first test for malaria detection but diagnostic accuracy depends on film quality and expertise of laboratory personnel. Effective treatment of malaria requires precise laboratory diagnosis of the four different *Plasmodium* species (*P. falciparum*, *P vivax*, *P ovale* and *P malariae*). A nineteen year old male had recently emigrated from Afghanistan came to the Emergency department with complaints of fever and chills associated with fatigue and generalized myalgia. Physical examination was remarkable for a temperature of 103F and pallor. Initial labs showed hemoglobin of 11.5 g/dL, white blood cell count of 3.4 x 109/L and platelets were 44 x 109/L. Total Bilirubin was 4.1 mg/dL and rest of the blood chemistry was normal. Initial Malaria smear was reported positive for *P Ovale* by both the Hospital Microbiology Lab and State Public Health Laboratory. However, Polymerase chain reaction (PCR) testing of the blood sample later identified the species as *P Vivax*. He was treated with the appropriate dose of Chloroquine followed by primaquine to eliminate latent hypnozoites. The patient responded well to the treatment and was discharged home. In conclusion, successful treatment of Malaria necessitates accurate diagnosis of the offending *Plasmodium* species. Cure of *P vivax* and *P ovale* mandates treatment to eradicate liver hypnozoites where as *P falciparum* infection can result in multiorgan failure requiring parenteral treatment. Microscopy which is quick and cheap can sometimes misidentify the *Plasmodium* species. PCR is a useful complement to microscopy in order to reliably identify the different *Plasmodium* species especially in situations where there is low level of parasitaemia, mixed infections and when there is lack of trained laboratory personnel.

563

QUALITY OF ARTEMISININ-BASED COMBINATION THERAPY PRESCRIPTION AND DISPENSING IN BAMAKO, MALI, WEST AFRICA

Mahamadou Diakite, Eve Sangaré, Samba Diop, Benoit Dembélé, Seidina A. Diakité, Saibou Doumbia, Karim Traore, Drissa Konaté, Sory Ibrahima Diawara, Mory Doumbia, Seydou Doumbia, Sékou Fantamady Traore

University of Bamako, Bamako, Mali

Increasing resistance of malaria parasites to chloroquine has pushed many African countries to adopt artemisinin-based combination therapy (ACT) for the treatment of uncomplicated malaria. Correct use of the ACT strategy is imperative to guarantee the effectiveness of treatment and avoid the spread resistance to ACT. We have conducted this study in order to assess the quality of the prescription and the dispensation of ACTs in randomly selected health centers across Bamako. Our study was a cross-sectional study conducted between April and July of 2008. An interview, via questioner, was administered to patients presenting at the clinic for malaria and to physicians, pharmacists, and other health workers who give prescriptions of antimalarials or work in the pharmacy. In total, 52 prescribers, 72 dispensers and 92 patients were included. Our study has shown that the ACT constituted the primary malaria treatment of choice among prescribers (75%) and dispensers (78.8%). 59.7% of dispensers and 73.1% of prescribers were reported that they were aware of the ACT recommendations by the National Malaria Program (NMCP). The majority of the prescribers (71.15%) and of the dispensers (84.72%) followed the ACT recommendations of the NMCP. However, 57.61% of the prescriptions against malaria did not contain ACT. Many patients (41.30%) did not understand the dosing of the prescribed ACTs which may increase likelihood of emergence of resistance to ACT. Almost all of the prescription containing ACT was a generic drug (97.72%; n = 44). The prices of the ACTs varied between 140 and 3.380 FCFA with an average of 750 FCFA (1 dollar = 500 FCFA). According to prescribers and dispensers, ACT constitutes their first choice (75% of prescribers and 78.8% of the dispensers). However, 57.61% of the prescriptions against malaria did not contain any ACTs. The majority of prescribers (71.15%) and dispensers (84.72%) were favorable to the NMCP's recommendations of malaria treatment in Mali.

564

LOW GAMETOCYTE DENSITIES RESTRICT THE DEVELOPMENT OF *PLASMODIUM FALCIPARUM* WITHIN *ANOPHELES GAMBIAE* WITH IMPLICATIONS FOR THE HUMAN RESERVOIR OF INFECTION AND PARASITE ELIMINATION

Thomas S. Churcher[1], Teun J. Bousema[2], Chris J. Drakeley[2], Caroline Harris[3], Anna Cohuet[4], Isabelle Morlais[5], Dina Vlachou[1], Emma J. Dawes[1], María-Gloria Basáñez[1]

[1]Imperial College London, London, United Kingdom, [2]London School of Tropical Hygiene and Medicine, London, United Kingdom, [3]Institut de Recherche pour le Développement, Montpellier, France, [4]Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso, [5]Organisation de Coordination de la lutte contre les Endémies en Afrique Centrale, Yaounde, Cameroon

Density-dependent processes regulating the development of the malaria parasite within the mosquito may influence parasite transmission and could have important implications for the control and the elimination of the parasite. Data from mosquito feeding experiments conducted on naturally found parasite-vector combinations from across Africa were collated to generate a dataset of more than 12,000 mosquitoes which had been fed on blood from a total of 327 different human patients. Gametocytemia was estimated by either microscopy or quantitative nucleic acid sequence-based amplification. Mosquito infectivity was assessed by both the presence of viable oocysts and the number of oocysts identified in infected mosquitoes. A range of mathematical techniques was used to show that the relationship between gametocytemia and oocyst presence and density was best described by a sigmoidal curve, indicating that sporogonic development is restricted at both low and high gametocyte densities. Gametocytemia surveys conducted in Burkina Faso are used to illustrate how these density-dependent regulatory processes will influence the contribution of children to overall transmission. The implications of the results for prospects of malaria elimination are discussed.

565

IMPACT OF INFRASTRUCTURE DEVELOPMENT SUPPORT ON CLINICAL TRIALS CAPACITY DEVELOPMENT IN AFRICA: INDEPTH-NETWORK-MALARIA CLINICAL TRIALS ALLIANCE

Rita Baiden[1], Bernhards Ogutu[1], Peter Smith[2], Fred Binka[1]

[1]INDEPTH Network, Accra, Ghana, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

The Malaria Clinical Trials Alliance (MCTA), a programme of INDEPTH-Network, was launched in 2006 with two broad objectives: to facilitate the timely development of a network of centres in Africa with the capacity to conduct clinical trials of malaria vaccines and drugs under conditions of Good Clinical Practice (GCP); and to support, strengthen and mentor the centres to facilitate their progression towards self-sustaining research centres. Sixteen research centres or sites in 10 African malaria-endemic countries that were already working with the Malaria Vaccine Initiative or the Medicines for Malaria Venture were selected. Assessment visits based on a standard questionnaire were conducted for all the sites to assess their strengths and requirements for research capacity strengthening, in order to conduct a phase lll malaria vaccine and drug trials. Assessments were made of the needs for infrastructure strengthening and short-term

human capacity development together with the long term sustainability of the centres. Support provided by MCTA included: construction and refurbishment of clinical trial and laboratory facilities, provision of major laboratory and clinical equipment for trials and clinical care, strengthening of the data and financial management systems, GCP and malaria microscopy networking including accreditation and microscopy external quality programmes. Sites were mentored and supported to develop strategic plans for long term sustainability. In 4 years, MCTA strengthened 13 sites to perform internationally acceptable GCP-compliant drug and vaccine trials, including 11 centres that are conducting a very large phase III malaria vaccine trial. The key improvements at the sites, including short and long term impact on the activities of the sites, will be presented. In conclusion, MCTA has demonstrated that clinical research capacity development in Africa is feasible and with modest resources, research centres in Africa can be brought up to GCP compliance standard to conduct research to an internationally acceptable standard.

566

TRENDS IN MALARIA MORBIDITY AMONG HEALTH CARE-SEEKING CHILDREN UNDER AGE FIVE IN MOPTI AND SÉVARÉ, MALI BETWEEN 1998 AND 2006

Seydou Doumbia

Malaria Research and Training Center, University of Bamako, Bamako, Mali

In Mali, malaria is the leading cause of death and the primary cause of outpatient visits for children under five. The twin towns of Mopti and Sévaré have historically had high under-five mortality. We investigated the changing malaria burden in children under five in these two towns for the years 1998-2006, and the likely contribution of previous interventions aimed at reducing malaria. We conducted a retrospective analysis of daily outpatient consultation records from urban community health centers (CSCOMs) located in Mopti and Sévaré for the years 1998-2006, and assessed risk factors for a diagnosis of presumptive malaria using logistic regression and trends in presumptive malaria diagnostic rates using multilevel analysis. Between 1998-2006, presumptive malaria accounted for 33·8% of all recorded consultation diagnoses (10,123/29,915). The monthly presumptive malaria diagnostic rate for children under five decreased by 66% (average of 8 diagnoses/month per 1,000 children in 1998 to 2·7 diagnoses/month in 2006). The multi-level analysis related 37% of this decrease to the distribution of bednet treatment kits initiated in May of 2001. Children of the Fulani (Peuhl) ethnicity had significantly lower odds of a presumptive malaria diagnosis when compared to children of other ethnic groups. In conclusion, presumptive malaria diagnostic rates have decreased between 1998-2006 amongst health-care seeking children under five in Mopti and Sévaré, and a bednet treatment kit intervention conducted in 2001 is likely to have contributed to this decline. Our results corroborate previous findings suggesting that the Fulani ethnicity is protective against malaria. Our findings are useful to encourage dialogue around the urban malaria situation in Mali, particularly in the context of achieving the target of reducing malaria morbidity in children younger than five by 50% by 2011 as compared to year 2000 levels.

567

EPIDEMIOLOGY OF *PLASMODIUM FALCIPARUM, P. VIVAX,* AND ZOONOTIC *P. KNOWLESI* IN SOUTHERN MINDANAO, THE PHILIPPINES

Mary Grace B. Dacuma[1], Rachel Hallett[2], Judeline Dimalibot[3], George Ugaddan[3]

[1]London School of Hygiene and Tropical Medicine and University of the Philippines Los Banos, London, United Kingdom, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom, [3]University of the Philippines Los Baños, Los Baños, Philippines

The Philippines has set the goal to be malaria-free by 2020. However, *Plasmodium falciparum* and *Plasmodium vivax* cases continue to cause significant morbidity in several areas of the country. In Southern Mindanao, transmission 'hot spots' and drug sensitivity, particularly of *P. falciparum*, has not been well studied. Such information is needed to contribute to the overall aim of malaria elimination in this region. We plan to determine whether *P. falciparum* parasites from Southern Mindanao are resistant to chloroquine, sulfadoxine-pyrimethamine, and artemether-lumefantrine using established and proposed molecular markers for drug resistance. We will also determine the transmission intensity of malaria using serological markers of infection and check for the presence of human *P. knowlesi* infection as this has been reported in Palawan in 2008 and in neighbouring Southeast Asian countries. We will conduct a cross-sectional survey in three provinces of Southern Mindanao namely Sarangani and South Cotabato (Region XII), and Tawi-Tawi (Autonomous Region for Muslim Mindanao) from June to July 2010. Consenting participants will answer a questionnaire survey covering personal, demographic and socio-cultural information as well as clinical history of malaria. We will collect a finger prick blood sample from each participant on a rapid diagnostic test strip Falcivax® to check for presence of *P. falciparum* and *P. vivax* malaria, and blood spots on filter paper to be transported to LSHTM for laboratory analyses. *Plasmodium* species presence and *P. falciparum* drug resistance markers will be assessed using molecular methods. We will also use the blood spots to detect and measure antibodies to merozoite surface proteins MSP1 and MSP2, and apical membrane antigen (AMA) using indirect ELISA.

568

MALARIA IN THE CONTEXT OF PREGNANCY: PRELIMINARY RESULTS FROM A QUALITATIVE STUDY IN THE EJISU-JUABEN DISTRICT, CENTRAL GHANA

Arantza Meñaca[1], Nana A. Afrah[2], Lianne Straus[1], Christopher Pell[1], Erin V. Andrew[1], Harry Tagbor[2], Robert Pool[1]

[1]CRESIB, Barcelona, Spain, [2]Department of Community Health, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

This presentation discusses how pregnant women integrate malaria into everyday knowledge and experiences of pregnancy and pregnancy care. The data presented are drawn from a wider anthropological study on the social context of malaria in pregnancy (MiP) in different settings where MiP clinical trials are underway. Data collection included free-listing and sorting exercises, in-depth interviews, group discussions and case studies with pregnant women, their relatives, biomedical and traditional health providers, opinion leaders and other community members. In the Ejisu-Juaben District, pregnancy is regarded as a period when women's bodies are weaker than usual "because of the baby", which is considered to take part of its mother's "blood"/ strength. In this setting, malaria is usually perceived as more frequent and serious in pregnant women than in non-pregnant women. However, knowledge of the risks of MiP, and awareness of malaria infection differ depending on a woman's age, and previous MiP experiences. In order to care for their pregnancies, women attend ANC and use traditional medicines. Nevertheless, biomedicine is the principal choice for the treatment of malaria, and although self-medication is common outside pregnancy, there is a consensus that pregnant women must only take the medicines given to them in the hospital or the clinic. In conclusion, malaria is considered one of the health problems associated with pregnancy, and pregnant women mainly seek treatment for malaria from ANC and maternity wards. Adolescents' lack of accurate knowledge about MiP compounds the vulnerability of pregnant women in this age group.

569

MALARIA AND ANEMIA AT DELIVERY IN HONIARA, SOLOMON ISLANDS

Bridget Appleyard[1], Lyndes Wini[2], Junilyn Pikacha[2], Jeffrey Hii[3], Makiva Tuni[2], Judith Seke[2], James McCarthy[1], Levi Hou[2], Albino Bobogare[2], Anna Maria van Eijk[4]

[1]Queensland Institute of Medical Research, Brisbane, Australia, [2]Ministry of Health and Medical Services, Honiara, Solomon Islands, [3]World Health Organization, Manila, Philippines, [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Malaria and anaemia are common problems in pregnancy in developing countries in tropical zones. From May 2009, a cross-sectional survey was undertaken among women who delivered at the National Referral Hospital, Honiara, Solomon Islands to investigate these problems. Information was collected by interview, from obstetric records, and blood was obtained for a maternal smear and haemoglobin assessment. A placental smear was also obtained. By January 2010 1995 women had been enrolled which comprised 57% of all deliveries in the hospital. The prevalence of anemia (Hb< 11 g/dl) was 49.6% and of moderate to severe anemia (Hb< 8 g/dl) 8.2%. Malaria was detected in 5.0% (28/557) of the maternal smears verified to date, mostly *falciparum* (22/28:78.6%); 3 of these women had a temperature > 37.5oC. Malaria was detected in 2.0% (18/866) of the placental smears verified. Risk factors for anaemia included first or second pregnancy (OR 1.2, 95% CI 1.01-1.44, P=0.03); aged under 25 (OR 1.23, 95%CI 1.02-1.48, P=0.03); complicated delivery (OR: 1.9, 95%CI 1.20-3.06, P=0.01); infrequent use of iron and folate supplements (OR 1.28, 95%CI 1.03-1.58, P=0.02); living outside of Honiara (OR 1.27, 95%CI 1.05 to 1.53, P=0.01); and being Polynesian or Micronesian (OR: 1.63;95%CI:1.10-2.37;P=0.01) compared to Melanesian. Use of malaria prevention interventions was common: 44.4% (594/1337) had screened windows, 53.5% (1064/1990) used a bed net during the pregnancy, and 88.5% (1763/1992) reported weekly use of chloroquine prophylaxis. Knowledge of the cause of malaria transmission was high (1631/1907: 85.5%) as was knowledge that malaria is more dangerous for pregnant compared to non-pregnant women (1820/1892: 96.2%). Ante-natal clinic attendance and use of iron and folate supplements was high (1942/1980:98.1%, and 1539/1985:77.5%, respectively). Albendazole was received by 59.4% (1147/1930) of women who attended ante-natal clinic. Final results will be presented at the meeting. In conclusion, while malaria was uncommon at delivery, anaemia was highly prevalent in this population, and requires further study to explore ways to improve this.

570

ANEMIA AND MALARIA IN THE DEMOCRATIC REPUBLIC OF CONGO

Carla C. Hand[1], Steve Taylor[2], Kashamuka Mwandagalirwa[3], Jeremie Muwonga[4], Augustin Okenge[4], Atua Matindii[5], Antoinette K. Tshefu[3], Steven R. Meshnick[1]

[1]University of North Carolina-Chapel Hill, Chapel Hill, NC, United States, [2]Duke University, Durham, NC, United States, [3]Ecole de Sante Publique, Faculte de Medecine, University of Kinshasa, Kinshasa, The Democratic Republic of the Congo, [4]Programme National de Lutte contre le SIDA, Kinshasa, The Democratic Republic of the Congo, [5]Programme National de Lutte contre le Paludisme, Kinshasa, The Democratic Republic of the Congo

Malaria and anemia are both highly prevalent in the Democratic Republic of Congo; however there are many causes of anemia and the importance of malaria remains unclear. We used molecular results from the 2007 Demographic and Health Survey to assess the relationship between malaria and anemia in 4,574 women throughout the country. Blood was collected for on-site hemoglobin testing and stored as dried blood spots, from which genomic DNA was extracted for testing in real-time PCR assays for *Plasmodium falciparum*, *P. malariae*, and *P. ovale*. The prevalence of parasitemia was 28.5% (n=1303). The prevalence of mild (hemoglobin [Hgb] < 11.5 g/dL), moderate (Hgb < 9g/dL), and severe anemia (Hgb<7g/dL) was 33.95%, 14.9% and 1.2% respectively. In bivariate analyses, anemia (defined as Hgb <11 g/dL) was more prevalent in patients living in rural settings compared with urban (32% v. 26%), in poorer compared with wealthier patients (31% v. 26%), in patients with lower body mass indices, in pregnant compared with non-pregnant patients (44% v. 27%), and in patients infected with HIV (47% v. 29% uninfected) or with malaria parasites (32% v. 28% uninfected; all p < 0.01). In a logistic regression model, malaria parasitemia (OR 1.2; 95% C.I. 1.03 - 1.38), HIV infection (OR 2.7; 95% C.I. 1.69 - 4.31), pregnancy (OR 2.3; 95% C.I. 1.91 - 2.66), rural residence (OR 1.4; 95% C.I. 1.13 - 1.62), and low BMI were independently associated with anemia (all p<0.02). Among multi- and mono-species infection, only *P. falciparum* monoinfection was independently associated with anemia (OR 1.2; 95% C.I. 1.05 - 1.42; p < 0.01); combination or monoinfections with P. malariae or P. ovale were not significantly associated with anemia. Independent of other measured correlates, *P. falciparum* is an important contributor to anemia in women the Democratic Republic of Congo.

571

GENES POLYMORPHISMS (CYTOKINE, HBB, G6PD AND TNF) IN A HIGH AND SEASONAL MALARIA TRANSMISSION AREA OF BURKINA FASO

Edith C. Bougouma, Alfred Tiono, Issiaka Soulama, Alphonse Ouedraogo, Amidou Diarra, Amadou T. Konaté, Issa Nébié, Sodiomon B. Sirima

Centre National de Recherche et de Formation sur le Paludisme (CNRFP), Ouagadougou, Burkina Faso

Genetic studies showed that genes polymorphism such as Haemoglobin, cytokine, G6PD and ABO are associated with susceptibility to malaria in Africa. These SNP showed powerful selective pressure of malaria (Kwiatkowski and al;, 2000). In this study we aimed to assess genes polymorphism genetic variation of HbS, G6PD, ABO and Cytokine genes according to malaria indicators in children less than five years in a site being characterised for future malaria vaccine trials. The study was carried out in rural villages close to Ouagadougou. We performed a transversal survey during the high malaria transmission season (August). During the survey, we examined 817 children. Blood smears were taken for thick and thin films and a venous sampling was taken for human genetic tests (Cytokine, HbS and ABO). HbAS and HbAC phenotypes were present respectively in 174(19,5%), and 78(8,7%) of 817 subjects. The prevalence of the G6PD genotype was 28,5% for females and 20% for males. IL1A, IL1B were found in 142 (28.7%) and 170 (21.5%) children. IL10 (IL10_232424450), IL10 (hIL-10-1082), IL10 (hIL-10-3533) were found in 395 (50.4%) and 170 (21.5%) subjects. Genotype distribution of children with IL4, IL13 and IL17 was 33.4 %, 28.3% and 48.9% respectively. TNFα376: TNFα308, TNFα238 were respectively 1.5% and 23%. Significant difference was found for Cytokine and TNF (P= 0,001) in term of malaria infection. In conclusion, these results revealed a high frequency of Haemoglobin, cytokines, and G6PD in the study population. These findings suggest that the presence of SNP (Cytokine, G6PD and HBB) could reduce malaria infection. This should be taken into account in the interpretation of malaria trials results.

572

VARIATION IN THE CIRCUMSPOROZOITE PROTEIN OF *PLASMODIUM FALCIPARUM*: IMPLICATIONS FOR MALARIA VACCINE DEVELOPMENT

Kavita Gandhi[1], Mahamadou A. Thera[2], Drissa Coulibaly[2], Karim Traore[2], Ogobara K. Doumbo[2], Shannon Takala-Harrison[1], Christopher V. Plowe[3]

[1]Center for Vaccine Development, University of Maryland, Baltimore, MD, United States, [2]Malaria Research and Training Center, University of Bamako, Bamako, Mali, [3]Howard Hughes Medical Institute, Baltimore, MD, United States

The leading malaria vaccine candidate RTS,S/AS01 is based on immunogenic regions of *Plasmodium falciparum* circumsporozoite protein (CSP) from the 3D7 strain, and has limited efficacy against clinical disease in African children. It is unclear, however, what aspect of the immune response elicited by the vaccine is protective and whether polymorphism in CSP affects efficacy. Better understanding of how diversity in CSP T-cell and B-cell epitopes relates to clinical immunity is needed to evaluate and improve the efficacy of vaccines based on CSP. The goal of this study is to measure diversity in these immunogenic regions and to identify associations between variation in amino acid sequences in CSP and the risk of infection and clinical disease caused by *P. falciparum*. A prospective cohort study was conducted to measure the age-specific incidence of malaria infection and disease in children and young adults living in Bandiagara, Mali. For this study, a subset of 100 children contributed 2,309 samples of finger-stick blood spots collected in asymptomatic monthly surveys and during acute clinical malaria episodes. Amplified T-cell and B-cell regions of the cs gene are being subjected to 454 sequencing, a powerful method for detecting diversity in complex infections. Cox proportional hazards models are being used to determine the effect of sequence variation in individuals' consecutive infections on the time to new infection and new clinical malaria episode. Preliminary analyses revealed that with >500X average coverage (range ~200-1,000X), 454 sequencing of 45 randomly selected samples had a high degree of variation in the T-cell regions Th2R and Th3R in the form of single nucleotide polymorphisms (SNPs). 24 SNPs were found in Th2R and 14 in Th3R, all non-synonymous, resulting in 72 unique Th2R haplotypes and 14 Th3R haplotypes. Only three of 45 samples (7%) are identical to the 3D7 vaccine strain in both epitopes, raising the possibility that parasite genetic diversity may limit efficacy of CSP-based vaccines, if protection is strain specific.

573

IS THE RISK OF *PLASMODIUM FALCIPARUM* MALARIA INCREASING IN VERY YOUNG CHILDREN WHO ARE VFRS? ARE THEIR PARENTS CONSCIENTIOUS OF THIS RISK?

Antonio Soriano Arandes, Olga Calavia Garsaball, Frederic Gómez Bertomeu, Clara Bras Boqueras, Sergi César Díaz, Meritxell Gellida Royo

Hospital Universitari Joan XXIII, Tarragona, Spain

Plasmodium falciparum malaria is a highly endemic disease in most of the sub-Saharan countries. Nigeria has the largest population of Africa and their emigrants to Tarragona (Spain) represent the second African number of inhabitants in this region. It is known that immigrant people returning home to visit friends and relatives (VFRs) are the highest risk traveling population for contracting malaria because they lost their pre-existing acquired immunity against *P. falciparum* and they also assume that they are "immune" for the infection leading to a lower compliance of the anti-malarial prophylaxis. The epidemiological patterns of VFRs are changing and, as a consequence of better socioeconomic and life conditions in the country of residence, more immigrant families are now traveling with their children to the African countries. We describe three pediatric cases of malaria diagnosed at the University Hospital Joan XXIII, Tarragona (Spain), member of the *TropNet Europ* for imported infectious diseases surveillance, in a one-year period 2009-2010. They all were born in Tarragona and diagnosed after traveling with their family as VFRs to Nigeria with a mean stay of 83.3 days. Two children received pre-travel counselling and anti-malarial prophylaxis with mefloquine but none of them achieved a good compliance. The youngest case was unable to receive mefloquine because of his age (4-month old) and the no seeking pre-travel health counseling. Ages ranged from 4 to 17 months old. Day of onset of the fever ranged from 6 to 13 days with a mean of 9.7 days after returning of the travel. In spite of being visited twice at emergency department of the hospital, diagnosis of malaria was delayed in two patients for 7 days. One of them was classified as a complicated malaria (parasitemia of 5%) complying criteria established by the WHO. PCR technique was positive for *P. falciparum* in the three cases. Lowest hemoglobin was 5.13gr/dL and lowest hematocrit was 14.6%. All of them were treated as inpatient cases with intravenous quinine and clindamycin and two also received antibiotic for associated bacterial pneumonia. The purpose of this study is to show these pediatric malarial cases to sensitize the medical professionals working in Tropical Medicine for the increasing importance of very young children travelling as VFRs and having a high risk to contract malaria in their familial origin countries.

574

ACTIVE SURVEILLANCE OF MALARIA IN MILITARY AREAS OF OPERATION (AOS) ALONG NORTHERN THAI-MYANMAR AND THAI-NORTHERN CAMBODIA BORDERS DURING 2004-2009

Narupon Kuttasingkee, Toon Ruang-areerate, Pradith Kaewsatien, Kiatisak Somsri, Khwananong Youngpakool, Bungauang Indontri, Chaiya Chanchu, **Jariyanart Gaywee**

Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Military areas of operation (AOs) along Northern Thai-Myanmar and Thai-Cambodia borders are known as malaria endemic. Therefore, immune naive troops who are deployed to these areas are at risk for malaria infection. Upon infection, soldiers are evacuated from the AO causing a force reduction. Malaria surveillance is crucial for effective malaria prophylaxis and decreases disease non-battle injury (DNBI). We have conducted a continuous surveillance program to obtain the epidemiological information of malaria in these bordered AOs since 2004. Each fiscal year, area-deployed troops were monthly screened for malaria infection in peripheral blood using rapid test and microscopic confirmation. Additional surveillance data were also collected from the healthcare providers in the area. From 2004 to 2009, malaria infections in army troops deployed to AO along northern Thai-Myanmar border were 13.2%, 5.1%, 9.6%, 5.2%, 4.9% and 12.2%, respectively. Whereas in AO along Thai-northern Cambodia border, 8.2% 4.0% 7.4% 8.6% 4.2% and 21.3% of deployed troops were infected with malaria during the same period. Malaria cases occurred in two peaks every year from October to February and May to July. An interesting point was that the incidence of *P. vivax* increased each year and implied a shift to primary malaria infection. Active surveillance and additional data must be collected and studied to provide understanding and implementation of efficiency protective programs and thus reduction of DNBI.

575

PREVALENCE OF MALARIA INFECTIONS AND RELATED MORBIDITY AMONG SCHOOL CHILDREN IN PARTS OF THE IMO RIVER BASIN, NIGERIA

Ikechukwu N. Dozie[1], Ijeoma J. Dozie[2], Uchechukwu M. Chukwuocha[1]

[1]Federal University of Technology, Owerri , Nigeria, [2]Federal Medical Centre, Owerri, Nigeria

This study was designed to investigate malaria signs and rates of parasiteamia among school children in Ezinihitte Local Government Area in the Imo River Basin, Imo State, Nigeria.Malaria parasite and degree of

anaemia were assessed in 469 selected primary school children, using standard parasitological and haematological mthods of diagnosis. Clinical examination was done to determine spleen size. About 12.8% of the pupils were positive for malaria parasites, 48.6% were anaemic and 11.3% had spleen enlargement. Also 4.9% of the study pupils had all three of the symptom. This study ascertained a significant association between malaria infections, anaemia, and splenomegally and identified the study area as a high risk area for malaria.There is need to enhance malaria control efforts to reduce the level of morbidity among children in the study area so as to make them more effective at school.

576

CONSEQUENCES OF PREGNANCY-ASSOCIATED MALARIA ON FETAL GROWTH IN KOROGWE, TANZANIA

Christentze Schmiegelow[1], Birgitte B. Nielsen[2], Vibeke Rasch[3], Thomas Scheike[4], Mayke Österholt[5], Daniel Minja[6], Pamela Magistrado[1], Stephanie Böstrom[7], Lydia Massawe[8], Martha Lemnge[9], John Lusingu[9], Thor Theander[1]

[1]Center for Medical Parasitology at the Institute for International Health, Immunology and Microbiology, University of Copenhagen and Copenhagen University Hospital, Copenhagen, Denmark, [2]Department of Obstetrics and Gynaecology, Aarhus University Hospital, Skejby, Denmark, [3]Department of Obstetrics and Gynaecology, Odense University Hospital, and Department of International Health at the Institute for International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark, [4]Department of Biostatistics at the Institute of Public Health University of Copenhagen, Copenhagen, Denmark, [5]Department of Medical Microbiology, Radboud University, Nijmegen, The Netherlands, [6]National Institute for Medical Research, Tanga Medical Research Center, Tanga, United Republic of Tanzania, [7]Department of Immunology at the Wenner-Gren Institute University of Stockholm, Stockholm, Sweden, [8]National Institute for Medical Research Tanga Medical Centre, Tanga, United Republic of Tanzania, [9]National Institute for Medical Research, Tanga Medical Research Centre, Tanga, United Republic of Tanzania

Pregnancy-associated malaria (PAM) has detrimental effects on mother and fetus. PAM causes low birth weight due to intrauterine growth retardation (IUGR), but the time of onset of IUGR is unknown. Recent studies have shown a relation between PAM and development of hypertensive disorders (Pregnancy Induced Hypertension (PIH) and Preeclampsia (PE)), which themselves can affect the fetus. The interrelation between PAM and hypertensive disorders is not thoroughly investigated. Furthermore, a fetal growth chart representative for an East African population is currently not available. The objective of the study is to investigate the effects of PAM on fetal growth and on the development of hypertensive disorders. A longitudinal prospective study of 1000 pregnant women is conducted in Korogwe, North-eastern Tanzania. Using ultrasound investigation, the gestational age is estimated before 24 weeks of gestation. Fetal growth is assessed on at least three consecutive ultrasound investigations, enabling us to diagnose IUGR. In parallel, screening for malaria, PIH and PE is performed during pregnancy and at delivery. PAM is diagnosed using Rapid Diagnostic Tests and placental histology. Using data from offspring of healthy mothers not diagnosed with PAM, PIH or PE a normal Tanzanian cohort is generated and a growth chart is produced. The prevalence and time of onset of IUGR among fetuses carried by mothers suffering from PAM and/or hypertensive disorders is investigated and compared with this normal cohort. Follow-up is ongoing. Preliminary analysis indicates a relative high prevalence of IUGR among women who had parasiteamia during the pregnancy. The onset of malaria induced IUGR occurs earlier in pregnancy among the primigravidae than in the multigravidae. Hence, the effect on birth weight is more pronounced among primigravidae. Furthermore a correlation is seen between PAM and the development of hypertensive disorders..

577

ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTIONS IN NAVRONGO, NORTHERN GHANA: A NEW ANALYSIS METHOD SUGGESTS DIFFERENCES IN CLEARANCE OF INFECTIONS COMPARED TO MALARIA THERAPY DATA

Michael T. Bretscher[1], Wilson Sama[1], Nicolas Maire[1], Nakul Chitnis[1], Seth Owusu-Agyei[2], Tom Smith[1]

[1]Swiss TPH, Basel, Switzerland, [2]Kintampo Health Research Center, Kintampo, Ghana

To understand the likely impact of preventive measures, it is important to know how long untreated infections persist. Longitudinal genotyping data represents the main source of information on the dynamics of natural *Plasmodium falciparum* infections. However, statistical analysis of such data is not straightforward due to ongoing re-infection and the problem of imperfect detection of asymptomatic infections. Specialized analysis methods which simultaneously estimate force of infection, detectability, and duration of infections have therefore been developed.We have now extended these methods to not only measure a mean duration of infection, but rather use various survival distributions to describe clearance of infections. This is an important step in an iterative model-finding process, which ultimately leads to a better understanding of the within-host processes in naturally exposed populations. We have applied our method to msp2 genotyping data from a one-year longitudinal study on asymptomatics in all age groups, conducted in Navrongo, Northern Ghana. The results suggest pronounced differences in the distribution of infection durations compared to malariatherapy data. Part of the infections in the natural population appear to be of relatively short duration, with some infections persisting for a long time. By using age of the human host as a proxy for cumulative exposure, we were able to exclude acquired immunity as possible cause. This indicates that other factors, such as the genetics of human or parasite populations may be responsible for the observed differences between natural infections and malariatherapy data. Ongoing research investigates the robustness of these results with respect to more explicit modeling of additional features of within-host dynamics, such as the decrease of parasite densities over the time course of an individual infection, which lowers the probability of detection.

578

QUANTIFYING THE BURDEN OF PREGNANCY-ASSOCIATED MALARIA IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Steve M. Taylor[1], Jane Messina[2], Carla C. Hand[1], Kashamuka Mwandagalirwa[3], Antoinette K. Tshefu[3], Atua Matindii[4], Jeremie Muwonga[5], Michael Emch[2], Steven R. Meshnick[1]

[1]University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC, United States, [2]University of North Carolina Department of Geography, Chapel Hill, NC, United States, [3]Ecole de Sante Publique, University of Kinshasa, Kinshasa, The Democratic Republic of the Congo, [4]Programme National de Lutte contre le Paludisme, Kinshasa, The Democratic Republic of the Congo, [5]Laboratoire National de Reference SIDA et IST, Kinshasa, The Democratic Republic of the Congo

Pregnancy-associated malaria reliably contributes to poor birth outcomes, but its true prevalence is unknown. We report the first estimate of burden based on a nationally-representative survey. Malaria prevalence was measured by real-time PCR in 4,574 women aged 15-49 years old responding to the 2007 Democratic Republic of the Congo Demographic and Health Survey. 520 (11%) women were pregnant when surveyed, and a further 954 (21%) women had delivered in the previous year; overall, median gravidity was 2 (IQR 2-5). Only 32% of pregnant women possessed a bednet, and 24% of all pregnant women slept under a bednet the previous night. Overall, 1,253 women (27%) were parasitemic with *Plasmodium falciparum*; there was no significant difference in parasite prevalence between pregnant women (31%) and nonpregnant women (27%; p=0.18) or between pregnant women in different

trimesters. Additionally, there was no significant difference in parasite rate between those who slept under bednets the previous night that were treated with insecticides (27%) or untreated (19%) and those who used neither (27%; p=0.32). Gravidity was associated with parasite prevalence, with primigravidae (41%) more frequently infected than secundigravidae (32%) and multigravidae (27%; p=0.02). *P. malariae* and *P. ovale* infected < 2% of women, and there were no significant differences in parasite rates by pregnancy or trimester. An estimated 3 million pregnancies occur every year in the DRC; with at least 31% of pregnant women infected with malaria, over 1 million episodes of pregnancy-associated malaria may occur every year. There is an urgent need for interventions to prevent malaria in pregnancy in the DRC.

579

IMPACT OF SUBSIDIZED ARTEMETHER-LUMEFANTRINE (AL) IN THE RETAIL SECTOR ON COVERAGE OF PROMPT EFFECTIVE TREATMENT OF CHILDREN UNDER FIVE IN KENYA: A CLUSTER RANDOMIZED CONTROLLED TRIAL

Beth B. Kangwana[1], Sarah V. Osano[1], Abdisalan M. Noor[1], Victor A. Alegana[1], Andrew J. Nyandigisi[2], Jayesh Pandit[3], Manya Andrews[4], Mbogo Bunyi[4], Greg Fegan[1], Simon Brooker[1], Robert W. Snow[1], Catherine A. Goodman[1]

[1]Kenya Medical Research Institute Wellcome Trust Research, Nairobi, Kenya, [2]Division of Malaria Control, Ministry of Public Health and Sanitation, Nairobi, Kenya, [3]Pharmacy and Poisons Board, Nairobi, Kenya, [4]Population Services International, Nairobi, Kenya

With a low proportion of children receiving the first line treatment for suspected malaria, a study was implemented to evaluate the impact of providing subsidized AL delivered through trained retail providers, and supported by communications on the coverage of prompt effective anti-malarial treatment in children 3-59 months. We employed a pre-post randomized cluster controlled design with nine control and nine intervention sub-locations, equally distributed across three districts in western Kenya. Three clusters of villages were randomly selected within each sub-location using probability proportional to size sampling, and 42 homesteads were randomly selected per cluster to participate in a household survey on treatment seeking behavior. Data was collected using structured questionnaires and analyzed using a difference in difference approach based on cluster level summaries, comparing control to intervention areas. A total of 2,749 children aged between 3 and 59 months were recruited, of which 2,662 were followed up 12 months later. 29% of children experienced fever within two weeks prior to the interview. At follow up, the percentage of children with fever receiving AL had risen by 17.5% points in the control arm (9.80% (SD:8.27) to 27.30% (SD:15.22) and 45.95% points in the intervention arm (7.74% (SD:5.05) to 53.69% (SD:12.29). The percentage of children receiving AL in the intervention arm at follow up was significantly greater than in the control arm (p=0.0001). No significant differences were observed between arms in where caregivers sought treatment for their child's fever, or in the child's adherence to AL (p>0.05). Subsidizing ACT in the retail sector can significantly increase coverage of prompt and effective treatment of malaria in rural areas. The increase in coverage observed in the control areas probably reflected improved availability of AL in public health facilities, highlighting that ensuring health facility AL stocks is also essential for improving AL access.

580

RECONSTRUCTION OF INDIVIDUAL MULTILOCUS GENOTYPES FROM MIXED *PLASMODIUM* INFECTIONS

Jordan Kemere[1], Paul Chaffee[2], Inna Gerlovina[2], Curt Hansen[2], Bryan Greenhouse[1]

[1]University of California at San Francisco, San Francisco, CA, United States, [2]University of California, Berkeley, Berkeley, CA, United States

Obtaining multilocus genotypes of *Plasmodium* parasites is essential in numerous applications in population genetics, such as tracking drug resistant parasites and understanding transmission. Determining genotypes of individual strains is challenging when multiple strains co-infect a single patient, as it is difficult to know which allele to assign to which strain. As a result, these types of infections, which are common in areas of moderate or high transmission intensity, are often ignored in population genetic analyses requiring multilocus genotypes. We developed a heuristic algorithm based on maximum likelihood to address these challenges for semi-quantitative microsatellite genotypes. The algorithm first estimates the number of unique strains and relative proportion of each, then assigns alleles to each strain. We estimated the number of strains present in a sample as the maximum number of alleles present at any given locus across all loci measured. The proportion of each strain and the allele assignments were then iteratively estimated. For loci at which multiple strains share an allele, we used maximum likelihood to assign alleles at each locus to a particular strain, optionally using a priori information about the population frequency of particular alleles or estimating these iteratively from a large data set. To test the algorithm, we simulated data for 2, 3, and 4 strains present at 10 loci based on empiric microsatellite allele frequencies measured from patient samples in Uganda. Assuming an average quantification error of 1%, the algorithm correctly assigned 99%, 97%, and 93% of alleles for 2, 3, and 4 strains. Assuming an average quantification error of 3%, the algorithm correctly assigned 97%, 91%, and 81% of alleles. Laboratory experiments are in process to determine the accuracy of both the microsatellite quantification and the algorithm using real data from 201 mixtures of 9 laboratory strains. We have developed a novel algorithm which may enable accurate determination of multilocus microsatellite genotypes from mixed *Plasmodium* infections.

581

PRECLINICAL PRIORITIZATION OF BLOOD STAGE MALARIA VACCINE CANDIDATES

Julie Healer[1], Alex Uboldi[1], Ping Cannon[1], Tony Hodder[1], Jack Richards[1], Matt O'Neill[1], Alex Maier[1], Satoru Takeo[2], Thangavelu Arumugam[2], Motomi Torii[2], Takafumi Tsuboi[2], James Beeson[1], Brendan Crabb[3], Alan Cowman[1]

[1]Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia, [2]Ehime University, Matsuyama, Japan, [3]Burnet Institute, Melbourne, Australia

A licensed vaccine for use in humans against *Plasmodium falciparum* malaria is not yet available. Until a vaccine based solely on stimulating immune responses against pre-erythrocytic developmental stages is proven to be clinically effective, there remains a need for discovery of novel candidate antigens of all life cycle stages, which can be further assessed for their potency in inducing efficacious anti-malarial immunity. We have initiated a functional screening approach to prioritizing blood-stage candidates for clinical development. Using a genome-wide approach, a cluster of 11 genes that putatively encode merozoite antigens including the MSP3/MSP6 family of proteins have been identified in a 43 kb region of *P. falciparum* chromosome ten. Population genetics studies have highlighted members of this gene cluster as among the most diverse in the genome and it is believed that immune selection has played a major role in generating this diversity. In support of this, some of these antigens are known to be targets of protective immunity have been subject to investigation as potential vaccine candidates. To assess the functional role of these antigens in merozoite invasion and to determine their potential

utility as vaccine candidates, we have characterized all of these antigens with respect to their cellular localization and interaction with red blood cells, their ability to elicit protective antibody responses and the capacity of parasites lacking these antigens through genetic deletion to invade red blood cells.

582

ANALYSIS OF AN EXTENSIVE INTEGRATED SAFETY DATABASE OF PEDIATRIC PHASE II CLINICAL TRIALS WITH THE RTS,S/AS CANDIDATE MALARIA VACCINE

Johan Vekemans, Yolanda Guerra, Marc Lievens, Sarah Benns, Didier Lapierre, Amanda Leach, Tom Verstraeten

GlaxoSmithKline Biologicals, Rixensart, Belgium

The RTS,S/AS malaria vaccine candidate is currently in Phase III clinical development in Africa. Before progressing to Phase III, safety data from 9 pediatric Phase II trials were pooled. Serious adverse events (SAE) during the whole follow-up period, unsolicited adverse events (AE) within 30 days post vaccination and laboratory safety blood markers were assessed. Relative risks (RR; RTS,S/AS over control) were calculated by Poisson regression models adjusted for study, without adjustment for multiplicity of analyses. P-values were calculated using an exact stratified test conditional on the number of cases. 2981 children under 5 years, including 617 aged <12 weeks at first vaccination, received a total of 8860 doses. The mean follow-up was 23.3 months. RTS,S/AS vaccination was associated with a significantly lower reporting rate of any SAE compared to control (RR 0.77 95%CI: 0.68-0.87;p<0.001) and a 2-fold reduction of all fatal reports (15 RTS,S/AS vs 29 controls; RR 0.49 95%CI: 0.24-0.94;p=0.031). No SAE was reported more frequently after RTS,S/AS. RTS,S/AS vaccination was associated with a reduction in malaria (RR 0.70 95%CI: 0.57-0.85;p=0.003), *P. falciparum* infection (RR 0.70 95%CI: 0.53-0.92;p=0.011), severe malaria (RR 0.61 95%CI: 0.43-0.85;p=0.003) and pneumonia (RR 0.71 95%CI: 0.52-0.97;p=0.032). After excluding malaria-related SAE, RTS,S/AS recipients still recorded significantly fewer SAE (RR 0.81 95%CI: 0.69-0.95; p=0.008). Two cases of RTS,S/AS-related simple febrile convulsions were reported. With respect to AEs, RTS,S/AS recipients recorded more upper respiratory tract infections (27.1 vs 20.6%;p=0.002), dermatitis diaper (0.6 vs 0%;p=0.01) and rash (1.1 vs 0.5%;p=0.032) than controls. *P falciparum* infection (1.1 vs 1.3%;p=0.011) and rhinorrhoea (4.1 vs 5.1%;p=0.048) were reported less frequently. Abnormal laboratory values were infrequent and usually not clinically significant. Analysis of a RTS,S/AS vaccine safety database confirms the favorable safety profile of RTS,S/AS in children and infants, and supports further Phase III assessment.

583

HETEROLOGOUS ADENOVECTORED VACCINE REGIMENS ARE IMMUNOGENIC AND PROTECTIVE IN THE *PLASMODIUM YOELII* MOUSE MALARIA MODEL

Noelle B. Patterson[1], Maureen E. Stefaniak[1], Kalpana Gowda[2], Fouzia Farooq[1], Shannon McGrath[3], Svetlana Konovalova[4], Ping Chen[4], Elena Semenova[4], Richard Grier[4], Joseph T. Bruder[4], Keith Limbach[1], Thomas L. Richie[2]

[1]United States Military Malaria Vaccine Program, Naval Medical Research Center, Henry M. Jackson Foundation for the Advancement of Military Medicine, Silver Spring, MD, United States, [2]United States Military Malaria Vaccine Program, Naval Medical Research Center, Silver Spring, MD, United States, [3]United States Military Malaria Vaccine Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [4]GenVec, Inc., Gaithersburg, MD, United States

Adenovector serotype 5-based vaccines encoding two *Plasmodium falciparum* antigens (CSP and AMA1) developed and tested by our program induced sterile protection against sporozoite challenge in Ad5 seronegative humans primed with DNA plasmids encoding CSP and AMA1. However, the efficacy of this prime-boost regimen may not extend to humans with preexisting neutralizing antibody specific to Ad5 (Ad5NAb). To address this concern, we developed adenovectored malaria vaccines designed to circumvent preexisting adenovirus-specific neutralizing antibody, including alternate serotype-based (Ad28 and Ad35) and hexon/fiber-modified adenovectored vaccines. We used the *P. yoelii* circumsporozoite protein (PyCSP) as the model antigen to test this approach. The new Py adenovectors that grew well and robustly expressed PyCSP *in vitro* were evaluated for immunogenicity and protective efficacy. BALB/c mice were immunized with a heterologous two-dose regimen given 6 weeks apart of varying prime-boost combinations, utilizing DNA, Ad5, Ad28, and/or Ad35 vectors. Two weeks post-boost, mice were either utilized for immunogenicity testing (ELISA, intracellular cytokine staining for multifunctional T cells, and ELISpot responses to PyCSP) or challenged with *P. yoelii* sporozoites intravenously and monitored for malaria parasitemia via blood smears. Here we report that alternate serotype malaria vaccines, used in certain heterologous prime-boost combinations, induced malaria-specific T cell and antibody responses comparable to the DNA/Ad regimen. While several alternate serotype malaria vaccines provided protection against malaria, the Ad28/Ad5 and Ad35/Ad5 regimens were the most promising. Specifically, Ad28/Ad5 (protection 36-71%; n=28) and Ad35/Ad5 (43-50%, n=28) elicited better protection than Ad5/Ad5 (14%, n=14) or Ad5 single administration (0%, n=14), respectively. Ad28/Ad5- and Ad35/Ad5-induced protection was comparable to that of DNA/Ad5 (29-43%, n=28). Subsequent experiments will evaluate the ability of the successful regimens to avoid preexisting Ad5NAb.

584

PHASE 1 STUDY OF BSAM2/ALHYDROGEL+CPG 7909 IN MALARIA NAÏVE UNITED STATES ADULTS

Ruth D. Ellis[1], Donna Shafer[2], Yimin Wu[3], Kazutoyo Miura[4], Joan Aebig[3], Kelly Rausch[3], Daming Zhu[3], Laura Martin[3], Michael Fay[5], Carole Long[4], Louis Miller[3], Anna Durbin[2]

[1]NIAID, Rockville, MD, United States, [2]Johns Hopkins Center for Immunization Research, Washington, DC, United States, [3]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [4]Biostatistics Research Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [5]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

A Phase 1 dose escalating study was conducted in malaria naïve adults to assess the safety, reactogenicity, and immunogenicity of the blood stage malaria vaccine BSAM2/Alhydrogel + CPG 7909. BSAM2 is a combination of the FVO and 3D7 alleles of AMA1 and $MSP1_{42}$, with equal amounts of each of the four proteins mixed, bound to Alhydrogel, and administered with the novel adjuvant CPG 7909. 30 volunteers were enrolled in two dose cohorts, with 15 volunteers receiving up to three doses of 40 µg protein at Days 0, 56, and 180, and 15 volunteers receiving up to three doses of 160 µg protein on the same schedule. Most related adverse events were mild or moderate, but 4 volunteers experienced severe systemic reactions and two were withdrawn from vaccinations due to adverse events. Antibody responses were not significantly different in the high dose versus low dose groups, and did not further increase after third vaccination. *In vitro* growth inhibition was demonstrated and was closely correlated with anti-AMA1 antibody responses.

585

COMPARISON OF *PLASMODIUM BERGHEI* CHALLENGE MODEL FOR THE EVALUATION OF PRE-ERYTHROCYTIC MALARIA VACCINES AND THEIR EFFECT ON PERCEIVED VACCINE EFFICACY

Elke S. Bergmann-Leitner[1], Wolfgang W. Leitner[2], Evelina Angov[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]National Institute of Allergy and Infectious Diseases-National Institutes of Health, Bethesda, MD, United States

The immunological mechanisms responsible for protection against malaria infection vary between different *Plasmodium* species, host species and the developmental stage of the parasite and continue to be very poorly understood. Therefore, a challenge with live parasites remains the most relevant and meaningful approach to testing the efficacy of experimental malaria vaccines both in animal models and in clinical trials. In the mouse models of *P. berghei* and *P. yoelii*, parasites are most commonly delivered by intravenous injection. This route, however, is highly artificial and produces inconsistent challenge results due to variations in the quality, purity and virulence of the inoculum produced by different laboratories on different days. In this study, we first optimized the intravenous (IV) delivery challenge model and compared it to an optimized single-mosquito bite challenge model. The latter proved to be more reliable producing more consistent challenge results while using the natural route of parasite delivery, thus avoiding the potential mis-interpretation of vaccine efficacy as this route allows vaccine-induced antibodies to exert their effects on the parasites. A single infectious bite consistently infected mice having different genetic backgrounds without the risk of overwhelming vaccine-induced protective immune responses. Recognizing the main disadvantage of the bite challenge model, the higher labor intensity, we explored an alternative approach that still delivers sporozoites to the correct anatomical site, the subcutaneous injection model. Based on this comparative study we conclude that the frequently used IV challenge model, is highly variable and the variations in the virulence of the inoculum, if not properly monitored by the rigorous inclusion of sporozoite titration curves in each challenge trial, can lead to unacceptable variations in reported vaccine efficacies. Any conclusive evaluation of a pre-erythrocytic malaria vaccine candidate should require challenge through the natural anatomic target site of the parasite, the skin.

586

INACTIVATED *ESCHERICHIA COLI* EXPRESS PROPERLY DISULFIDE-BRIDGED *PLASMODIUM FALCIPARUM FVO* MSP1-42 FROM DIFFERENT CELLULAR LOCALIZATIONS

Heather Hosie[1], Elke S. Bergmann-Leitner[1], Jessica Trichilo[2], Clarissa Dake[2], Tim Alefantis[2], Paul Grewal[2], Vito G. DelVecchio[2], Evelina Angov[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Vital Probes, Inc., Mayfield, PA, United States

Plasmodium falciparum Merozoite Surface Protein-1 (MSP-1) is a lead vaccine candidate targeting the erythrocytic stage of malaria. MSP-1 is the major protein found on the surface of merozoites and is implicated in erythrocyte invasion by the parasite. The full length protein is 195-kDa which undergoes two proteolytic processing events, leading to four polypeptides (p82, p20, p45 and p42) that noncovalently associate with the merozoite surface. In this study, the C-terminal-most fragment, p42 from the p195, was used in an expression and delivery vaccine approach using inactivated *Escherichia coli* designated GeMI-Vax. The inactivated bacteria provided inherent pathogen associated molecular patterns (PAMPS) bypassing the requirement for the addition of adjuvant. The GeMI-Vax also served to express the target antigen to various bacterial intracellular localizations: i.e. the outer membrane and the periplasmic space. Studies investigating the types of immune responses induced by presenting antigen at different sites have taught us that the location of the antigen will direct the immune responses towards either primarily cellular or humoral responses. Antigen targeting to either the outer membrane or the periplasm required construction of fusion proteins with either peptidoglycan associated lipoprotein (PAL) or the maltose binding protein (MBP), respectively. Data will be presented demonstrating proper folding of the MSP1-42-fusion proteins using disulfide-dependent monoclonal antibodies on Western blots and that the proteins are targeted to the specified bacterial intracellular localization using immunofluorescence staining. In addition, antibody responses from rabbits immunized with periplasm and outer membrane MSP1-42 GeMI-Vax will be assessed. Results from measurements of antibody fine specificities and functional activities against the parasite will be evaluated by ELISA, a bead-based multiplex Luminex assay and by the pLDH GIA, respectively. This data will demonstrate that particle-based presentation; proper MSP1-42 folding and adjuvant characteristics are important for the induction of protective antibody responses to MSP1, and are provided by GeMI-Vax.

587

VAR2CSA DBL4 AND DBL5 DOMAINS AS VACCINE CANDIDATES FOR PLACENTAL MALARIA

Tracy Saveria[1], Andrew Oleinikov[1], Gladys Keitany[1], Richa Chaturvedi[1], Joe Lograsso[1], Michal Fried[1], Patrick Duffy[2]

[1]Seattle Biomedical Research Institute, Seattle, WA, United States, [2]National Institutes of Health, Bethesda, MD, United States

Placental malaria (PM) is characterized by infected erythrocytes (iRBC) that selectively bind to chondroitin sulfate A (CSA) and sequester in placental tissue. Var2CSA, a PfEMP1 protein family member, is expressed on the surface of placental iRBCs and mediates adherence to CSA on the surface of syncytiotrophoblast. Var2CSA is a 350kD transmembrane protein that contains 6 Duffy Binding Like (DBL) domains which might contribute to the specific adhesive properties of iRBC. Here we use 3d7 Var2CSA DBL domains expressed in *E.coli* to generate antibodies specific for this protein. We show that DBL4 and DBL5 protein bind selectively to CSA *in vitro*, but not to CSC or Hyaluronic Acid, and that this binding can be inhibited by competition with sera from multigravid women. Flow cytometry results show that antisera generated against DBL4, DBL5 and a double domain of DBL4&5 bind to maternal isolates and lab strains selected for CSA binding, but not to children's parasites. These antibodies also inhibit parasite binding to purified CSA, and at least partially inhibit binding to placental tissue. The ability to generate functional antisera to pregnancy parasites via a large-scale and efficient system such as *E.coli* is an essential asset to the design of a vaccine against PM.

588

OPENING THE DOOR: CRYOPRESERVED PRIMARY HUMAN HEPATOCYTES AND THEIR POTENTIAL USE IN *IN VITRO PLASMODIUM FALCIPARUM* LIVER STAGE INFECTION MODELS

Michael Zyzak[1], Xiaoyan Zou[1], Brent House[2], Thomas Richie[1]

[1]United States Military Malaria Vaccine Program, Navy Medical Research Center, Silver Spring, MD, United States, [2]Navy Medical Research Unit #3, Cairo, Egypt

Functional liver stage assays, such as the inhibition of sporozoite invasion (ISI) assay and the inhibition of liver stage development assay (ILSDA) assess the impact of immunological responses on malaria parasite development *in vitro*, and thus potentially could identify immunological correlates of protection. However, the traditional ISI and ILSDA assays using HepG2 and HC04 cell lines are limited by low sporozoite invasion rates and by the difficulty of accurately counting invaded parasites using microscopy. In early 2009, we identified a commercial source of cryopreserved primary human hepatocytes (CPHH) and have validated their usefulness in both assays using a quantitative real time PCR approach. Advantages: (a) CPHH provides a 7-13 fold improvement in invasion rates;

(b) CPHH should be more biologically relevant for measuring functional antibody or any other aspect of liver stage biology being studied, due to the loss of normal hepatocyte biological characteristics associated with hepatoma cell lines; (c) CPHH derived from a single human liver can be purchased in sufficient quantities to conduct thousands of assays, thereby providing a standardized reagent; (d) CPHH are available across a wide demographic: male/female, multiple ethnicities, and diverse age groups including infants. Thus, CPHH significantly improve liver stage infection models allowing quantification and standardization of functional assays when coupled with a PCR-based read-out.

589

PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN SYNTHETIC REPEAT PEPTIDE CONJUGATED TO OUTER MEMBRANE PROTEIN COMPLEX GENERATES A FUNCTIONAL IMMUNE RESPONSE IN MICE AND RHESUS MONKEYS

Craig Przysiecki[1], Bob Lucas[1], Robert Mitchell[2], Daniel Carapau[2], Zhiyun Wen[1], Hui Xu[1], Xin-Min Wang[1], Debbie Nahas[1], Robert Hepler[1], Elizabeth Ottinger[1], Jan ter Meulen[1], David Kaslow[3], John Shiver[1], Elizabeth Nardin[2]

[1]West Point Vaccines Research, Merck Sharp and Dohme Corp., West Point, PA, United States, [2]New York University School of Medicine, New York, NY, United States, [3]Research Medicine, Merck Sharp and Dohme Corp., Upper Gwynedd, PA, United States

Vaccines based on the repeat region of P*lasmodium falciparum* circumsporozoite protein (PfCSP) can elicit protective immunity in human and murine experimental hosts. The first pediatric malaria vaccine to enter Phase III trials, RTS,S which is comprised of a *Pf*CSP repeats and C-terminal fragment, elicits protection against clinical disease in immunized African children that was correlated with anti-repeat antibodies and possibly CD4+ T cell responses. The first repeat peptide-conjugate vaccine, (NANP)3-TT, elicited anti-repeat antibodies that could protect a few immunized volunteers; however titers were suboptimal. Outer membrane protein complex (OMPC) derived from *N. meningitidis* has been used successfully as a carrier for polysaccharide vaccines in infants and conjugation to OMPC increased immunogenicity of a malaria Pfs25 transmission blocking vaccine in mice and monkeys. We evaluated immunogenicity of an alum-adsorbed (NANP)6-OMPC vaccine in mice and Rhesus monkeys. BALB/c and C57Bl mice immunized with alum-adsorbed (NANP)6-OMPC developed high anti-repeat peptide ELISA titers and IFA titers using *P. falciparum* sporozoites, as well as anti-CSP reactivity with viable sporozoites. Murine immune sera inhibited invasion of human hepatoma cells by transgenic *P. berghei* sporozoites that express *P. falciparum* repeats. Vaccinated mice challenged by mosquitoes infected with transgenic parasites, demonstrated sterile immunity or delayed prepatent period and reduced parasite burden in the liver (>90% inhibition by real-time PCR). Monkeys immunized with two doses of (NANP)6-OMPC formulated with alum and a co-adjuvant developed anti-repeat antibodies that persisted at decreasing levels for 662 days. A third injection of (NANP)6-OMPC at day 662 boosted ELISA titers to peak levels observed post second dose. Rhesus sera obtained post second and third dose displayed sporozoite neutralizing activity in the parasite hematoma cell invasion assay. Results obtained in immunized mice of different MHC haplotype and a non-human primate species suggests that peptide-OMPC conjugates, based on a human acceptable carrier, may lead to new vaccine candidates.

590

EFFICACY OF FMP2.1/AS02A AGAINST GAMETOCYTEMIA IN 1-6 YEAR OLD CHILDREN IN BANDIAGARA, MALI: IMPLICATIONS FOR MALARIA ELIMINATION

Matthew B. Laurens[1], Mahamadou A. Thera[2], Drissa Coulibaly[2], Abdoulaye K. Kone[2], Ando B. Guindo[2], Dapa A. Diallo[2], Karim Traore[2], Amadou Niangaly[2], Amagana Dolo[2], Youssouf Tolo[2], Mahamadou S. Sissoko[2], Bourema Kouriba[2], Kirsten E. Lyke[3], Shannon Takala-Harrison[3], William C. Blackwelder[3], Yukun Wu[3], Johan Vekemans[4], Joe Cohen[4], David E. Lanar[5], Sheetij Dutta[5], Carter L. Diggs[6], Lorraine Soisson[6], D. Gray Heppner[5], Ogobara K. Doumbo[2], Christopher V. Plowe[1], Idrissa Traore, Issa Diarra, Modibo Daou, Mady Sissoko, Olivier Godeaux, Marie-Claude Dubois, Ripley Ballou, Darby Thompson, Tina Dube, Brent House, Jason W. Bennett

[1]Howard Hughes Medical Institute/Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, [2]Malaria Research and Training Center, University of Bamako, Bamako, Mali, [3]Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, [4]GlaxoSmithKline Biologicals, Rixensart, Belgium, [5]Division of Malaria Vaccine Development, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [6]Malaria Vaccine Development Program, United States Agency for International Development, Washington, DC, United States

Malaria vaccines could affect malaria transmission in a community by influencing gametocyte density in human hosts in either direction, but efficacy against gametocyte carriage is not typically measured in vaccine trials. The malaria vaccine candidate FMP2.1/AS02A was recently evaluated in Bandiagara, Mali, West Africa, showing limited overall efficacy against first and multiple clinical episodes but high allele-specific efficacy. To determine the potential impact of a blood stage vaccine on malaria transmission, we evaluated the efficacy of the FMP2.1/AS02A vaccine against gametocytemia. Four hundred healthy children aged 1-6 were randomized 1:1 to receive three doses of 50 µg of FMP2.1 in 0.5mL of AS02A or rabies vaccine, 30 days apart. Malaria smears were read for all participants at scheduled time points and at unscheduled clinic visits when children presented with any malaria symptom. *P. falciparum* gametocytemia rates will be compared among both vaccine groups at scheduled time points. As a measure of cumulative gametocyte density, data from all malaria smears will be used to calculate the median area under the curve of gametocyte as well as asexual parasite density. The median area under the curve of asexual parasite density was 168,577 per microliter in the malaria vaccine group and 376,863 per microliter in the control group in the intention-to-treat analysis, and 97,708 per microliter in the malaria vaccine group and 308,638 in the control group in the according-to-protocol data set (P=0.012 in both cases). Results for gametocytemia are being completed and will be presented. In conclusion, in the context of malaria elimination, malaria vaccine candidates should not only be evaluated for efficacy against clinical episodes, but also against malaria transmission in a community. Gametocytemia rates can serve as a surrogate for malaria transmission in a community and should be part of evaluation of malaria vaccine efficacy.

591

DRASTIC REDUCTION OF MALARIA TRANSMISSION AND SEVERE CASES IN DEPARTEMENT OF OUÉMÉ AFTER PROTECTION OF COMMUNITIES AGAINST PYRETHROID RESISTANT ANOPHELINES WITH FICAMR M (BENDIOCARB 800G/KG) IN INDOOR RESIDUAL SPRAYING (IRS) IN REPUBLIC OF BENIN

Akogbeto Codjo Martin, Padonou Gil, Yadouleton Anges, Kiti Eugène

Entomological Research Center of Cotonou, Cotonou, Benin

Despite numerous efforts to roll back malaria, this disease unfortunately remains the primary cause of morbidity and mortality in children under 5 years in Africa. Despite this result, the international community decided to increase technical and financial support for malaria elimination. This is why, since 2004, the American government, through "President's Malaria Initiative" (PMI), is giving an important support to National Malaria Control Programs (NMCP) for malaria control in Africa. A large scale of IRS has been implemented with PMI support since 2008 in Oueme region (Bénin), an area characterized by a high resistance of *Anopheles gambiae* to pyrethroids. The goal of this of study is to verify if the use of a non-pyrethroid for IRS can reduce malaria transmission by eliminating *An. gambiae* populations resistant to pyrethroids and induce a significant decrease of malaria incidence. Houses of more than 350,000 inhabitants were treated with FicamR M (bendiocarb 800g/kg) because of the presence of a high permethrin resistant population of *Anopheles gambiae* (kdr frequency > 0.70). Houses were treated by the Oueme local community after training by the RTI team. The monitoring evaluation of the operation was focused on acceptability of community for IRS, quality control of the spraying done by local community, residual effect of bendiocarb, dynamics of pyrethroid resistance in areas under IRS, dynamics of malaria transmission and evolution of severe malaria in health centres. Acceptability and adhesion of communitys to the strategy was total. People who refused the strategy during the first round of IRS were convinced by the lethal action of bendiocarb on resistant mosquitoes. The spraying carried out by the community was perfect. Two weeks after IRS, 100% mosquitoes were killed whatever the strain or the position of the cone-test on the treated walls. For malaria vectors, after IRS, the human bite rate (HBR) and the inoculation rate (EIR) have drastically decreased with 94.4% reduction. This reduction due to the lethal action of bendiocarb was observed in all districts. At the same period, the managers of health centres reported 70% reduction of severe cases of malaria.

592

MANAGING DENGUE VECTOR RESISTANCE TO TEMEPHOS WITH COMMUNITY SUPPORTED APPLICATIONS OF THE MICROBIAL INSECTICIDE, VECTOBACR WG (*BACILLUS THURINGIENSIS ISRAELENSIS* STRAIN AM 65-52)

Ngan Chantha, To Setha, **Doung Socheat**

National Malaria Center, Phnom Penh, Cambodia

Cases of dengue fever transmitted by the mosquito *Aedes aegypti* in Asia are growing, and now results in an average of 12,000 cases in Cambodia since 2006. Historically, applications of temephos to larval habitats provided acceptable dengue control; however, mosquito resistance to temephos has been observed in some localities of some provinces in the recent years in Cambodia. To better manage dengue situation, our center evaluated other vector control tools. A 4 year field study with WHOPES reviewed biolarvicide, VectoBac® WG (Bti strain AM 65-52) has shown that a single direct application at 8 g/1000 L in any water type during the low dengue vector season significantly reduced the adult mosquito density in the peak season for 3 months. In Kandal province, community acceptance of VectoBac® WG was measured by treating 461,693 containers in 64,241 household in 2007. A post treatment survey showed high household acceptance (96 %) because of the quick kill in the larvae and the treated waters did not have any physical change (odor and color). Excellent product efficacy & community acceptance in Cambodia, together with successful dengue control programs with VectoBac® WG in Asia and Brazil led the Ministry of Health to include this tool in the National Dengue Control Program this year. We report here the initial results of provincial-wide, community-supported program to reduce dengue cases through vector control using VectoBac® WG initiated in May 2010.

593

THE IMPORTANCE OF INSECTICIDE RESISTANCE MANAGEMENT IN THE CONTROL OF THE MOSQUITO VECTORS OF MALARIA

Mark A. Hoppé

Insecticide Resistance Action Committee, Stein, Switzerland

Insecticides have been extensively and successfully used since the 1940s to control the mosquito vectors of disease, and have been a vital component in the fight against malaria. Indeed, vector control is considered the mainstay of malaria control programmes. However, insecticide resistance has developed in populations of the major mosquito vector species to the classes of insecticide currently recommend for vector control. As insecticide resistance continues to develop and spread, there is a real danger that these valuable interventions will be lost. This paper outlines the principles of Insecticide Resistance Management (IRM), in the vector control context. Special emphasis is placed on the need to use insecticide resistance monitoring methods that provide information that enables the decision-makers within a vector control programme to choose the intervention that best fit both their circumstances, and the principles of IRM. Much energy is currently being spent with the aim of developing, or repurposing, insecticides with novel modes of action for vector control. A strategy must be put in place to preserve the long term utility of novel insecticides, as they are developed, and take steps to maintain the effectiveness of those insecticidal tools currently available. Recommendations for such strategies are outlined. With malaria elimination returning to the international agenda, it is argued that only through IRM can the sustainable use of insecticidal vector control interventions be maintained.

594

DEVELOPMENT OF A GRAVID-OVITRAP FOR COLLECTING *AEDES AEGYPTI*

Roberto Barrera, **Andrew J. Mackay**, Manuel A. Amador, Belkis Z. Caban, Veronica Acevedo, Gilberto Felix

Centers for Disease Control and Prevention, San Juan, PR, United States

The ovitrap has been used for many decades as a very sensitive and inexpensive tool for detecting the presence of *Aedes aegypti*. Ovitraps also have been used to reduce the reproductive potential of female mosquitoes entering the trap by providing a suitable substrate for oviposition, while preventing eggs deposited from producing adult mosquitoes. Sticky ovitraps and lethal ovitraps (ie. gravid-ovitraps) incorporate an additional mechanism to kill or capture gravid mosquitoes. To be a practical surveillance or control tool, a gravid-ovitrap must be effective, inexpensive, and shouldn't require frequent maintenance. Traps that don't utilize pesticides are more likely to be accepted by homeowners concerned with potential health/environmental risks associated these chemicals, and won't contribute to the development of insecticide resistance. Our objective was to develop a trap that incorporates simple, low-cost mechanisms for eliminating gravid *Ae. aegypti* and their progeny, that doesn't use a toxic insecticide, and that can remain efficacious for an extended period of time without servicing. We compared seven commercial adhesives for capturing adult *Ae. aegypti* and determined that the most promising adhesive can be used in a gravid-ovitrap for at least six weeks under field conditions without a significant loss of capture efficacy. We established that a gravid trap baited with hay infusion and a supplemental hay packet remains very attractive to gravid *Ae. aegypti* for at least three weeks. Data collected from field tests of gravid-ovitraps in metropolitan San Juan suggest an

optimal trap density of three traps per home, with a mean capture rate of 1.4 *Ae. aegypti* females per trap per day. We also evaluated a synthetic polymer that is highly attractive to gravid *Ae. aegypti* as an oviposition substrate, but prevents development of their progeny. Our results indicate that a gravid-ovitrap incorporating these components (adhesive, supplemental hay and artificial oviposition substrate) could be an effective tool for the surveillance and/or control of *Ae. aegypti*.

595

FIELD TRIALS OF A NEW GRAVID-OVITRAP FOR INTEGRATED AREA-WIDE CONTROL OF *AEDES AEGYPTI* IN PUERTO RICO

Roberto Barrera, Andrew J. Mackay, Manuel A. Amador, Belkis Z. Caban, Veronica Acevedo, Gilberto Felix

Centers for Disease Control and Prevention, San Juan, PR, United States

A new gravid-ovicidal trap was used in conjunction with source reduction and larviciding for integrated area-wide control of *Aedes aegypti* in an isolated urban community (295 houses) in southern Puerto Rico (trap intervention area; TIA). Mosquito population post-intervention was compared with pre-intervention value in TIA and with change in a nearby isolated urban community (423 houses) where only source reduction and larviciding were concurrently applied (no trap intervention area; NTIA). The trap used hay infusion and color contrast as attractants for gravid females that were then captured on internal adhesive surfaces. The trap also used a synthetic polymer as a substrate for egg laying instead of water, where hatching larvae fail to develop. Three traps were deployed for two months in each home in TIA. Source reduction consisted of a clean-up campaign and turning over containers that could not be disposed of or treated with a larvicide (three formulations of spinosad). Containers holding animal or human drinking water were left untreated. Water storage containers were not common in the study areas. The number of adult female *Ae. aegypti* in each community was monitored using 28 BG-Sentinel TM mosquito traps in TIA and 40 in NTI. These traps used BG-lure and were operated for three days a week during eight weeks, before and after the intervention. Average *Ae. aegypti* female post-intervention reduction was 43% in TIA and 21.7% in NTIA. Mosquito population reduction due to the gravid-ovicidal traps was 21%. Three gravid-ovicidal traps captured an average of 0.54 *Ae. aegypti* females per house per day (>95% gravid or parous). The number of eggs per captured female in the traps was 14.9. Increasing trap attractiveness is a next step in the development of this low-maintenance, inexpensive device that targets the epidemiologically important, gravid/parous *Ae. aegypti* females.

596

THE CARTAGENA PROTOCOL AND GENETICALLY MODIFIED MOSQUITOES

John M. Marshall

California Institute of Technology, Pasadena, CA, United States

The Cartagena Protocol on Biosafety is the fundamental document of the United Nations on the responsible use of genetically modified (GM) organisms. The Protocol applies to GM mosquitoes; however its terms were negotiated primarily with concerns over the safety and trade of GM crops in mind. We argue that, while the Protocol may be adequate for strains of GM mosquitoes intended for population suppression, it is inadequate for strategies intended to replace entire mosquito populations with disease-resistant varieties. In this latter strategy, gene drive systems are being considered that are capable of propagating transgenes within and across national borders. In its current form, the Protocol provides inadequate protection against an accidental release, notably due to the exemption of GM mosquitoes in transit or destined for contained use from the Advance Informed Agreement. At the same time, the conditions for an intentional release are almost impossible to satisfy, requiring unanimous approval from every country that the species inhabits. Furthermore, mosquitoes infected with non-transgenic *Wolbachia* bacteria are exempt from the Protocol, despite unknown consequences for the environment and human health. We encourage future regulation that addresses the unique biosafety concerns of modified mosquitoes and seeks a balance between the precautionary principle, respect for the sovereignty of states, and the ethical mandate to prevent disease on a global scale.

597

SUITABLE MATING COMPETITIVENESS OF INCOMPATIBLE *AEDES POLYNESIENSIS* MALES SUPPORTS LYMPHATIC FILARIASIS ELIMINATION STRATEGY FOR THE SOUTH PACIFIC

Limb K. Hapairai[1], Eric Chambers[2], Bethany Peel[2], Stephen Dobson[2], Hervé Bossin[1]

[1]Institut Louis Malardé, Papeete, Tahiti, French Polynesia, [2]Department of Entomology, University of Kentucky, Lexington, KY, United States

Despite the sustained mass drug administration (MDA) of anti-filarial treatments over several decades, lymphatic filariasis (LF) remains a leading cause of disability in the South Pacific. Recent epidemiological observations clearly demonstrate that MDA alone will not be enough to end the LF transmission cycle, at least in some Pacific island countries. Supplemental control strategies are thus much needed to ensure the success of the global LF elimination campaign. Obligate vector mosquitoes provide additional targets that can complement existing anti-filariasis approaches. However, due to the ubiquitous nature of mosquitoes, conventional methods have failed to successfully control the vector Aedes polynesiensis, the primary LF vector throughout most of the South Pacific. Such paucity in the arsenal of tools available to control Ae. polynesiensis has raised interest in the use of evolutionary genetics to fight vector-borne diseases. Replacing the endosymbiont *Wolbachia* present in Ae. polynesiensis with that from Ae. riversi through interspecific hybridization and introgression has led to the development of a laboratory strain (CP) which is bi-directionally incompatible with its wild counterpart, resulting in egg sterility. Laboratory assays demonstrated the equal competitiveness of CP males and established the proof-of-principle of population elimination following the introduction of incompatible males into wild type A. polynesiensis cage colonies. CP male competitiveness was assessed in a field cage trial. This bioassay demonstrated equal CP male mating competitiveness with their wild counterpart under semi-natural tropical conditions. These findings support the implementation of a large field trial to assess the efficacy of the *Wolbachia* -mediated mosquito suppression strategy as a supplemental strategy to curb LF prevalence in endemic regions of the South Pacific.

598

INSECT REPELLENTS: FROM MODE OF ACTION TO NEW APPLICATIONS IN VECTOR CONTROL

Cédric Pennetier[1], Bruno Lapied[2], Fabrice Chandre[1], Vincent Corbel[3]

[1]Institut de Recherche pour le Développement, Montpellier, France, [2]RCIM, Université d'Angers, Angers, France, [3]Institut de Recherche pour le Développement, Cotonou, Benin

With the spread of pyrethroid resistance in most mosquito vector species and the lack of alternative compounds for public health issues, the search for alternative control strategies effective against resistant vector populations has become a priority. In this weapons race, insect repellents, which have been used for some time via topical application to the skin, are becoming of greater interest for community protection against mosquito borne diseases. Here we used a multidisciplinary approach to investigate whether repellents could be used to limit the contact between mosquito vectors and humans. First, using electrophysiological, biochemical and toxicological methods, we described two modes of action of the gold standard repellent DEET: it is an acetylcholine esterase inhibitor and it exerts neurotoxic effects through an elevation of the intracellular calcium concentration. We also showed that repellants had strong synergistic effects with available insecticides and have great potential for use in insecticide for insecticide treated nets or indoor residual sprayings.

Moreover we showed in both the laboratory and field that different fabrics impregnated with repellents alone and combined with other biocides are highly effective against resistant mosquito vectors. We also showed that the impregnation of clothes with repellents is also a valuable opportunity for personal protection. Moreover repellents could be used for their primary activity, as an insect behavior modifier, in promising strategies like the push-pull strategy. To conclude, repellents are highly promising to better control pyrethroid resistant vectors. Although the volatility of these chemicals limits their immediate use on long lasting fabrics, overcoming this technological problem should be lot more easily achieved than finding insecticides with new modes of action.

599

DRY SEASON PILOT INDOOR RESIDUAL SPRAY (IRS) TARGETING RIVERBANK HAMLETS IN SUDAN SAVANNA AREAS OF MALI

Nafomon Sogoba[1], Moussa Keita[1], M'Bouye Diallo[1], Ibrahima Baber[1], Massiriba Kone[1], Nicholas C. Manoukis[2], Ellen Dotson[3], Sekou F. Traore[1], Seydou Doumbia[1]

[1]MRTC/FMPOS University of Bamako, Bamako, Mali, [2]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

In the Sudan savanna of Mali there is marked seasonality in malaria transmission, a consequence of very low densities of mosquito vectors during the dry season, and so control efforts are generally focused on the rainy season. However, there are parts of that environment, such as the riparian parts of the Niger River, where there is perennial breeding of *Anopheles gambiae* s.l. together with dry season malaria transmission. Thus control measures targeting adult mosquitoes during the dry season in these areas are of interest, and may decrease the size of mosquito population and transmission in the subsequent rainy season. This study aims to explore the effectiveness and potential impact of a dry season IRS in hamlets along the Niger River where mosquitoes continue breeding in the dry season and describe ways in which such an approach might limit malaria transmission during the rainy season. Entomological parameters of malaria transmission were monitored using PSC before and after the IRS in 3 sets of hamlet-inland villages in 2008 and 2009, respectively. Mosquito density and entomological inoculation rate (EIR) in 2009 (after IRS) were lower than that of 2008 (before IRS) in the 3 hamlets under study (Bozokin, Fourda and Somonosso). The geometric mean number of mosquitoes per house during the rainy season showed a reduction of 40.0% in Bozokin [1.5 (0.6_2.4) vs 0.9 (0.3_1.5)]; 8.3% in Fourda [1.2 (0.5_1.8) vs 1.1 (0.6_1.6)], and 33.3% in Somonosso [1.8 (1.2_2.5) vs 1.2 (0.5_1.8)]. The same pattern was observed in EIR, measured as the number of infective bites per person per season, with a reduction rate of 79.4% (0.34 vs 0.07), 36.4% (1.87 vs 1.19) and 42.9% (0.28 vs 0.16) respectively in Bozokin, Fourda and Somonosso. Mosquito density and EIR decreased between 2008 and 2009 in hamlets where the IRS was performed. However excepting in Bozokin, this reduction was < 50%. Additional dry season IRS intervention may be required to observe any significant reduction in malaria transmission in subsequent rainy season.

600

IMPACT OF LONG-LASTING DELTAMETHRIN-TREATED CONTAINER COVERS ON *AEDES AEGYPTI* OVIPOSITION

Daniel Kline[1], Gary G. Clark[1], Joyce Urban[1], Frances Golden[1], Michael B. Nathan[2], Tessa Knox[3]

[1]United States Department of Agriculture/ARS, Gainesville, FL, United States, [2]World Health Organization, Geneva, Switzerland, [3]Vestergaard Frandsen, Nairobi, Kenya

USDA researchers are studying novel methods to control *Aedes aegypti*. One approach focuses on prevention of *Ae. aegypti* oviposition. In collaboration with Vestergaard Frandsen Ltd., factory-treated long lasting deltamethrin PermaNet® Container Covers (jar lids) were evaluated with 55-gallon drums with and without covers. Exclusion efficacy was measured with sticky ovitraps and oviposition substrates placed on the inner wall of the drums. Tests were performed in 1,800 ft2 outdoor screened cages (30 ft wide, 60 ft long, 16 ft high gabled to 18 ft) at the USDA CMAVE facilities in Gainesville, Florida. In test 1, there was 1 drum per cage with either an untreated or deltamethrin-treated cover, or an uncovered drum (untreated control). In test 2, there were 4 drums per cage: 3 covered and 1 uncovered drum, 1 covered and 3 uncovered drums, or 4 uncovered drums (untreated control). Test 3 was similar to test 2 but with a different version of the Container Cover. For each test, 200 gravid *Ae. aegypti* were released into each cage. The drum(s) were 2/3 full of well water and lined with absorbent germination papers to detect female oviposition. Container Cover efficacy was measured 24 hrs post-release of females with 5 widely distributed sticky ovitraps (containing a 10% 7 day-old hay infusion) placed in each cage as alternative oviposition sites for gravid females. Sticky ovitraps were examined after 48 hrs and egg (germination) papers were removed after 72 hrs. Drums with untreated covers yielded a similar number of females to those with no cover, whereas treated covers resulted in a 64% reduction in females. With 1 of 4 drums with treated covers, there was a 45-65% reduction in females and a 42-52% reduction in oviposition. With 3 of 4 drums with treated covers, there was a 67-100% reduction in females and a 75-100% reduction in oviposition. The presence of treated Container Covers of either version significantly reduced female oviposition. Container Covers present a potential tool for the control and prevention of dengue virus transmission.

601

MONITORING THE DURABILITY OF LONG-LASTING INSECTICIDAL BEDNETS IN RURAL ETHIOPIA

Stephen C. Smith[1], Aprielle Brackery[2], Gedeon Yohannes[3], Patricia M. Graves[2], Tekola Endeshaw[3], Estifanos B. Shargie[3], Aryc W. Mosher[2], Paul M. Emerson[2], Teshome Gebre[3]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]The Carter Center, Atlanta, GA, United States, [3]The Carter Center, Addis Ababa, Ethiopia

Following the distribution of 3 million PermaNet™ 2.0 long-lasting insecticidal nets in Ethiopia in February - May 2007, a total of 609 nets were collected from households at three time intervals: 2007, 2008, and 2009; to assess physical damage and insecticide loss, and to develop practical methods to quantify deterioration. Nets were collected from 19 sites and time-in-use ranged from 3 to 32 months. Collected nets were confirmed as being from the 2007 campaign by using the batch number printed on each net's label. Physical deterioration was first assessed by counting and measuring the size and location of each hole, and later by counting the number of holes falling into each of three size categories. Insecticide retention was measured by x-ray fluorescence spectrometry, and confirmed by testing a subset of nets using high-performance liquid chromatography. Hole formation began early: over 40% of nets used for 3 months had at least one hole >0.5 cm in diameter. The number of holes per net (hole rate) followed a highly skewed distribution, with many nets having few holes and a few nets having many holes. Median hole rate increased from 1 for nets used for 3-6 months to 10.5 for nets used for 17-21 months. Pairwise analysis showed that nets collected from 5 pairs of sites differed significantly ($p<0.002$) in hole rates after 17-21 months of use. The distribution of hole sizes was highly skewed and, although hole rate increased with time-in-use, the ratio of large to small holes remained unchanged from 3-6 months to 17-21 months. Repairs were rare, suggesting that net lifetime could be increased significantly by improved user care.

Insecticide analysis indicated that 96% (192/200) of the nets retained sufficient (>10mg/m2) deltamethrin after 28-32 months of use. The distribution of insecticide level was very broad with 0.5% (1/189) having inadequate deltamethrin after 3-6 months and 6% (12/200) after 17-21

months. Pairwise analysis of the insecticide levels of nets used for 17-21 months found that 3 pairs of collection sites had nets with significantly different levels of insecticide ($p<0.002$).

602

THE GENETIC BASIS OF PYRETHROID RESISTANCE IN THE MAIN AFRICAN MALARIA VECTOR *ANOPHELES GAMBIAE* S.S.

Claudia Witzig[1], Strode Clare[1], Rousseau Djouaka[2], Charles Wondji[1], Hilary Ranson[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]IITA Cotonou, Cotonou, Benin

Vector control measures for malaria rely heavily on insecticides. Increasing usage of insecticides, has led to the emergence of resistance to all of the classes of insecticides currently available for public health. This is threatening the success of vector control programmes. Quantitative trait loci (QTL) mapping is being used to identify the mechanisms responsible for pyrethroid resistance in *Anopheles gambiae* in Benin. F2 populations of *An. gambiae*, raised directly from field collected mated females, have been analysed to identify the major loci controlling resistance. New genetic markers within candidate genes have been identified to complement the existing sets of SNP and microsatellite markers. Preliminary analysis identified one QTL on chromosome 3R near a P450 cluster. Advanced Intercross Line (AILs) are now being used to finesse the mapping. Both target site and metabolic resistance have been reported in the M form of *An. gambiae* in Southern Benin and this genetic approach will enable us to determine the relative contribution of different alleles to the resistance phenotype. Identification of responsible factors will hopefully lead to a better understanding of the resistance mechanism and enable the development of more powerful insecticides as well as suitable screening assays to detect resistance in the early stages of development.

603

HEALTH IMPACT ASSESSMENT OF DEVELOPMENT PROJECT: IMPACT OF SARDAR SAROVAR NARMADA PROJECT ON MOSQUITO-BORNE DISEASES IN GUJARAT, INDIA

Harish Chandra Srivastava[1], R.M. Bhatt[2], R.S. Yadav[3]

[1]National Institute of Malaria Research, Nadiad, Gujarat, India, [2]National Institute of Malaria Research, Field Station (Near MHI Hospital), Raipur, Chhatisgarh, India, [3]Vector Ecology and Management, Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland

Sardar Sarovar Narmada in Gujarat is a multipurpose water resources development project on river Narmada in Narmad district. Assessment of the impact of this water development project on mosquito borne diseases has been undertaken in command area of Phase I and II districts. We studied geographical reconnaissance of mosquito breeding habitats, bionomics of vectors assessment of the incidence of malaria, dengue and filarial and links between Agro-ecosystems in above said areas. In general malaria in villages in command area remained low as compared to non-command areas in 2009. The mosquito breeding habitats was surveyed along the main canal across 6 districts in phase I and II. *Anopheles culicifacies* which is the main malaria vector in the plains was found to breed in almost all the habitats surveyed. Breeding of filariasis vector *Culex quinquefasciatus* and vector of Japanese Encephalitis *Cx. vishnui* group was also detected from different habitats. All three important malaria vectors viz., *An. culicifacies, An. stephensi* and *An. fluviatilis* were collected resting indoor from the indicator villages. Dengue vectors *Aedes aegypti* and *Ae. albopictus* were collected in very low numbers in adult collections. *An. culicifacies* was predominantly a zoophilic species represented by sibling species A, B and C of which B was predominant (88%). Paddy cultivation in command areas has increased after commencement of irrigation in phase I districts. The depth of water has begun rising in theses areas. The study is in progress in 3 more districts in phase II command area. The lessons learned from this study would be useful for incorporating health safeguards in the development of future project in the Gujarat state.

604

MALARIA TRANSMISSION IN MINERAL PROSPECTION AREAS IN THE BRAZILIAN AMAZON REGION

Izis M. Sucupira, Marinette M. Povoa

Instituto Evandro Chagas, Ananindeua, Brazil

The mineral prospection projects established in Brazilian Amazon promotes the intensity of human migration and can causes environmental changes that modify the transmission profile, since these modifications increase the vector-man contact. Our aims were to identify anopheline mosquitoes species and to determine which species were implicated on malaria transmission in two study areas. The study was performed on the municipalities of Juruti (bauxite extraction - Juruti Project) and of Marabá/Parauapebas (iron extraction - Salobo project) both in Para state. The first project was implemented on 2003 while the second one is older than 20 years. The mosquitoes collection were carried out during 12 hours (from 6 p.m to 6 a.m), 2 or 3 times/year, from 2005 to 2008. All collected mosquitoes were identified by morphological characters and used for detection of malaria parasites by ELISA, and 10% of them were dissected for reaching the parity tax. In Juruti project we had collected 976 mosquitoes of 8 species and the most important for malaria transmission, was the *Anopheles albitarsis s.l.*, which had showed a parity tax of 9.6% and was the only one found infected by *Plasmodium vivax* (infection rate = 0.1%), which is the main specie circulating among the human population in Brazil. In Salobo project we collected 746 adults mosquitoes of 11 species. From those, *An. darlingi* and *An. albitarsis s.l.*, that are incriminated as main vector in Brazil, had showed parity tax of 8,6% and 6,1%, respectively. Furthermore they were the only species founded infected by malaria parasites (*An. darlingi*: *Plasmodium falciparum* and *P. vivax* and *An. albitarsis*: *P. vivax*), resulting on a infection rate of 0.8%. Based on the results it is possible to deduce that the longer is the project more stable is the malaria transmission

605

HARMONIC CONVERGENCE AND THE SEXY SONS HYPOTHESIS IN *AEDES AEGYPTI*

Lauren Cator, Ron R. Hoy, Laura C. Harrington

Cornell University, Ithaca, NY, United States

In order to improve mosquito control programs, it is important to understand mating biology. In particular, little is known about whether mate assessment occurs and what characteristics are important for fitness. Several medically important mosquito species mate on the wing in aerial swarms. Flight tone is important for male orientation to females. Recently, we determined that variation in flight tone is perceived by both males and females. Males and females altered their flight tone to converge on common harmonic frequencies and this harmonic convergence was important for the formation of a successful copula. The studies presented here, build upon our earlier work on harmonic convergence. We recorded acoustic interactions of male and female *Aedes aegypti* prior to mating. We then followed females throughout their lifetime and measured both direct benefits to females (longevity and daily egg laying counts) and indirect benefits manifested in their offspring. Our results are consistent with the predictions of the sexy son hypothesis: male offspring of pairs that converged prior to mating had higher mating success. In addition to direct and indirect benefits of convergence behavior, we will present data on the heritability of convergence. By understanding the signals used in mating interactions, we will be better able to understand the mating system of *Ae. aegypti* as a whole.

606

FIELD EVALUATION OF THE BEHAVIORAL DIFFERENCES ASSOCIATED WITH TWO GEOGRAPHICALLY ISOLATED POPULATIONS OF *ANOPHELES DARLINGI*

Paige Sachs, Nicole Achee, Diana Riner, John Grieco

Uniformed Services University of the Health Sciences, Bethesda, MD, United States

Anopheles darlingi is a major vector for malaria in Central and South America. A review of the literature shows that a number of independent studies have documented behavioral differences in this species across its range including differences in biting activity, feeding preference and propensity for endophagy. Previous work has also shown that *A. darlingi* can be categorized into two genotypes. Genotype 1 is found in Amazonia and southern Brazil and genotype 2 is found in Belize, Guatemala, Colombia and Venezuela. It is not known whether these different genotypes affect mosquito behavior which could contribute to differences in vector competence. The current work represents the first study to incorporate a multi country evaluation of the genetic diversity of this species with a thorough evaluation of behavioral variations in the two populations. Using an experimental hut design, the entrance and exit behavior of *An. darlingi* from two locations; Iquitos, Peru, representing genotype 1 and Cayo district, Belize, representing genotype 2, were evaluated. Differences in the endophagic behavior of this species across its range can translate into differences in its ability to transmit malaria. These behavioral differences can also impact the timing and potential impact of interventions such as long lasting bed nets and other personal protective measures (i.e. mosquito coils and insecticide treated materials).

607

MODELING *CULEX TRITAENIORHYNCHUS* MOSQUITOES TO PREDICT THE GEOGRAPHIC DISTRIBUTION OF JAPANESE ENCEPHALITIS IN THE REPUBLIC OF KOREA

Penny M. Masuoka[1], Terry A. Klein[2], Heung-Chul Kim[2], David M. Claborn[3], Nicole Achee[1], Richard Andre[1], Judith Chamberlin[1], Jennifer Small[4], Assaf Anyamba[4], Dong-Kyu Lee[5], Suk H. Yi[2], Michael Sardelis[6], John Grieco[1]

[1]Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]65th Medical Brigade/United States Army MEDDAC-Korea, Seoul, Republic of Korea, [3]Center for Homeland Security, Missouri State University, Springfield, MO, United States, [4]Hydrological and Biospheric Sciences Laboratory, NASA's Goddard Space Flight Center, Greenbelt, MD, United States, [5]Department of Health and Environment, Kosin University, Busan, Republic of Korea, [6]National Center for Medical Intelligence, Fort Detrick, MD, United States

Japanese encephalitis (JE) is a serious mosquito-borne disease that results in approximately 35,000 cases reported worldwide each year. The disease occurs throughout Southeast Asia, India, parts of China, Japan, and Korea. The primary JE vector, *Culex tritaeniorhynchus*, is associated with rice fields and other low-lying grassy areas that flood. Large water birds are the primary reservoir, while pigs are amplifying hosts of the virus. Partners of the Armed Forces Health Surveillance Center (AFHSC), Division of Global Emerging Infections Surveillance (GEIS) and Response System Operations are working to develop predictive models for JE surveillance for the purpose of developing mitigation strategies in Asia. As a first step in this project, an ecological niche modeling program, Maxent, was used to determine the potential distribution of *Cx. tritaeniorhynchus* in the Republic of Korea (ROK). Input data for the model included mosquito occurrence locations from field collections throughout the ROK and environmental data, including land cover, climate variables, normalized difference vegetation index (NDVI), and elevation. A probability map produced by the model predicted low probabilities of *Cx. tritaeniorhynchus* in the forested, mountainous areas and high probabilities associated with rice paddies and low-lying areas. A jackknife test demonstrated that land cover, elevation, summer NDVI, summer minimum temperature, and maximum winter and fall temperature contributed to the model for the presence of *Cx. tritaeniorhynchus*. The model was validated using traditional statistical methods, and reported JE cases from 2001 to 2009 fell within the higher probability areas on the map. Ecological niche modeling of *Cx. tritaeniorhynchus* was shown to be a useful tool for identifying areas of greater risk of transmission of JE virus. Although reservoir and amplifying hosts are necessary for transmission of JE virus, the prediction of disease outbreak occurrences may be more dependent upon mosquito abundance rather than presence. Future work will examine the use of real-time satellite data to determine if mosquito abundance can be predicted using NDVI or climate variables.

608

ASYMMETRICAL INTERSPECIFIC COMPETITION BETWEEN *CULEX RESTUANS* AND *CX. PIPIENS* IN ILLINOIS

Joel A. Morris, Robert J. Novak

University of Alabama-Birmingham, Birmingham, AL, United States

Competitive interactions between sympatric West Nile virus vectors, *Culex restuans* and *Cx. pipiens*, were evaluated in Champaign-Urbana, IL in 2007 using a replacement series in which field collected mosquito larvae were placed in rearing pans with different ratios of each species (100%:0%, 75%:25%, 50%:50%, 25%:75%, and 0%:100%). Each larval combination was repeated at biologically relevant temperatures (22°C, 26°C, and 30°C) and densities (0.05, 0.10, 0.20, and 0.40 larvae/ml). Results suggest that overall survival of *Cx. restuans* was greater than *Cx. pipiens* across all treatment variables. However, each species responded to survival and fitness (body mass) tradeoffs quite differently. Survival of both species was primarily limited by increasing density, but survival of *Cx. restuans* benefited from increasing competition with *Cx. pipiens* while survival of *Cx. pipiens* benefited from intermediate temperatures. In contrast, while fitness of both species primarily decreased with increasing densities, only *Cx. pipiens* mass benefited from increasing competition with *Cx. restuans*. Thus, *Cx. restuans* placed emphasis on survival at the expense of fitness, while *Cx. pipiens* placed emphasis on fitness at the expense of survival rate. These species yielded identical total biomasses by sex, which suggests that superior competition for *Cx. restuans* may be countered by superior fitness for *Cx. pipiens*. This would allow robust populations *Cx. pipiens* to rapidly colonize opened niches as *Cx. restuans* enter diapauses in July.

609

CLIMATE AND LAND COVER INFLUENCES ON *CULEX TARSALIS* (DIPTERA: CULICIDAE) POPULATIONS IN SIOUX FALLS, SOUTH DAKOTA

Ting-Wu Chuang[1], Michael B. Hildreth[2], Denise L. VanRoekel[3], Lon Kightlinger[4], Michael C. Wimberly[1]

[1]GISc Center of Excellence, South Dakota State University, Brookings, SD, United States, [2]Department of Biology and Microbiology, South Dakota State University, Brookings, SD, United States, [3]Sioux Falls Health Department, Sioux Falls, SD, United States, [4]South Dakota Department of Health, Pierre, SD, United States

West Nile virus (WNV) first invaded the Northern Great Plains (NGP) in 2002, and the incidence of human cases in this region has remained high compared to the rest of the United States. *Culex tarsalis* is the most important vector of WNV infection in the NGP, and the abundance of mosquitoes is a key factor in the amplification and transmission of WNV. However, the effects of land cover and climatic variability on vector populations in the NGP are not well understood. This study compared *Cx. tarsalis* populations and examined their relationships with land cover types, temperature, and precipitation in Sioux Falls, South Dakota from 2005 to 2008. Between 20 and 40 CDC CO2-baited light traps were set annually in Sioux Falls from May to September of 2005 through 2008,

and the number of *Cx. tarsalis* was identified by seasonal staff. Land cover characteristics were acquired from the 2001 National Land Cover Dataset (NLCD) and the percentages of selected land cover types were calculated within a buffer zone around each trap determined by the flying range of *Cx. tarsalis*. Temperature and precipitation were summarized from local weather stations. Land cover analysis indicated that wetland and cultivated crops were usually positively correlated with mosquito populations but the strength and seasonality of these correlations varied by year. Developed land showed consistent negative associations through the whole study period. Both temperature and rainfall showed lagged effects on mosquito populations. In general, higher temperature and precipitation in different week lags were associated with higher mosquito populations in the current week after adjusting for spatial autocorrelation. The early emergence of vector abundance in 2007 was associated with a high number of WNV human cases in early summer. This study demonstrated the associations among *Cx. tarsalis* populations, land cover types, and seasonal climate patterns in Sioux Falls. These results can be used to improve vector control strategies and disease prevention efforts.

610

BLOOD MEAL ANALYSIS OF MALARIA MOSQUITO VECTORS, EQUATORIAL GUINEA

Vasiliki Pappa[1], Hans Overgaard[2], Michael Reddy[1], Jeff R. Powell[1], Adalgisa Caccone[1]

[1]Yale University, New Haven, CT, United States, [2]Medical Care Development Inc./Marathon Oil Corp., Bioko, Equatorial Guinea

Host feeding patterns and longevity serve as important parameters in determining the capacity of a mosquito population to act as a vector of disease. Mosquitoes such as *Anopheles gambiae* are considered highly anthropophilic and endophilic. However, in endemic regions, vector populations consist of more than one species that may vary in terms of host seeking behavior. More importantly, in regions where vector control programs have been in effect for years, vector populations have been observed to prefer non-human hosts. The degree of anthropophily can be assessed by estimating the Human Blood Index (HBI). This index is then used to estimate the vectorial capacity in an area. In this study we used a multiplexed PCR assay to identify the bloodmeal source of the vector population in Equatorial Guinea, in order to estimate the HBI. All the vectors showed high anthropophily, with HBI greater than 0.5. Bloodmeal analyses are essential to determine vector host choice and feeding behavior changes, which highlights the importance of such studies for evaluating malaria control interventions. As a result, these analyses should be incorporated in malaria control programs.

611

DIFFERENTIAL IMPACT OF LONG-LASTING INSECTICIDE TREATED NETS ON ANOPHELINE VECTOR POPULATIONS AND MALARIA TRANSMISSION IN PAPUA NEW GUINEA

Lisa Reimer[1], Edward Thomsen[1], Gussy Koimbu[1], Absalom Mai[1], August Kapa[1], John Keven[1], Henry Dagoro[1], Ivo Mueller[2], Peter Siba[2], James Kazura[3], Manuel Hetzel[2], Peter Zimmerman[3]

[1]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [2]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea, [3]Case Western Reserve University, Cleveland, OH, United States

In Papua New Guinea (PNG) members of the Punctulatus Group, including *Anopheles punctulatus*, *An. koliensis*, *An. farauti s.s.*, *An. farauti 4* and *An. hinesorum* (formerly *An. farauti* 2), exhibit heterogeneities in distribution, biting behavior and malaria infection levels. The PNG National Department of Health recently launched a nation-wide long-lasting insecticide treated net (LLIN) program. This study aimed to evaluate the impact of the campaign on anopheline species density, composition, feeding behavior and malaria infectivity. Eight sentinel sites were chosen from 6 provinces of PNG representing coastal, riparian, inland and highland regions. Entomological surveys were conducted prior to and one year post-LLIN distribution. Two of these sites were chosen for an intensive monthly entomological evaluation beginning one year prior to distribution in August 2009 until August 2010. Host-seeking anophelines were collected by the landing catch method from 6pm to 6am (N=46,000). Adults were identified to morphospecies and confirmed by PCR-RFLP of the internal transcribed spacer 2 rDNA. Malaria infectivity was determined by circumsporozoite ELISA for *P. falciparum*, *P. vivax* 210 and *P. vivax* 247. Overall man-biting rates for each species were reduced following the LLIN campaign. The reduction ranged between five-fold and ten-fold, and was greater in species with late night biting habits such as *An. punctulatus* and *An. koliensis* than the Farauti complex which has a tendency to bite in the early evening. Within 6 months of LLIN distribution, peak biting times for both *An. farauti 4* and *An. punctulatus* shifted significantly ($p<0.001$). The proportion of *An. farauti 4* biting between 6pm and 10pm was significantly higher after bednets, while the peak biting times for *An. punctulatus* shifted from 12am-3am pre-LLIN to 11pm-1am post-LLIN. Preliminary data show that members of the Farauti complex have lower rates of infectivity to *Plasmodium* than *An. punctulatus* and *An. koliensis*. Differences in biting times and susceptibility to infection will impact the success of LLIN campaigns, and the behavioral shift to earlier biting may alter malaria transmission dynamics.

612

LATE BITING OF *AEDES ALBOPICTUS* IN CHIANG MAI PROVINCE, NORTHERN THAILAND, CHANCE FOR PREVENTION AND MOSQUITO CONTROL

Wannapa Suwonkerd[1], Nantawan Suwannachote[1], Theeraphap Chareonviriyaphap[2]

[1]Office of Disease Prevention and Control, Ministry of Public Health, Chiang Mai, Thailand, [2]Department of Entomoloy, Kasetsart Univesrity, Bangkok, Thailand

Aedes albopictus is widely distributed throughout Thailand and play an importance role in transmitting of viral diseases i.e dengue fever and chikungunya in Thailand. It breeds in natural container as well as human made container, however little is known about its behavior particularly the biting pattern. The study was conducted in rural of Chiang Mai, northern Thailand. 6 households located at head, middle and at the end of the village were selected to be the collection sites. In each household one pair of collector collecting the mosquito by using the sweeping net and aspirator, collecting mosquitoes inside and outside a house of 10 minutes interval between indoor, outdoor and brake, as a total 40 minutes collecting time in each household. The mosquito collection started from 6.00 am to 23.00 pm, two days per month. The study was conducted from January to April and May to August 2010 representing dry and wet season respectively.

In dry season (January to April) Ae. albopictus showed long day biting from 06.00 hr to 23 hr with sharp peak from 15.00-18.00. However after sunset, 18.00-23.00 hr. this mosquito showed the same number of mosquito collected between 12.00-15.00 hr. The raining season study is on going and it will be further discuss later.

613

MULTIPLE MATING IN *AEDES AEGYPTI*: SPERM TRANSFER AND USAGE PATTERNS

Prasit Deewatthanawong, Laura C. Harrington

Cornell University, Ithaca, NY, United States

Mosquito mating behavior including mating frequency is an important factor affecting mosquito population structure and genetic control efforts. Our previous work has detected a small but potentially significant frequency of multiple mating in the laboratory and field for *Aedes aegypti*. The objective of this study was to investigate the frequency of multiple mating and more specifically, the frequency of sperm transfer and

female utilization of sperm from more than one mating. In this study, we report our results of female sperm usage patterns using a combination of approaches including PCR-based detection of sperm genotypes and screening of female reproductive output.

614

BEHAVIOURAL PATTERN OF THE MALARIA VECTORS AND VECTOR CONTROL INTERVENTIONS IN LUANGWA VALLEY, SOUTHEAST ZAMBIA

Aklilu Seyoum[1], Chadwick Sikaala[2], Javan Chanda[2], Dingani Chinula[2], Neil Lobo[3], Frank Collins[3], Gerry F. Killeen[4]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]National Malaria Control Centre, Lusaka, Zambia, [3]University of Notre Dame, South Bend, IN, United States, [4]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania

Insecticide treated nets and Indoor residual spraying are the principal vector control interventions in Africa. Understanding of the behaviour of malaria vectors in different vector control intervention areas is important for effective implementation of the control programs. Human landing catches conducted both indoor and outdoor allowed us to survey the preferred feeding patterns of mosquitoes while the human behavior data collected through the household surveys have allowed us to estimate actual patterns of human exposure. *Anopheles funestus* seems equally predisposed to bite indoor or outdoor, regardless of intervention treatments. However, *Anopheles gambiae* s.l exhibited its classically documented endophagic tendency, particularly in blocks where both insecticide treated nets and indoor residual spraying applied in combination. Data on the hourly mean catches of the malaria vectors indicates the peak biting of *An. gambiae* s.l was just after the average time that residents go to bed at approximately 20 hrs. Contrary to *An. gambiae*, the peak biting time of *An. funestus*, the predominant vector in the area was in the late hours of the night, well after people go to sleep in both intervention areas. The highest catch, regardless of the intervention treaments was between 4 and 5 hrs. Because residents typically go to indoors and into bed at 20 hrs and get up at 6hrs, rude estimates of the proportion of human exposure occuring indoors was high for both species, with mean values of 0.90 and 0.94 for *An. gambiae* and *An. funestus*, respectively, that are essentially unchanged by intervention status.

615

SEXUAL PERFORMANCE OF *AEDES ALBOPICTUS* (DIPTERA:CULICIDAE) MALES IN THE FRAME OF A STERILE INSECT TECHNIQUE PROGRAM

Sebastien Boyer

Institut de Recherche pour le Développement, Sainte Clotilde, Réunion

Aedes albopictus was described as a vector of at least 22 arboviruses. This species can transmit Alphavirus (Chikungunya, equine fever) and Flavivirus (4 dengue serotypes, yellow fever, west nile fever). This species is now considered as the most efficient vector of Chikungunya virus and second for the dengue. *Ae. albopictus* is well established in Asia (native area), North and South America, Africa, Europe and Australia. Its high vector competence is combined with an efficient spreading behaviour. Current mosquito control methods against this species consist of chemical or biological treatments. The major problem besides the persistence of insecticides in the field and their impact on non-targeted species is the rapid acquisition of insecticide resistance. In the context of an area-wide integrated vector management, the Sterile Insect Technique (SIT) could be suitable in La Reunion Island. The insularity creates a geographical isolation and the existence of cool and dry seasons bring a decrease in mosquito populations. The sexual performance of wild males of targeted populations needed to be investigated in a SIT strategy. The mating ability of males *Ae. albopictus* was tested with batches of females and different cage sizes under laboratory conditions (colony from Saint-Pierre, La Réunion). One male was able to inseminate an average of 9.5 females and filled an average of 15.5 spermathecal capsules. One male encaged with 2 females removed and replaced every 24 h for 12 days inseminated 5.3 females and filled 8.6 spermathecal capsules. One male with 10 females removed and replaced every 24 h for 14 days inseminated 8.6 females and filled 12 spermathecal capsules. In the last two experiments, a significant decrease of mated females was observed over time. The high number of mated females by one male is encouraging for a SIT control of mosquitoes. The duration of the male activity is also a good new, in spite of its decrease over time. These two results will be used to model the release of males Ae. alobpictus.

616

DEVELOPMENT OF A NEW BIOMARKER OF EXPOSURE TO *ANOPHELES* BITES BASED ON HUMAN ANTIBODY RESPONSES TO SALIVARY PROTEINS: FROM THE CONCEPT TO THE APPLICATIONS

Anne Poinsignon[1], Papa-Makhtar Drame[2], Badara Samb[3], Sylvie Cornelie[1], Cheikh Sow[4], Ibrahima Dia[5], Gilles Riveau[4], Lassana Konate[6], Cheikh Sokhna[7], **Franck J. Remoue**[2]

[1]IRD-UR016, Montpellier, France, [2]IRD-UR016/CREC, Cotonou, Benin, [3]University Cheikh Anta Diop, Dakar, Senegal, [4]NGO Espoir pour la Santé, Saint-Louis, Senegal, [5]Institut Pasteur, Dakar, Senegal, [6]Université Cheikh Anta Diop, Dakar, Senegal, [7]IRD-UMR198, Dakar, Senegal

Human antibody (Ab) IgG response to whole saliva of An. gambiae could be an epidemiological biomarker of exposure to An. gambiae bites. In the objective to increase the specificity to Anopheles exposure, the second step is to identify the salivary proteins i) specific to Anopheles genus and ii) antigenic in children exposed to malaria. First, the identification of immunogenic salivary proteins of An. gambiae by an immuno-proteomic approach was assessed. The second step was to design peptide sequences, from the selected An. gambiae gSG6 antigen using a bioinformatic approach, taking into consideration i) their potential antigenic properties and ii) the absence of cross-reactivity with other arthropods/organisms. The specific IgG Ab levels were then evaluated in Senegalese children in different context of malaria.

From five gSG6 peptides, one gSG6-P1 peptide presented all criteria to be an optimal candidate biomarker for evaluating exposure to An. gambiae bites. Indeed, in addition to high specificity to Anopheles genus, the anti-gSG6-P1 IgG level was associated with the intensity of exposure to An. gambiae bites. In addition, complementary studies indicated that gSG6-P1 represents a specific tool for detecting low exposure to An. gambiae and also one biomarker for evaluating the level of An. funestus bites. This new "salivary" biomarker of Anopheles exposure could be used as a geographic indicator for mapping the risk of malaria transmission and especially in low Anopheles density conditions, where entomological methods are limited in sensitivity (dry season, altitude or urban malaria). It could also represent a direct criterion of efficacy in the evaluation of anti-vector strategies.

617

THE EFFECTS OF SUBLETHAL PESTICIDE EXPOSURE ON VECTORIAL CAPACITY OF BITING INSECTS

Lee Cohnstaedt, Sandra Allan

United States Deparment of Agriculture, Gainesville, FL, United States

Vectorial capacity is the efficiency of an arthropod to transmit disease to susceptible hosts. The Ross-Macdonald mathematical model identifies the parameters determining disease transmission efficiency and can be used to determine the role of insecticide use in reducing vector-mediated disease transmission (as reported previously). The frequent contact of disease vectors with insecticides via aerial spraying, residual spraying in houses or on barriers, bed nets, and larval treatments will reduce disease transmission but the sublethal aspect of insecticide exposure may also increase the surviving insect's vectorial capacity and contribute to the

evolution of insecticide resistance. Sublethal exposures to insecticides has been known to change the biting activity, longevity, host seeking ability and possibly the intrinsic incubation period if the arthropod's physiology is changed. Sublethal effects must also be considered in disease resistance evolution because in addition to the altered vectorial capacity, the surviving mosquitoes will have a fitness advantage in the presence of pesticides. Therefore, sublethal effects of insecticides may be altering the vectorial capacity and increase the rate of insecticide resistance evolution.

618

FIRST DATA ON *AEDES ALBOPICTUS* DISPERSAL IN ITALY

Francesca Marini[1], Beniamino Caputo[1], Marco Pombi[1], Manuela Travaglio[2], Gianfranco Tarsitani[1], Fabrizio Montarsi[3], Andrea Drago[4], Alessandra della Torre[1]

[1]University of Rome "Sapienza", Rome, Italy, [2]University of Padua, Padua, Italy, [3]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padua, Italy, [4]Entostudio snc, Padua, Italy

We here present the results of *Aedes albopictus* Mark-Release-Recapture (MRR) experiments in which sticky-traps (STs) were used to collect released females. Three MRRs were carried out in a 250 m radius area within the campus of Sapienza University in Rome (RM, central Italy) in summer 2008, releasing about 500 blood-fed females and employing 55 STs (≈1 ST/3,600 m2) in each replicate. Other 3 MRRs were carried out in a 500 m radius area in a rural/periurban site in the Province of Padova (PD, northern Italy) in summer 2009, releasing about 1.000 blood-fed females and employing 94 STs (≈1 ST/8,400 m2) in each replicate. Recaptures were carried out for 16 days after releases. The recapture rates obtained ranged between 3.3 and 5.1% in RM and 3.4 and 13.4% in PD. Most re-captured females were collected at the gravid stage in the first 8 days after releases. This observation - coupled with the results of single oviposition experiments carried out in PD simultaneously to the releases - allows to conclude that our results mainly refer to the dispersal of females looking for an oviposition site after having completed a single gonotrophic cycle triggered by the blood-meal provided before releases. The females were mostly recaptured at 50-200 m and 0-150 m from the release sites in RM and PD, respectively. Single females flew up to 230 and 464 m in 4 days in RM and PD, respectively. In both sites the females reached the limit of the study areas, indicating that they may probably fly even further away. The cumulative mean distance travelled was 105, 121, 139 m in RM and 110, 77, 68 m in PD. These results will be discussed with reference to the ecological characteristics of the two study areas. These data represent the first evaluation of *Ae. albopictus* movements in an European area and are instrumental to plan control activities and to determine appropriate control limits necessary to interrupt pathogen transmission in case of possible arbovirus epidemics in Europe, such the Chikungunya outbreak occurred in northern Italy in 2007.

619

COMBINED SEWAGE OVERFLOW WATER QUALITY AND SEASONALITY EFFECTS ON *CULEX SPP* OVIPOSITION

An T. Nguyen, Luis F. Chaves, Uriel Kitron

Emory University, Atlanta, GA, United States

Larvae of *Culex quinquesfasciatus*, the main vector of West Nile virus in the southern US, are often found in combined sewage overflows (CSOs) in the city of Atlanta, GA. Semi-natural experiments were conducted to study the association of mosquito oviposition with fluctuating levels of chemical nutrients and seasonal weather variability in pools containing CSO water in Tanyard creek, Atlanta, GA. Semi-natural habitats were created in artificial isolated pools to compare oviposition frequency and intensity in protein-enriched CSO water with pools containing unenhanced CSO water. Water nutrients, dissolved organic N and P, in the isolated pools and the main creek, and oviposition rates in the isolated pools were compared over time. The addition of nutrients to these systems increased organic matter concentration and the oviposition rate. Water temperature and relative humidity changes in the environment had a direct impact on the number of oviposited egg rafts. These results are relevant to understand the spatial and temporal abundance of mosquito vectors and West Nile Virus transmission risk in urban settings.

620

A "CONSUMER PRODUCT" STUDY FOR USE OF INSECTICIDE TREATED CURTAINS TO REDUCE MOSQUITOES AND DENGUE INFECTIONS IN HOMES

María Alba Loroño-Pino[1], Julián García-Rejón[1], José Arturo Farfán-Ale[1], Arturo Losoya[2], Lyla Aguilar[2], José Trapaga-Barrientos[2], Saul Lozano-Fuentes[3], Lars Eisen[3], **Barry J. Beaty**[3]

[1]Universidad Autónoma de Yucatán, Merida, Mexico, [2]Bayer Environmental Science - Bayer de Mexico, Mexico City, Mexico, [3]Colorado State University, Fort Collins, CO, United States

Novel control strategies are essential to reduce vector-borne diseases. Empowering individual home owners to complement and enhance governmental control efforts is an attractive and innovative approach for vector control. A "Casa Segura" study was conducted in Merida, Mexico to determine if individual home owners could utilize insecticide treated curtains (ITCs), provided by Bayer de Mexico/Acytex Internacional, to reduce the number of vector mosquitoes (*Aedes aegypti*) and the risk for becoming infected with dengue virus in their own homes. The study included 411 homes and 2,042 participants. Paired homes were randomly selected to receive ITCs or non-treated curtains (NTCs). They were located 80-100 m apart (*Ae. aegypti* rarely disperses > 50 m in urban settings) to prevent any spill over effect of the ITCs. Epidemiological outcomes (including dengue virus infection and seroconversion) and entomological outcomes (including *Ae. aegypti* and *Culex quinquefasciatus* abundance in the homes) were monitored. Further, ITC killing efficacy over time was characterized in bioassays (WHO cone assay), and "consumer" acceptance, usage and satisfaction with the ITC product was determined. Preliminary results indicate a reduction in dengue infections in participants in homes with ITCs versus NTCs. Reductions in *Ae. aegypti* and *Cx. quinquefasciatus* abundance were initially detected in ITC homes, and other entomological outcome trends, e.g., presence of dengue virus infected *Ae. aegypti* females, also suggest a protective effect of the ITCs. The great majority of infected mosquitoes were collected from bedrooms. Finally, social scientist interviews conducted as part of the study revealed extensive use of aerosol, mosquito coil, and insecticide emanators in households in Merida to reduce indoor mosquito biting.

621

EVALUATION OF ZEROVECTOR® DURABLE LINING (DL) - RESTING AND IRRITANCY PATTERNS OF *AEDES AEGYPTI* UNDER VARYING SURFACE AREA COVERAGE

Joseph Wagman, Luana Arce, Pankhil Shah, Truc Vy Nguyen, Tarra Foggie, John Grieco, Nicole Achee

Uniformed Services University of the Health Sciences, Bethesda, MD, United States

Maximum success from a particular vector control strategy results from optimizing the intervention. Such outcomes depend on characterizing species-specific behavioral responses of vectors against the product under evaluation. Recently, a *D*urable *Activated R*esidual *T*extiles (DART) product has been developed to replace the traditional indoor residual spray (IRS) strategy. The function of this durable-lining (DL) material is to transfer toxic doses of deltamethrin to vectors resting on the surface of the material thereby reducing overall populations and biting pressures to human hosts. The DL is fixed to the interior wall surfaces of homes in malaria endemic areas. As house dimensions vary, there are areas of the wall that are left exposed (i.e., no DL) thereby creating "safe-sites" where vectors may rest without making contact with chemical. The contribution of these safe-sites to overall product success - mortality and/or escape prior to biting was evaluated using laboratory methodologies. We present results

describing the resting preference, knock down (KD) and 24 h mortality responses of unfed *Aedes aegypti* females (THAI strain) exposed to varying surface area coverage of DL (100, 75, 50 and 25%) under laboratory conditions. In addition, rates of assay escape were quantified to determine time (i.e., probability of man-vector contact under field conditions) and density (i.e., total reduction of potential biting vectors) responses to DL material. For all assays in which treated DL was applied, there was less resting response overall - even on safe sites (i.e., metal surfaces) within the assay device- and significant increases in the proportion of test cohorts flying and exhibiting KD compared to matched controls. This indicates an agitation response from the chemical active that was true even at a 25% coverage ratio. When test cohorts were evaluated for escape response in subsequent movement assays, there was significant increase in percent escape compared to matched control assays at the 25, 50 and 75% DL coverage ratios after correcting for movement in control tests. Combined, these results suggest minimal negative effects of safe sites to the overall efficacy goals of the DL product. Similar studies will be repeated against blood-fed *Ae. aegypti* test cohorts in preparation for field validation.

622

SPATIAL SCALE OF DENGUE VIRUS TRANSMISSION IN IQUITOS, PERU

Kelly A. Liebman[1], Steven T. Stoddard[1], Brett M. Forshey[2], Amy C. Morrison[1], Tadeusz Kochel[2], Thomas W. Scott[1]

[1]University of California, Davis, Davis, CA, United States, [2]United States Navy Medical Research Center Detachment, Lima, Peru

Understanding spatial patterns of dengue virus (DENV) transmission is critical for the design of improved surveillance and control strategies. Although clinically apparent DENV infections are known to occur in households with similar onset of illness, a key unanswered question is at what scale transmission occurs beyond the home. Mosquito mark-release-recapture studies indicate that the DENV vector *Aedes aegypti* typically disperses only short distances (<100 m), leading to the prediction that cases will cluster within a 100 m radius. We carried out Bernoulli spatial analyses of DENV infections in a prospective longitudinal cohort of >4,000 people from 1999-2005 in Iquitos, Peru. Infection status was determined by seroconversion (neutralizing antibody) in paired blood draws taken every 6 months. Date of infection was assigned as the mid-date between paired samples, and each year was divided into 3 distinct seasonal periods of DENV transmission (Jan 1 - Apr 30, May 1 - Aug 31, Sept 1 - Dec 31). We identified serotype-specific spatial clusters at maximum radii of 100, 300, 600 and 900 meters, during each trimester, for all DENV serotypes circulating in Iquitos [5 DENV-1 clusters (1999, $p \leq 0.001$; 2002, $p=.008$), 1 DENV-2 (2000, $p=0.025$), and 15 DENV-3, beginning in 2001 when DENV-3 was first introduced (2001, $p<0.022$; 2002, $p<0.05$; 2003, $p<0.026$; 2004, $p=0.003$)]. The number of cases per cluster ranged from 3 to 39 with radii of 0 - 740 meters. Our results indicate that (1) interventions need to extend beyond a person's home to substantially reduce their risk of infection, (2) human movement beyond the flight range of *Ae. aegypti* is important for defining the spatial scale of DENV transmission, and, (3) if clusters of cases can be operationally and cost effectively identified, spatially targeted intervention strategies should be considered as a cost-effective way to prevent disease.

623

AN EVALUATION OF NON-TOXIC APPROACHES FOR DENGUE VECTOR CONTROL

Hortance Manda, Luana Arce, Pankhil Shah, Nicholas Martin, Truc Vy Nguyen, Tarra Foggie, John Grieco, Nicole Achee

Uniformed Services University of the Health Sciences, Bethesda, MD, United States

Research shows that killing insects through direct chemical means may not be necessary for effective vector control. Other approaches to reduce man-vector contact at the house level exist and might be sufficient. This includes initiating a spatial repellent effect, preventing house entry; and/or contact irritant effect, causing an escape response prior to mosquitoes biting humans indoors. Such non-toxic approaches are currently being evaluated in this research project as proof-of-principle for a Push-Pull strategy for *Ae. aegypti* control. We are targeting preferred house entry portals and/or indoor resting sites to make them unsuitable, using minimum effective dose at minimum surface area coverage to reduce densities of mosquitoes inside homes, a site of disease transmission. A component to the overall success of the program is the ability to correlate chemical concentration in the surrounding air space of a treatment source with vector behavior over distance. This quantification will be used to describe behavioral thresholds of non-toxic control strategies and help to clarify the mode of action of target chemicals. The overall goal is to drive the development of innovative and cost-effective vector control strategies. This study reports on the contact irritancy and spatial repellency responses of two geographically distinct female *Ae. aegypti* strains (Thailand and Peru). Entry (i.e., repellency), escape (i.e., irritancy), and mortality rates were quantified in response to different doses of chemical and surface area coverage of standard vector control compounds under laboratory conditions. Air space at various distances away from the treatment source was sampled for chemical, and its concentrations quantified and correlated to the entrance and exit behavior of test mosquito populations. Experiments were also validated under field conditions using experimental huts. Results of this study will guide the development of air sample testing protocols as it relates to vector ecology and ultimately guide the optimal conditions for a Push-Pull strategy trial.

624

EVALUATION OF THE SPECIES COMPOSITION AND RELATED ECOLOGY OF BLACK FLIES (DIPTERA: SIMULIDAE) FROM BELIZE, CENTRAL AMERICA

Cecilia L. Coscaron-Arias[1], Nicole Achee[1], Russell King[2], Ireneo Briceno[2], John Grieco[1]

[1]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]Ministry of Health, Belmopan, Belize

Across the Americas, over 500,000 people live in areas at risk for the transmission of Onchocerciasis and over 180,000 are infected in endemic areas. It is well documented that this disease is transmitted by black flies in the family Simulidae. The members of this family occupy a diverse range of environments and habitats including man-made sites such as dams and irrigation canals. Black flies from Guatemala and Mexico have been studied extensively due to the endemic foci of onchocerciasis found in these countries. However, the simulids from Belize, a neighboring country, are not well known and current information for the country only exists for the most southern regions. Information that does exist suggests that five of the thirteen species found in Belize are known to vector onchocerciasis. In addition, two other species are known to vector carate or mal de pinto and Venezuelan Equine Encephalitis. Due to the lack of information from this region of Central America with regard to the potential risk for the transmission of onchocerciasis, this study evaluated the species distribution and related ecology of black flies from Belize. The resulting data was incorporated into a GIS platform to display the areas at risk for the dominant vector species in the region. The resulting risk maps can be used to guide surveillance and control efforts in Belize.

625

CONTEXT-DEPENDENT OVIPOSITION STRATEGIES BY *AEDES AEGYPTI*

Jacklyn Wong, Yui Yin Chu, Steven T. Stoddard, Helvio Astete, Amy C. Morrison, Thomas W. Scott

University of California, Davis, Davis, CA, United States

Effectiveness of *Aedes aegypti* surveillance and control strategies depends on assumptions about oviposition behavior. Sensitivity of ovitraps for detecting *Ae. aegypti* presence and efficacy of lethal ovitraps for reducing

adult populations may be overestimated if skip oviposition occurs less frequently than anticipated. Effectiveness of targeted source reduction is based on the assumption that females egg-saturate all available oviposition sites. Removing a portion of containers must result in a net reduction in adult population because productivity in remaining containers is already at maximum (as reported previously). In this study, we tested the hypothesis that *Ae. aegypti* egg allocation strategies are context-dependent. We predicted that given a choice between large containers with organic debris and small containers with clean water individual females would concentrate their eggs in one large container, leaving many small containers unoccupied. If large containers were removed, we predicted that females would switch to an alternate strategy, distributing their eggs widely among several small containers. We released into a field enclosure 12- 20 F1 females that had mated with known males in the laboratory and collected their eggs daily from eight potential oviposition containers. Our experiment was repeated three times with both large and small containers available, and twice with only small containers present. Using 10 microsatellite markers, we are assigning progeny to parental pairs (Probmax version 1.2, as reported previously) and tracking when and where each female laid her eggs over 7-10 days after release. We expect that removal of the most productive containers will not lead to a simple, proportional reduction in mosquito population size. Detailed studies of where wild females allocate their eggs and how they respond to targeted control measures will test fundamental assumptions of this frequently recommended control strategy and provide insights into improved dengue surveillance and control.

626

INSECTICIDE SUSCEPTIBILITY OF *PHLEBOTOMUS PAPATASI AND P, SERGENTI* (DIPTERA: PSYCHODIDAE) FROM TWO GEOGRAPHICAL REGIONS OF EGYPT USING CDC BOTTLE AND MICROPLATE ASSAYS

Abdelbaset B. Zayed[1], Daniel E. Szumlas[1], Hanafi A. Hanafi[1], David F. Hoel[1], Barry D. Furman[1], Peter J. Obenauer[1], Hanaa M. Mahmoud[2], Reham M. Abolwafa[1]

[1]United States Naval Medical Research Unit Number Three, Cairo, Egypt, [2]Faculty of Science, Al-Azhar University (Girls Branch), Cairo, Egypt

Phlebotomine sand flies vector cutaneous leishmaniasis (CL), is a serious disease infecting 1,500,000 persons per year over throughout the world. According to WHO 2008, the Middle East harbors about 15% of the global leishmaniasis burden. In Egypt, the North Sinai is the most CL epidemic area. Evaluation of insecticide resistance is an important component in any integrated vector control program. Field trials were conducted from two regions in Egypt to evaluate insecticide resistance from two populations of *Phlebotomus papatasi* and *P. sergenti*. Insecticide susceptibility of both species was observed in samples collected from North Sinai (desert area, 450 Km north east Cairo) and Aswan (agriculture area, 950 Km south Cairo) governorates. Insecticide resistance was detected using the CDC bottle assay and confirmed by biochemical mircoplate assays. First generation of sand flies from both species were exposed to seven insecticides at the following doses (µg/ bottle): permethrin (150), resmethrin (250), lambdacyhalothrin (3), deltamethrin (5), cypermethrin (5), malathion (150) and fenitrothion (120). Time-dose mortality curves demonstrated that, *P. papatasi* from Sinai were more susceptible than those collected from Aswan to lambdacyhalothrin, deltamethrin, cypermethrin, malathion and fenitrothion. While both populations were resistant to resmethrin and permethrin, *P. sergenti* demonstrated low resistance to deltamethrin and lambdacyhalothrin, cypermethrin and resmethrin. *P. papatasi* population collected from Aswan show high level of enzymes activity in AChE, insensitive AChE, EST, GST and Oxidase compared with the laboratory population, while Sinai populations demonstrated a high level of EST, EChE and Oxidase. No increased levels in detoxified enzymes were observed in the *P. sergenti* population collected from Sinai. These results confirmed insecticide susceptibility for this population.

627

SURVEY OF RICKETTSIAL VECTORS AND RESERVOIR HOSTS IN MILITARY AREAS OF OPERATION (AOS) ALONG NORTHERN THAI-MYANMAR AND THAI-CAMBODIA BORDERS

Pimmada Sakpaisal[1], Toon Ruang-areerate[1], Narupon Kuttasingkee[1], Nopadol Sangjun[1], Kiatisak Somsri[1], Jittasak Koewsathit[2], Khwananong Youngpakool[1], Pradith Kaewsatien[1], Chaiya Chanchu[1], **Jariyanart Gaywee**[3]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Faculty of Sciences, Chulalongkorn University, Bangkok, Thailand, [3]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Rickettsioses have been reported in military troops deployed to military areas of operation (AOs) along northern Thai-Myanmar and Thai-Cambodia borders. Causative agents, rickettsiae are intracellular bacteria maintained in nature via small mammalian hosts and blood sucking arthropod vectors. To better understand how these pathogens are transmitted to humans, we investigated these AOs for the presence of rickettsial reservoir hosts and vectors. Wild rodents were captured and there ectoparasites were collected. We also collected ectoparasitic arthropods from livestock, pet animals and humans in these AOs. *Rickettsia-Orientia* duplex nested PCR as well as sequencing were utilized to detect and identify rickettsial agents. From April 2008 to December 2009, we have collected and tested 133 clones of arthropods including twelve species of fleas, lice, and ticks from different hosts and locations. Rickettsial genes were detected in 80.7% and 74.8% of arthropods from Northern Thai-Myanmar and Thai-Cambodia border areas, respectively. Species of pathogenic rickettsiae identified by 17 kDa sequence analysis were *Rickettsia japonica*, *R. rickettsii* and *R. massiliae*. Those rickettsiae were detected in ticks (*Dermacentor* sp., *Rhipicephalus* sp. and *Haemaphysalis* sp.) collected from humans and pet dogs. Spotted fever group *Rickettsia* sp. similar to *Rickettsia* sp. Cf 1, Cf 5 and SE 313 were also detected in fleas (*Ctenocephalides canis*, *C. felis* and *Echidnophapga gallinacean*) and lice (*Liperus caponis*, *Menopon gallinae* and *Haematopinus asini*) collected from dogs, cats, cattle and chickens. *Orientia tsutsugamushi* DNA was not detected. Using ISE6 tick cell culture, 2 rickettsial isolates were obtained from *Dermacentor* and *Haemaphysalis* ticks collected from dogs in AOs along the Thai-Cambodia border. Specific species identification of theses isolates is ongoing. These findings indicate these AOs are endemic foci for rickettsioses. This information is crucial to establish an effective disease prevention and control strategy specific to such areas.

628

WOLBACHIA INFECTION AND *PLASMODIUM* DEVELOPMENT IN *ANOPHELES GAMBIAE*

Grant L. Hughes, Jason L. Rasgon

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

Recently, *Wolbachia pipientis,* an endosymbiotic bacterium of insects and nematodes, has been shown to interfere with pathogen development in stably transinfected *Aedes aegypti,* possibly due to stimulation of the mosquito innate immune system. Moreover, it can confer a fitness advantage to the host in naturally infected *Drosophila* when challenged with a virus. In these systems, *Wolbachia* acts against a broad range of pathogens, including viruses, bacteria, nematodes and *Plasmodium*. *Wolbachia,* which has not been detected in any species of *Anopheles,* has never been transinfection into this genus, despite numerous attempts to establish a stable line. However, transient somatic infections in *Anopheles* can be established. Here, we investigate the effect of somatic infections with multiple *Wolbachia* strains on *Plasmodium* development in *Anopheles gambiae*. In somatically infected *Anopheles*, *Wolbachia* is ubiquitously disseminated throughout the mosquitoes, however is noticeably absent

from the ovaries. After adult microinjection, normalized *Wolbachia* titers initially decrease, presumably due to the host immune response clearing the bacteria, but then increase dramatically approximately 2 weeks post-injection. Microarray analysis of *Wolbachia*-infected cell lines identified a suite of regulated host genes, with a range of immune-related genes both up and down regulated. In somatically infected wMelpop mosquitoes, qPCR identified down regulation of immune genes 15 days post injection. The wAlbB *Wolbachia* strain (from *Aedes albopictus*) induced both up and down regulation of immune genes. Our initial data indicates that somatically-infected *An. gambiae* do not have resistance to *Plasmodium falciparum* or *Plasmodium berghei*, suggesting that the interplay between *Wolbachia* and *Anopheles* differs from previously observed interactions. These observations may be related to why the *Anopheles* genus is uninfected in nature, with implications for developing a stably-infected *Anopheles* line.

629

DIPETALODIPIN, A NOVEL SALIVARY PLATELET AGGREGATION INHIBITOR THAT DISPLAYS HIGH-AFFINITY BINDING TO TXA_2

Teresa C. Assumpção, Patricia H. Alvarenga, José M. Ribeiro, John F. Andersen, Ivo M. Francischetti

National Institutes of Health, Rockville, MD, United States

Dipetalodipin (DPTL) is an 18 kDa protein from the salivary gland of hematophagous triatominae *Dipetalogaster maximus*. It belongs to the lipocalin superfamily of proteins and shows sequence similarity to pallidipin, a lipocalin from *Triatoma pallidipennis*, claimed as a specific inhibitor of collagen-induced platelet aggregation. Recombinant DPTL was found to inhibit platelet aggregation triggered by collagen, without interfering with neither GPVI nor integrin $\alpha2\beta1$-mediated platelet adhesion. DPTL also prevented platelet responses induced by U-46619 and arachidonic acid, without affecting aggregation induced by ADP, convulxin, PMA, and by ristocetin. This inhibitory profile suggested that DPTL targets a secondary mediator released from activated platelets. An assay based on incubation of DPTL with small molecules (e.g. prostaglandins, leukotrienes and biogenic amines) followed by gel-filtration chromatography, and mass spectrometry was optimized in an attempt to identified DPTL-bound ligand(s). Results indicated the presence of a ligand with molecular mass of 351-352 which is compatible with prostaglandins. Identification of the compound was attained by isothermal titration calorimetry which demonstrated that DPTL binds with high affinity to cTXA2, TXA2-mimetic (U-46619) and structurally related prostaglandins such as PGH2, PGF2α, and PGD2. Consistent with its binding properties, DPTL prevents rat aorta contraction stimulated by U-46619, and its effect was abolished when collagen-induced platelet aggregation was attenuated with SQ29,548, an antagonist of TXA2 receptor. A 3D model for DPTL is presented where the putative binding site is indicated. Our results demonstrate that Dipetalodipin, and presumably pallidipin, are platelet aggregation inhibitors with unique specificity to TXA2.

630

PERCEIVED UTILITY OF TICK IDENTIFICATION FOR CLINICAL MANAGEMENT OF TICK-BORNE DISEASES

Rachel E. Tsui[1], Jon E. Rosenblatt[2], Bobbi S. Pritt[2]

[1]St. George's University of London, London, United Kingdom, [2]Mayo Clinic, Rochester, MN, United States

Ticks are globally important ectoparasites that transmit multiple pathogens. They are routinely submitted to many clinical parasitology labs for identification although the extent of characterization is highly variable and the clinical relevance is not well described. The goal of this study was to define the role that tick characterization plays in clinical management, and determine which features are perceived as being clinically important. A 5-question email survey was sent to all Minnesota-based Mayo Clinic primary care physicians and nurse practitioners. Information regarding the use of lab tick identification services and disease prophylaxis was obtained. Of the 1008 surveyed, 153 (15.2%) responded. 74 reported seeing ticks in their practice, of which 29% would submit a tick to the lab for identification when removed in the office, and 31% would submit a tick if brought in by the patient. 24% would only submit select ticks based on patient symptoms or request, and 16% would not submit a tick to the lab under any circumstances. 36% would administer prophylaxis for tick-borne disease based solely on the presence of a tick, regardless of lab identification, whereas 20% would only administer prophylaxis if the laboratory identified the tick as a potential disease vector. The remaining 44% would base prophylaxis decisions on several factors. Of the information provided by the lab, species, degree of engorgement, presence/absence of mouthparts, gender, and life cycle stage were perceived as useful by 32, 11, 9, 6 and 6 respondents, respectively. PCR for tick-borne pathogens performed on the tick was perceived to be useful by 43 respondents. 60% of respondents would routinely submit ticks to the lab for identification if they were found on or by the patient, but only 20% required identification of a known disease vector before providing prophylaxis. Tick speciation was perceived as being the most useful morphologic feature although more respondents considered PCR for pathogens most useful, even though this is not supported by clinical recommendations.

631

SARCOPTES SCABIEI EXTRACT INFLUENCES EXPRESSION OF NFκB (NUCLEAR TRANSCRIPTION FACTOR KAPPA B) THAT CONTROLS EXPRESSION OF IL-8 GENES

B. Laurel Elder, Larry G. Arlian, Marjorie S. Morgan

Wright State University, Dayton, OH, United States

Previous experiments in our laboratory have indicated that exposure of human dermal microvascular endothelial cells to an extract made from *Sarcoptes scabiei* mite bodies results in a decrease in detectable interleukin-8 in the endothelial cell supernatant. We attempted to elucidate the mechanism that might be involved in the suppression of this IL-8 secretion. Several cellular pathways are proposed that lead to IL-8 secretion but in the final stage all utilize up- or down-regulation of nuclear transcription factor kappa B (NFκB) to control expression of IL-8 genes. NFκB is activated and able to enter the nucleus when its inhibitor IκB is phosphorylated and degraded. We hypothesized that scabies extract might inhibit the phosphorylation of IκB resulting in suppression of IL-8 genes. We found that TNFα-stimulated endothelial cells increased expression of nuclear NFκB as expected (positive control). Cells stimulated with scabies extract decreased NFκB expression as postulated but also expressed some other low molecular weight proteins that were bound by antibody to NFκB. Cells stimulated with mite extract expressed more cytoplasmic IκB (the NFκB inhibitor) implying that a component of the extract may be interfering with the phosphorylation of IκB. The meaning of these results is not yet clear but the data suggest that some component or components in scabies extract may act at this level of the pathway to influence the secretion of IL-8.

632

NATURAL INFECTION OF *LEISHMANIA* SPECIES IN *SERGENTOMYIA INGRAMI* AND *S. HAMONI* IN AN OUTBREAK AREA IN THE HO DISTRICT OF GHANA

Chukwunonso O. Nzelu[1], Shirley C. Odoom[1], Kwame Desewu[2], Naiki Puplampu[1], Karl C. Kronmann[3], Anita Ghansah[1], Michael D. Wilson[1], Daniel A. Boakye[1], Delphina Gomez[4]

[1]Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana, [2]Ghana Health Service, Accra, Ghana, [3]United States Naval Medical Research Unit 3, Ghana Detachment, Legon, Accra, Ghana, [4]Atomic Energy Commission, Accra, Ghana

Following the leishmaniasis outbreak in the Ho district of Ghana in 2004, studies have been going on to determine the causative parasite, the reservoir hosts and the sandfly vector species. The parasite species have been reported to be *Leishmania major* and a yet to be characterized species indicating a complex epidemiology for this outbreak than anticipated. Sandfly samples collected from 2005 to 2008 also recorded almost 100% Sergentomyia species. In 2009 blood meal analysis from blood-fed flies collected in human habitats indicated anthropophily of 3 *Sergentomyia* species: *S. ingrami, S. africana africana* and *S. simillima*. As a follow up on that study, we examined non-blood fed populations of these 3 species as well as others collected at the same time as the blood-fed ones for the presence of *Leishmania* parasites. A total of 951 sandflies belonging to 9 different *Sergentomyia* species were identified based on morphology. The major ones were *S. africana africana* (30.0 %); *S. ingrami* (22.7 %); *S. dissimillima* (20.8 %); *S. simillima* (19.6 %), and *S. hamoni* (5.9 %). Female sandflies were pooled in groups of 10 for DNA extraction and PCR for infection with *Leishmania* parasites. Thirty-four pools composed 7 *S. africana africana*, 8 *S. ingrami*, 7 *S. dissimillima*, 6 *S. simillima* and 6 *S. hamoni* were obtained. Two of the *S. ingrami* pools and one of *the S, hamoni* pools were positive. These results show for the first time the natural infection of *Sergentomyia* species (*S. ingrami* and *S. hamoni*) with *Leishmania* parasite in Ghana, and builds on earlier data indicating the possibility of *Sergentomyia* species as vectors of cutaneous leishmaniasis in Ghana similar to the suspicion of *S. ingrami* as potential vectors of *L. major* in Kenya.

633

WOLBACHIA INFECTION DYNAMICS IN TSETSE FLY POPULATIONS ACROSS UGANDA

Uzma Alam[1], **Corey Brelsfoard**[1], Oleg Kruglov[1], Yineng Wu[1], Loyce Okedi[2], Serap Aksoy[1]

[1]Yale University, New Haven, CT, United States, [2]National Livestock Resources Research Institute, Tororo, Uganda

Tsetse flies (*Diptera Glossinidae*) are the single vector for the protozoan parasite African trypanosomes, which are the causative agents of Human African trypanosomiasis (HAT), or sleeping sickness in humans and nagana (AAT) in animals. The incidence of HAT is geographically separated along the line of the Great Rift Valley with *Trypanosoma brucei rhodesiense (Tbr)* present in East and Southern Africa and *Trypanosoma brucei gambiense (Tbg)* in West and Central Africa. The disease epidemiology in Uganda is unique considering that it is the only African country known to have both *Tbr* and *Tbg*. *Glossina fuscipes fuscipes* transmits both parasite species, with *Tbg* transmission occurring in the northwest region and *Tbr* in the southeast limited to areas close to the shores of Lake Victoria. This segregation had held in place despite the movement of cattle and people. Recently the presence of *Tbr* in the mid-western part of Uganda has been confirmed in a three patients. This merger of the two disease belts is feared and stands to create a major public health crisis given the differences in diagnosis and treatment options. Based on mtDNA haplotypes, we have shown that tsetse populations across Uganda are highly differentiated. Based on this finding we hypothesized that a contributing factor to the high levels of differentiation observed could be circulating *Wolbachia* genotypes in the different populations. We used the *Wolbachia groEl* gene to screen *G. f. fuscipes* populations across Uganda for the presence of parasitic infections and *Wolbachia* infection types. Using a multiple locus sequence typing (MLST) approach we demonstrate that natural tsetse populations are infected with multiple *Wolbachia* types. We present a spatial and temporal map of *Wolbachia* infection dynamics and discuss their potential impact on tsetse population structures observed.

634

DETECTION OF *BLA*$_{CTX\text{-}M\text{-}15}$ EXTENDED-SPECTRUM-LACTAMASE GENES IN *E. COLI* FROM HOSPITAL PATIENTS IN NIGERIA

Adekunle O. Olowe[1], Lothar Wieler[2], Mirjam Brobbel[2], Antina lube-Becker[2], A. Fruth[3]

[1]Ladoke Akintola University of Technology, Osogbo, Osun State, Nigeria, [2]Institutes for Microbiology and Epizootics, Freie University, Berlin, Germany, [3]Robert Koch Institute, Wernigerode Branch, Germany, Wernigerode, Germany

The aim of this study was to investigate the occurrence and relatedness of CTX-M type extended spectrum -Lactamases (ESBL) in *Escherichia coli* isolates from patients of a Nigerian Hospital. The study included 116 *E. coli* isolated from inpatients and outpatients from January 2006 to January 2007 at the Ladoke Akintola University Teaching Hospital in Osogbo, Nigeria. The phenotypical confirmation test revealed 12 ESBL positive isolates, which were investigated for the presence of *bla*CTX-M genes. Nine of these *E. coli* contained *bla*CTX-M group 1 genes and additionally harbored *bla*TEM and *bla*OXA group 1 genes. Pulsed field gel electrophoresis (PFGE) of these 9 strains revealed 6 clonal groups, as four of the isolates revealed identical PFGE patterns while the other five showed no relatedness. Sequencing of the *bla*CTX-M gene of one isolate from each clonal group always identified CTX-M 15. At present there are no published data about the genetic background of ESBL-producing *E. coli* in Nigeria. To our knowledge, this is the first report of *E. coli* carrying *bla*CTX-M-15, *bla*TEM, and *bla*OXA genes in Nigeria. Further studies are ongoing on blaCTX-M enzymes situation in zoonotic isolates as it relates to man.

635

IN VIVO EFFICACY OF CIPROFLOXACIN, CEFTRIAXONE AND DOXYCYCLINE ALONE AND IN THEIR COMBINATION AGAINST *VIBRIO VULNIFICUS* INFECTION

Ganesh-Prasad Neupane, Dong-Min Kim

Division of Infectious Diseases, Department of Internal Medicine, Chosun University, College of Medicine, Kwangju, Republic of Korea

Ciprofloxacin, doxycycline and ceftriaxone; highly recommended drugs for the treatment of invasive *Vibrio vulnificus* infection were evaluated in high dose bacteria inoculated (1 × 108 CFU) iron loaded ICR mice (n=10). Mouse survival rate was calculated by Kaplan- Meier survival curve after monitoring for 48-h, and a highly sensitive quantitative PCR assay was performed to evaluate the number of DNA copies from viable bacterial cell in mouse blood drawn immediately after died or moribund by cardiac puncture. DNA binding dye Ethidium bromide monoazide (EMA) was used to differentiate viable and dead cells. The efficacies of ciprofloxacin, cefotaxime and doxycycline (oral and ip) route was compared with different combination therapy. The number of DNA copies was found decreased with increase of survival time of mice (1 × 104 - 1 × 105 CFU at 24h in dead mice). However, the number of DNA copies was 1 × 102 CFU or below than this level at 24 h survival mice in either monotherapy or combination therapy. Ciprofloxacin was the most effective drugs for monotherapy with high survival rate of 25 % at 48h. In combination therapy, a single dose doxycycline (i.p.) plus ceftriaxone was sufficient to reduce the mortality by 50 % in high dose *Vibrio* inoculated iron loaded mice in contrast to survival rate of 40 % in doxycycline oral plus ciprofloxacin treatment groups at least in our mouse

model infection. Furthermore, out of three combinations only doxycycline i.p. plus ceftriaxone showed significant ($P < 0.05$) versus doxycycline oral plus ceftriaxone treated group. Similarly, doxycycline i.p. plus ciprofloxacin ($P = 0.056$), and ciprofloxacin plus ceftriaxone ($P = 0.9$) did not showed significant result versus doxycycline oral plus ceftriaxone. Hence, in conclusion, cefotaxime plus doxycycline (i.p.) combination therapy might be the best treatment option among monotherapy or other combination therapy for lowering the high mortality rate of *Vibrio vulnificus* infection.

636

ANTIBIOTIC SUSCEPTIBILITY OF ENTEROBACTERIACEAE ISOLATED FROM COSTEÑO ARTISAN CHEESE SOLD IN MONTERIA DEPARTMENT OF CORDOBA, 2009

Linda M. Chams, Alberto Mestra, Maria F. Yasnot

Grupo de Investigaciones Microbiologicas y Biomédicas de Córdoba, Universidad de Córdoba, Monteria, Colombia

Infections caused by *Enterobacteriaceae* with multiple resistance to antimicrobial drugs, are a serious problem which must be faced daily by Physician, Veterinarians and Microbiologists. The use of these antibiotics, often indiscriminately, brings with it the selection of resistant mutants. The incidence of different species as well as the level of resistance to antibiotics most commonly used is a phenomenon with its own characteristics so it is essential to monitoring the evolution of resistance in microorganisms. The objective of this study was to determine the susceptibility of *Enterobacteriaceae* isolated from costeño artisanal cheese, to commonly used antibiotics in the treatment of humans and cattle. Descriptive exploratory study was conducted in 25 retail store in the city of Monteria, capital of the department of Cordoba, Colombia, duly registered with the Secretariat of Health of the department of Cordoba in 2009. 3 samples were taken from store, one every two months for a total of 75 samples, which were evaluated on color, smell and consistency. To isolate and to identify Enterobacteriaceae producing antibiotic resistence, we used the method of the FDA and used TSI, LIA, CITRATE, MR / VP, UREA and SIM. Serology was performed with the Kauffman-White scheme and sensitivity by the agar diffusion method under the international standards issued by the CLSI, 2008. The results were analyzed with the statistical software EXCEL. *E. coli* were isolated in the 75 samples (100%) tested, 7 of the 75 samples (9,3%) showed *Salmonella spp.*, and in 68 of the 75 samples (90,7%) analyzed was isolated Citrobacter spp. All strains isolated were resistant to amoxycillin for 100%, tetracycline 87.5%, Gentamicin and Chloramphenicol 70% c/u and 62.5% to amikacin. In conclusion, given that the costeño cheese is a food prepared from raw milk, a fact which get worse by deficiencies in sanitation and hygiene of outlets that sell this high regional consumption product, the high resistance percentage of Enterobacteriaceae isolated in these product to antibiotics is a concern, and turn on the alarms on their indiscriminate use, a fact that may cause the emergence of multiresistant strains to transfer this resistance to commensal and to pathogenic bacteria in food and bacteria belonging to the gastrointestinal flora of the consumer, making a serious public health problem.

637

TYPHOID FEVER WITH NEUROLOGIC FINDINGS -- MALAWI-MOZAMBIQUE BORDER, 2009

Emily C. Lutterloh[1], Andrew Likaka[2], James Sejvar[3], Tadala Khaila[2], Jeremias Naiene[4], Eric Mintz[3], Robert Manda[2], Michael Humphrys[3], Abel Phiri[2], Rudia Lungu[2], James Kaphiyo[2], Deborah Talkington[3], Kevin Joyce[3], Beth Tippett Barr[3], Austin Demby[3], Linda Capewell[3], Sara A. Lowther[3], David Townes[3], Kashmira Date[3], Yanique Redwood[3], Joshua Schier[3], Steve Monroe[3], Malawi-Mozambique Outbreak Investigation Team[3]

[1]New York Department of Health, New York, NY, United States, [2]Malawi Ministry of Health, Lilongwe, Malawi, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Mozambique Ministry of Health, Maputo, Mozambique

Salmonella enterica serovar Typhi is the agent of typhoid fever, which often presents with fever and abdominal pain and is transmitted by the fecal-oral route. Annually, an estimated 22 million cases and 216,500 deaths occur worldwide. We investigated an outbreak of unexplained febrile illnesses with neurologic findings, determined to be typhoid fever, in villages along the Malawi-Mozambique border.

Methods: Ill persons were identified through Malawi Ministry of Health surveillance. We gathered demographic and clinical information on ill persons for March-November 2009 by interview, examination, and chart review. Classification as a suspect case required fever and ≥1 other finding (e.g. headache, abdominal pain); a probable case required fever and a positive rapid IgM antibody test for typhoid (TUBEX® TF); a confirmed case required isolation of Salmonella serovar Typhi from blood or stool. Isolates underwent antimicrobial susceptibility testing. Local springs used for drinking water were tested for total coliform bacteria and Escherichia coli with presence-absence broth. We identified 204 suspect, 47 probable, and 37 confirmed cases from 18 villages. Median age was 21 years (range: 1-81 years); 56% were female. Forty-three patients had neurologic signs including ataxia, hyperreflexia, and clonus. Of these 43 patients, 16 (80%) of 20 had positive rapid typhoid tests, and 4 (67%) of 6 blood cultures yielded Salmonella serovar Typhi. All 27 isolates that were tested were resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole; 3 were also resistant to nalidixic acid. All three village springs tested were positive for total coliform bacteria and E. coli. In conclusion, the unusual neurologic manifestations of certain patients during this typhoid outbreak initially posed a diagnostic challenge. Rapid typhoid antibody testing in the field supported the diagnosis. Culture confirmation with antimicrobial susceptibility testing guided treatment. Recommended control measures include improvements in water quality, sanitation, and hygiene.

638

ANTIGEN SPECIFIC MEMORY B-CELL RESPONSES IN BANGLADESHI ADULTS AFTER ONE OR TWO DOSE OF ORAL CHOLERA VACCINATION, AND COMPARISON WITH NATURAL INFECTION

Mohammad Murshid Alam[1], Md. Asrafuzzaman Riyadh[1], Kaniz Fatema[1], Mohammad Arif Rahman[1], Nayeema Akhtar[1], Tanvir Ahmed[1], Mohiul Islam Chowdhury[1], Fahima Chowdhury[1], Stephen B. Calderwood[2], Jason B. Harris[2], Edward T. Ryan[2], Firdausi Qadri[1]

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

Natural infection with *Vibrio cholerae* O1 induces a robust systemic and mucosal immune responses that result in protective immunity against subsequent disease that lasts for at least 3-10 years. In contrast, protective immunity following vaccination with the whole cell killed oral cholera vaccine containing recombinant CtxB (Dukoral, Crucell, Sweden) is of shorter duration. The development of memory B cell responses, which may be important mediators of protective immunity, have not been

previously studied in vaccinees. In this study, we examined memory B cell responses in adult Bangladeshi individuals who received one (n=30) or two (n=30) doses of Dukoral, and we compared responses in 41 adult cholera patients. Vaccination induced vibriocidal antibody, and CtxB and lipopolysaccharide (LPS)-specific antibody responses in plasma within 3 days of the first dose of the vaccine ($P<0.001$) in both the groups of vaccinees. At 30 and 90 days after immunization, the responses (both magnitude and response rates) were comparable in both vaccine cohorts. Vaccinees developed significant CtxB-specific IgG and IgA memory B cell responses by day 30 post-immunization, and the CtxB-specific IgG memory B cell responses persisted for three months. The response to LPS was lower. In comparison to vaccinees, patients infected with wild type *V. cholerae* O1 mounted higher vibriocidal, CtxB and LPS-specific antibody responses at day 30. The memory B cell responses to CtxB were similar between patients and vaccinees; the LPS-specific responses were similar but lower in both groups as well. The vaccine induced an anamnestic response that was detected within 3 days, suggesting that protection can be induced very rapidly in a previously exposed population. Both a single and a two dose vaccine regimen resulted in a similar longevity of antibody and memory B cell responses. Thus, in settings where cholera is common, a single dose of the vaccine may induce sufficient immune responses to possibly confer protection.

639

EPIDEMIC CHOLERA IN KAKUMA REFUGEE CAMP, KENYA: THE IMPORTANCE OF SANITATION AND SOAP

Abdirahman Mahamud[1], Jamal Ahmed[1], Raymond Nyoka[1], Erick Auko[1], Vincet Kahi[2], James Ndirangu[2], Margaret Nguhi[2], John Burton Wagacha[3], Bosco Muhindo[3], Robert Breiman[1], Rachel Eidex[1]

[1]Centers for Disease Control and Prevention-Kenya, Nairobi, Kenya, [2]International Rescue Committee-Kenya, Nairobi, Kenya, [3]United Nations High Commission for Refugees, Nairobi, Kenya

Refugee camps are vulnerable to cholera outbreaks due to limited resources, poor sanitation and overcrowding. Kakuma Refugee Camp in Kenya has experienced recurrent cholera outbreaks. We report on findings from a cholera outbreak from September-December, 2009 with 224 cases and four deaths. We conducted a case control study in December 2009. Cases were identified by reviewing the hospital registry for patients meeting the WHO case definition for cholera. Two controls were selected per case; one was matched to the case based on area of the camp (the camp is separated into four areas) and age group (<5, 5-14, 15-24, and >25 years), and the second was an unmatched control selected by three-stage sampling method. A questionnaire focusing on potential risk factors was administered to cases and controls. A total of 93 cases, 93 matched and 126 unmatched controls, were enrolled into the study. In a multivariate model including cases and matched controls, washing hands with soap was protective against cholera (Adjusted Odds Ratio [AOR] =0.25; p<0.01) while presence of dirty water storage containers was a risk factor (AOR=4.4; p=0.03). In the multivariate model including cases and unmatched controls, using a latrine consistently was protective against cholera (AOR=0.13; p<0.01), whereas children not using a latrine (AOR=2.8; p=0.02) and living in Area A (AOR=10.23; p<0.0001) were risk factors. In conclusion, provision of soap, along with education on hand hygiene may be considered, as an affordable intervention to prevent cholera. Additional education may be helpful on importance of cleaning water storage containers, and latrine use. Areas with higher disease burden should be prioritized for these interventions.

640

BACTERIOLOGICAL AND PHYSICAL QUALITY OF LOCALLY PACKAGED DRINKING WATER IN KAMPALA CITY, UGANDA

Ali Halage, David Guwatudde, John Ssempebwa, David K. Ssemwanga

Makerere University College of Health Sciences, School of Public Health, Kampala, Uganda

Drinking water packaged in bottles and polythene bags has become a common consumer product in Kampala City, however, the quality of packaged water is unknown. The study aimed at assessing quality of locally packaged water sold for public consumption in Kampala city. We carried out a descriptive cross-sectional study, during January - March 2009. We collected 60 samples of bottled water from ten brands and 30 samples of sachet water from 15 brands. Bacteriological quality analysis used the membrane filtrate method with m lauryl sulphate broth as culture medium at Kasangati Public Health Laboratory. A quarter of all samples were sent to a reference laboratory (National Water and Sewerage Corporation) for validation. The samples were analyzed for total and faecal coliform organisms per 100 ml and reported in terms of cfu/100 ml. The sign test test and odds ratios were used to measure the difference in total coliforms between bottled and sacket water with the level of significance taken to be p<0.05.

Consumer perceptions towards packaged water were assessed from 423 respondents obtained by simple random sampling from 12 parishes in 3 divisions of Kampala. Total coliform significantly above the acceptable level of zero cfu was detected in 15% (9/60) of the bottled samples (p=0.004); and 70% (21/30) of sachet water (p=0.000). There was significantly higher prevalence of total coliform in sachet water compared to the bottled water (OR=13.2, 95% CI: 4.12-43.58). Also, more than half of the respondents, 56 % (237/423) preferred bottled to sachet water for drinking, because they perceived the latter as unsafe. In conclusion, about 15% of bottled water and 70% of sachet water samples in the retail outlets in Kampala city are likely to be contaminated with total coliform. Sachet water had significantly higher prevalence of total coliform compared to bottled water. Findings emphasize the need for repeated testing of packaged water at different processing levels at frequent intervals during the shelf life, community sensitization about recommended packaged water standards to improve their participation in quality surveillance and strengthening safety surveillance by Uganda National Bureau of Standards (UNBS).

641

FAECAL LACTOFERRIN, INFANT FOOD AND DIARRHEAL DISEASES IN A COHORT STUDY AMONG CHILDREN IN NORTHEASTERN BRAZIL

Aldo A. Lima[1], Alberto M. Soares[1], Noélia L. Lima[1], Leah J. Barrett[2], Richard L. Guerrant[2]

[1]Federal University of Ceara, Fortaleza, Brazil, [2]University of Virginia, Charlottesville, VA, United States

Lactoferrin is an iron-binding glycoprotein present as a major component of the secondary granules of polymorphonuclear neutrophils and also secreted by most mucosal membranes. Aim. The aim of this study was to determine the association of infant food and diarrheal diseases with faecal lactoferrin. A 45-month prospective cohort study, with infant food history and diarrheal diseases surveillance, was conducted in 184 children (0-3 years old) in an urban community in northeastern Brazil. Faecal lactoferrin (FL) was evaluated in 253 (23%) out of 1,091 specimens collected, using a latex agglutination test (LEUKO-TEST, TechLab, Blacksburg, VA). The test was considered positive for a title < or = 1:50 faecal dilution. Results. A total of 167 (66%) out of 253 samples was positive for FL. Children on exclusive breastfeeding (BF) had 100% (14/14; vs other food p=0.0003; Fisher's exact test), mixed BF 87% (78/90; vs other food p<0.0001; Chi-square test) and other food 52% (73/140), positive for FL. Children on BF plus mixed BF (any BF) had 88% (92/105; p<0.0001; Chi-square

test) FL positive compared to 51% (73/143) of children on other food. Children without any BF but with diarrheal diseases had 64% (58/90) FL positive compared to 30% (15/50; p<0.0001; Chi-square test) control children without any BF or diarrhea. Acute diarrhea episodes (AD; 2-6 days duration), prolonged diarrhea (Pro-AD; 7-13 days) and persistent diarrhea (PD; 14 days or more) had 63% (29/46; p=0.0012 vs control; Chi-square test), 65% (20/31; p=0.0023) and 69% (9/13; p=0.0095) FL positive, respectively. In conclusion, these data suggested an association of diarrheal diseases with intestinal inflammation. In addition, the results showed that any BF might influence on FL positive specimens, which can over estimate positive results. Further study of quantitative fecal lactoferrin concentrations may help distinguish low expected concentrations with breastmilk from higher expected concentrations with inflammatory diarrhea.

642

IN VITRO UNDERNUTRITION MODEL OF INTESTINAL EPITHELIAL CELL PROLIFERATION AND MIGRATION USING IEC-6 CELLS

Jefferson S. Oliveira[1], **Jones B. Neto**[1], Cirle A. Warren[2], James K. Roche[2], Aldo A. Lima[1], Richard L. Guerrant[3]

[1]Federal University of Ceara, Fortaleza, Brazil, [2]University of Virgina, Charlottesville, VA, United States, [3]University of Virginia, Charlottesville, VA, United States

Glutamine (Gln) is the preferred fuel source for enterocytes and is important in the maintenance of mucosal growth. Previous studies suggest that Gln modulates the response of the small intestine to systemic injury and infection. Enteroaggregative Escherichia coli (EAEC) has been implicated as a cause of persistent diarrhea among young children in developing countries. We hypothesize that the withdrawal of Gln and a decrease in fetal bovine serum (FBS), which contains amino acids important for cell viability including Gln, will cause the intestinal cells *in vitro* to be under severe stress that would be comparable to the animal model of malnutrition and would facilitate adherence of EAEC, thereby, increasing the severity of infection. To test this hypothesis we developed an *in vitro* model of "malnutrition". Rat intestinal epithelial cells (IEC-6) were cultured in media with or without Gln and supplemented with either 1% or 5% FBS. Cell proliferation and migration were measured after 6, 24, and 48 hours of exposure to the different media using WST1 colorimetric assay and cell counting software, respectively. IEC6 cells grown in 1% FBS or Gln-free media have significantly decreased proliferation at 6h, 24h, and 48h (p<0.05) compared with cells grown in media with 5% FBS and Gln 4mM (regular media). The optimal proliferation occurred at 24h (WST-1 absorbance was: 2.006 ± 0.071 SD, Gln(+) 5%FBS (positive control); 1.749 ± 0.087 SD, Gln(+) 1%FBS; 1.050 ± 0.085 SD, Gln(-) 5%FBS; 0.537 ± 0.055 SD, Gln(-) 1%FBS (negative control)). Furthermore, migration by cells in 1% FBS/Gln-free media was significantly less at 24h and 48h (p<0.05) (after 24h the mean of the count was: 2119.66 ± 384.07 SD; 1579.12 ± 117.142 SD; 1469.79 ± 55.664; 1220.78 ± 48.207, for the same groups respectively). These findings suggest our *in vitro* IEC-6 cell model may be applied in to the assessment of the impact of nutritional deprivation and glutamine intervention in *in vitro* models of bacterial adherence, such as EAEC infection.

643

EFFECTS OF ALANYL-GLUTAMINE IN IEC-6 CELLS CHALLENGED WITH *CLOSTRIDIUM DIFFICILE* TOXINS

Raphael S. Rodrigues[1], **Jones L. Neto**[1], Aldo A. Lima[1], Cirle A. Warren[2], Glynis Kolling[2], Richard L. Guerrant[2]

[1]Federal University of Ceara, Fortaleza, Brazil, [2]University of Virginia, Charlottesville, VA, United States

Clostridium difficile is the leading cause of antibiotic-associated diarrhea and pseudomembranous colitis, contributing to increased morbidity, mortality and cost because of prolonged hospitalization and illness. The bacteria produce toxins that causes necrosis and apoptosis in the intestinal epithelium through signaling pathways such as the Rho GTPase glycosylation and increases production of ERK. Glutamine is the most abundant amino acid in plasma and fuels the enterocyte to enable mucosal growth. Glutamine-induced cell signaling includes increases in antiapoptotic proteins (ERK, PKD) that inhibit cell death. The dipeptide alanylglutamine (AG) is more stable, 20-fold more soluble, and enters cells better than glutamine alone. Thus we examined the effects of AG when cells are exposed to the toxin A or B using IEC-6 cells. Migration and proliferation (WST-1) were assessed. Toxin A (10ng/ml) permits migration of cells but not in the same level of the control group. Toxin A at 1000ng/ml or 100ng/ml were largely lethal to these cells. Toxin B at 10ng/ml, 1ng/ml and 0,1ng/ml was cytotoxic in all groups.Toxin A (10ng/ml) inhibited both proliferation and migration by 20-40% and was chosen as the dose for further study. For Toxin B, 0,001ng/ml and 0,01ng/ml reduced migration and proliferation respectively by 20-40% and was used for further study. Toxins A or B reduced proliferation at 6 hours. AG given with toxin was protective on proliferation (p<0.05 in 18h and 24h) and migration (p<0.05 in 24h and 48h). AG given 24 hours before toxins had an earlier effect on proliferation (6h ; p<0.05). These studies suggest that AG supplementation may ameliorate *C. difficile* toxin-induced intestinal epithelial damage and may have a role in non-antimicrobial approaches to treat *C. difficile* infection such as the treatment with AG.

644

ANTIMICROBIAL SUSCEPTIBILITY, MECHANISMS OF RESISTANCE AND VIRULENCE FACTORS OF *SHIGELLA* STRAINS ISOLATED FROM PERUVIAN CHILDREN

Angela M. Lluque[1], Susan G. Mosquito[1], David Durand[1], Erik Mercado[1], Ana M. Prada[1], Carmen Contreras[1], Theresa J. Ochoa[1], Joaquim Ruiz[2]

[1]Instituto de Medicina Tropical Alexander Von Humboldt Universidad Peruana Cayetano Heredia, Lima, Peru, [2]CRESIB, Barcelona, Spain

Several reports showed that Shigella have been progressively acquiring resistence to commonly used and inexpensive antimicrobials. The aim of the study was to determine the antimicrobial susceptibility, mechanisms of resistance and virulence genes of Shigella strains isolated from Peruvian children under 5 years of age with and without diarrhea. 65 Shigella spp. isolates were assessed; 49 from diarrhea cases and 16 from healthy controls, from a cohort study in Lima, Peru. The isolates were serologically identified as 41 S. flexneri, 13 S. boydii, 8 S. sonnei and 3 S. dysenteriae. The susceptibility to five antimicrobial agents was tested by disk diffusion; the mechanisms of resistance and virulence genes were searched by PCR. A high proportion of Shigella isolates were resistant to cotrimoxazol (Sxt) (88%), tetracycline (Tet) (63%), ampicillin (Amp) (55%), and chloramphenicol (Chl) (45%). Only one isolate was resistant to nalidixic acid. Multi-drug resistance was present in 41% of S. flexneri, 8% S. boydii and in all S. sonnei isolates. Amp resistance was related to the presence of blaOXA(11/36), blaTEM (6/29) and blaCARB (9/36), no blaSHV were detected. Chl resistance was mainly related to cat (24/29), only one isolate presented cmlA and no floR were detected. Resistance to Tet was principally related to tetB (37/41) while tetA was founded in 2 isolates. SXT resistance was mostly related to sul2 (52/57), sul1 was only present in 3 isolates.

In the case of virulence genes, 97% (63/65) isolates present ipaH, the 2 isolates without this gene were S. boydii from the diarrhea group. The ipaBCD gene was detected in 56% of S. flexneri, 39% S. boydii, 13% S. sonnei and in the 3 isolates of S. dysenteriae. In conclusion, there is a high frequency of antimicrobial resistance to commonly used antibiotics, with multiple mechanisms of resistance. Quinolones remain as the drug of choice for the treatment of Shigella infections in Peru; however, development of resistance should be closely monitored.

645

COMPARISON OF SECRETED PROTEINS AND ACTIN POLYMERIZATION AMONG DIARRHEA AND CONTROL ENTEROPATHOGENIC *E. COLI* (EPEC) STRAINS ISOLATED FROM PERUVIAN CHILDREN

Carmen A. Contreras[1], Victor H. Bustamante[2], Theresa J. Ochoa[1], Maribel Riveros[1], Claudio F. Lanata[3], Thomas G. Cleary[4], Jose Luis Puente[2]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Universidad Nacional Autonoma de Mexico, Cuernavaca, Mexico, [3]Instituto de Investigacion Nutricional, Lima, Peru, [4]University of Texas School of Public Health, Houston, TX, United States

EPEC pathogenesis includes the production and translocation of bacterial proteins through a "needle complex" via a type III secretory system, and actin polymerization-associated intimate attachment and pedestal formation. The aim of this study was to compare the secretion of *E. coli* secreted proteins (EspA, EspB/D and EspC) and actin polymerization evaluated by FAS (Fluorescent actin stain) among diarrhea and control EPEC strains. 69 representative EPEC strains (46 diarrhea and 23 controls) isolated from children under 1 year of age living in Lima, Perú were analyzed for: Protein secretion (EspA, EspB/D and EspC) by SDS-PAGE and FAS test, evaluated in HEp-2 cells. The characterization of typical EPEC (tEPEC) and atypical EPEC (aEPEC) were determined by the presence of *bfp*A gene evaluated by PCR. EspB/D was the most frequently protein recovered in diarrhea (25/46, 54%) and control (17/23, 74%), as well as in tEPEC (10/13, 77%) and aEPEC (26/56, 46%). EspA was found with similar frequency in diarrhea and controls (22/46, 48% and 10/23, 43% respectively). EspA was more frequently found in tEPEC than in aEPEC (9/13, 69 vs 23/46, 50%). EspC was found with similar frequency among diarrhea cases and controls. The strains that secreted EspA and EspB/D proteins were more common in diarrhea cases than controls (19/46, 39% vs. 2/23, 9%, $p<0.05$). FAS was present in 16/46 (35%) of diarrhea vs. 4/23 (17%) of control samples, and in 9/13, 69% tEPEC vs. 11/56, 20% of aEPEC ($p<0.05$). In conclusion, our findings indicate that there is high heterogeneity among EPEC strains isolated from Peruvian children. Few EPEC strains secrete all 3 type III secretory proteins (EspA and EspB/D); however it correlates with diarrhea cases. The small frequency of actin polymerization among the isolated strains is due to the small frequency of tEPEC in our population.

646

ASSOCIATION OF TYPICAL ENTEROPATHOGENIC *ESCHERICHIA COLI* WITH TOTAL DIARRHEA EPISODES BUT NOT SUBTYPES OF EPISODES DURATION AMONG CHILDREN FROM FORTALEZA, CEARA, BRAZIL

Ila F. Lima[1], Josiane S. Quetz[1], Alexandre Havt[1], Eunice B. Carvalho[1], Alberto M. Soares[1], Noélia L. Lima[1], Rosa R. Mota[1], Richard L. Guerrant[2], Aldo A. Lima[1]

[1]Federal University of Ceara, Fortaleza, Brazil, [2]University of Virginia, Charlottesville, VA, United States

Enteropathogenic E. coli (EPEC) is a leading cause of infantile diarrhea in developing countries. Our recent data have shown and defined that prolonged acute diarrhea (Pro-AD; 7-13 days duration) and persistent diarrhea (PD; 14 or more days) episodes are risk factors for increased diarrhea burden in children. The aim of this work was to determine the prevalence of EPEC and its association with Pro-AD, PD or both types of episodes in children with (cases) and without diarrhea (controls) from urban areas in Fortaleza, Brazil. We analyzed stool samples collected from 249 children aging 2-36 months. Mothers provided information about the occurrence and duration of diarrhea episodes in the antecedent 14 days. Stool DNA was extracted by QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA). Diagnosis of EPEC was done by multiplex polymerase chain reaction (PCR, Qiagen) by the detection of eae (chromosomal, encodes intimin) and bfpA (located in eaf plasmid, encodes bundle-forming pili) genes. Approximately 30.1% (75/249) of studied children were positive for eae, 13.7% (34/249) for bfpA, and 35.3% (88/249) were positive for both genes. EPEC (eae+ and/or bfpA+) was significantly more detected in cases (89.2%, 74/83) than in controls (74.1%, 123/166) ($p=0.0076$). Typical EPEC (eae+ and bfpA+) was found in 65.1% (54/83) of cases and in 20.5% (34/166) of controls, and atypical EPEC (eae+ or bfpA+) was identified in 24.1% (20/83) of cases and in 53.6% (89/166) of controls ($p<0.0001$). There were no significant differences between EPEC pathotype neither between typical nor atypical EPEC regarding to duration of diarrhea episodes ($p<0.05$). In conclusion, typical EPEC strains were significantly associated with total diarrhea episodes in the studied population and no association was seen with EPEC diagnosis and the duration of diarrhea episodes. Therefore, EPEC strains seem not a risk factor for Pro-AD, PD or both.

647

CHOLERATOXIN AUGMENTS GENE EXPRESSIONS OF GLUTAMINE AND ALANYL-GLUTAMINE TRANSPORTERS IN RABBIT SMALL INTESTINE: THE ROLE OF GLUTAMINE RICH SMALL PEPTIDES-BASED ORAL REHYDRATION SOLUTIONS

Aldo A. Lima[1], Sandra S. Nunes-Monteiro[2], Alexandre Havt[1], Ila F. Lima[1], Eduardo E. Silva[1], Alberto M. Soares[1], Manuel C. Monteiro[1], Sean R. Moore[1], Richard L. Guerrant[3]

[1]Federal University of Ceara, Fortaleza, Brazil, [2]Federal University of Rio Grande do Norte, Natal, Brazil, [3]University of Virginia, Charlottesville, VA, United States

Glutamine (Gln) and alanyl-glutamine (AG) are potential candidates as nutritional supplement and replacing glucose in the oral rehydration and nutrition therapy for malnourish children. The aim of this study was to determine the effects of choleratoxin (CT) on genes transcriptions of glucose, glutamine and alanyl-glutamine transporters in rabbit small intestine and the role of glutamine rich small peptides-based oral rehydration solutions (ORS). Following RNeasy Mini Kit® protocol (Qiagen-Valencia, CA), total RNA was extracted from New Zealand rabbit small intestine. The relative genes transcriptions for the following intestinal transporters were determined with and without treatment with CT: PEPT1 (AG transporter); SGLT-1 (glucose transporter); SN-1 (glutamine transporter); and SN-2 (glutamine transporter). Intestinal electrolytes and water transports were evaluated in perfused intestinal loops and Ussing chamber (UC) experiments. The transcription of SGLT-1 was down-regulated, about 19 fold reduction, in tissue previously treated with CT. PEPT-1, SN-1 and SN-2 were up-regulated by 5, 213 and 124 fold increase in the same tissue treated with CT. UC results showed consistent improvement of sodium/hydrogen absorption induced by Gln, AG in CT treated tissues. Intestinal perfused experiments using Gln-, AG- or Gln rich peptides based ORS were also consistent with electrolytes and water (Gln = 183%; AG = 228%; Gln rich peptides = 165%) greater absorption in tissue treated with CT. In conclusion, these data suggested up-regulation of CT on genes transcription for Gln, AG and Gln rich small peptides in rabbit small intestine. The data also showed a consistent functional effect of electrolytes and water absorption using these substrates-based ORS in secretory diarrhea induced by choleratoxin.

648

A NEW MALNOURISHED APOE KO MURINE MODEL OF JM221 ENTEROAGGREGATIVE *ESCHERICHIA COLI* (EAEC) INFECTION

Jones B. Lima Neto[1], Jefferson S. Oliveira[1], Reinaldo Oriá[1], Aldo A. Lima[1], James P. Nataro[2], James Roche[3], Richard Guerrant[3]

[1]Federal University of Ceara, Fortaleza, Brazil, [2]University of Maryland, Baltimore, MD, United States, [3]Center for Global Health, University of Virginia, Charlottesville, VA, United States

Enteroaggregative Escherichia coli (EAEC) has been increasingly recognized as a cause of diarrhea in the developing and industrialized countries since its description in 1987, and the infection is characterized by watery, mucoid and secretory diarrhea. We compared Charles River C57Bl/6 wild type mice with ApoE knockout mice in order to assess the contribution of genetic lineage and nutritional status on the development of infection with the EAEC strain JM221. Mice were started on a low protein diet (2%) malnutrition protocol at day 21 of life. After 4 to 6 days of low protein diet, the malnourished (MN) mice were challenged with either the JM221 (virulent), media or HS (non-virulent) E. coli as negative controls. Bacteria were grown overnight (approximately 18 hours) in 1mL of Dulbecco's Modified Eagles Media (DMEM) and each mouse was challenged with 108 organisms in 100µL. Outcome measures were weight and shedding of the organism in the stools as determined by qPCR. Preliminary results show that MN ApoE knockout mice are more susceptible to EAEC strain JM221 (3 of 5, 60%, died) than to the negative control HS (1 of 4, 25%, died) in less than 48 hours after infection. In addition, JM221 also caused more diarrhea (2/5 vs 0/4 with HS) and tended to have impaired weight gain in the wild type animals in comparison with the HS challenged controls. Histology showed destruction of the epithelial architecture, as well as increased numbers of goblet cells in the colon in the JM221 infected animals that died. Malnourished wild type mice had transient weight loss with JM221, but no diarrhea or death. Quantitative PCR studies of fecal shedding and intensity of intestinal infection in this new model are underway.

649

SPOTTED FEVER GROUP RICKETTSIOSES IN MOROCCO

M'hammed Sarih[1], Najma Boudebouh[1], Touria Fatihi[2], Abdelfettah Chakib[2], Abdelfettah Chakib[2], Mohammed Hassar[1], Philippe Parola[3], Didier Raoult[3]

[1]Institut Pasteur du Maroc, Casablanca, Morocco, [2]Service des maladies infectieuses. CHU Ibno Rochd, Casablanca, Morocco, [3]WHO Collaborative Center for Rickettsial Reference and Research, Marseille, France

Rickettsioses are emerging infectious disease caused by rickettsiae in association with arthropods. There have been only few reports about epidemiology and clinical aspects of rickettsioses in this region. We report here a prospective study of clinical characteristics and course of 94 cas of rickettsioses diagnosed clinically in CHU Ibn rochd, Casablanca, Morocco, between May 2007 and December 2009. Sera and skin biopsies tested by reference methods including IF serology, Western immunoblotting combined with cross-adsorption, and molecular tools. A survey on the vector has been achieved in differents régions of Morocco. 679 specimens of various species of hard ticks colleted by direct removal from domestics animals (livestock, cattle, dogs) and by flannel flags dragged over vegetation have been analyzed by molecular methods on the presence of *Rickettsia sp.* The results show the presences of eight rickettsiae of the spotted fever group were identified, including 5 pathogens in ticks but that only the infection by *Rickettsia conorii* were detect among patients. These results increased our knowledge about the prevalence of Rikettsial pathogens in Morocco and provided information to understand the epidemiology of tick-borne diseases and may help to implement measures to control transmission to humans and animals in this region.

650

RAPID ASSESSMENT METHODS FOR TUNGIASIS AND PEDICULOSIS IN IMPOVERISHED COMMUNITIES

Liana Ariza

Federal University of Ceará, Rio de Janeiro, Brazil

Pediculosis and tungiasis are common in many resource-poor communities throughout the world. In these settings, despite the considerable burden caused by them, both neglected ectoparasitic diseases have been regarded by policy makers and health professionals as a problem of low priority. Consequently, prevalence and distribution of the disease are not documented in most endemic areas. To fill up this, gap rapid assessment methods were developed to estimate the prevalence and severity in endemic communities.

Recent studies from endemic areas in Brazil and Nigeria have shown that asking individuals about their pediculosis infestation status would give a highly accurate diagnosis. However, the accuracy varies greatly with geographic location and populations and is lower in developed market societies. For tungiasis, we have recently developed a rapid assessment method of high accuracy, based on identification of embedded Tunga penetrans fleas in the periungual sites of the feet. Considering the dynamic nature of the morphology of embedded jigger fleas, even lay people can diagnose the ectoparasitosis correctly. In areas where head lice infestations and tungiasis are endemic estimation of prevalence and severity of disease can be be based on simple and rapid approaches, and there is no need for resource-intensive and complex diagnostic procedures made by health professionals.

651

EFFECTS OF ROUTE OF INOCULATION OF *EHRLICHIA* ON LEVELS OF BACTEREMIA

Tais B. Saito, Lucas S. Blanton, Nagaraja Thirumalapura, Donald H. Bouyer, David H. Walker

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

Animal models have been developed for many emergent and re-emergent vector-borne diseases. For example, ehrlichial pathogenesis has been studied using the *Ehrlichia muris* or *Ixodes ovatus* ehrlichia (IOE)-C57BL/6 mouse model, but those models utilize intraperitoneal inoculation, which does not accurately reflect the natural route of transmission that occurs via tick feeding. The purpose of our study was to determine if the route of infection had an effect on the bacterial blood load in infected animals. In order to determine if the route of infection, intradermal (i.d.) versus intraperitoneal (i. p.), played a significant role, C57Bl/6 mice were inoculated with two *Ehrlichia* species, *E. muris* and IOE. The animals were infected via i.p. and i.d. inoculations using the same dose of bacteria, and blood samples were collected for analysis of bacteremia. For the *E. muris* infection, blood was collected on days 3, 6, 9, 12, 15, 20, and 30. Blood was collected from the IOE-infected mice on days 3, 5, 7, 9, and 12. The blood samples were processed for DNA purification and quantification by real time PCR using primers and probes specific to the *Ehrlichia dsb* gene. Our results indicated that after i. p. *E. muris* infection, ehrlichiae were first detected on day 3 p.i., and the highest level of bacteremia occurred on day 9, with decreased load on days 20 and 30 although persistent bacteremia was still detected. The *E. muris* i.d. infection model was less consistent, with the animals showing a wide variation of occurrence and concentration of bacteremia on days 12-15. IOE intradermal infection was similar to *E. muris*, showing an inconsistent infection pattern with bacteremia in only one animal in which IOE was detected on day 10, the same day as the animal became ill. In the IOE i. p. model of infection, the bacteria were detected on day 3 p. i. with a peak on day 5. The bacterial load decreased on day 7, 12 hours before they became ill. These results suggest that the route of infection can influence the bacteria load and course of infection.

652

SURVEY OF TICKS AND TICK-BORNE PATHOGENS IN NORTH DAKOTA

Nathan M. Russart, Jay A. Schroeder, **Jefferson A. Vaughan**

University of North Dakota, Grand Forks, ND, United States

Outdoor enthusiasts in North Dakota acknowledge that ticks can be overwhelming during certain times of the year and within certain areas. However, there has been never been a systematic survey of ticks in North Dakota. Thus the abundance and distribution of different tick species within the state are ill-defined. Similarly, there are no data on the occurrence, distribution and prevalence of tick-borne pathogens in North Dakota ticks. With an increasing number of hunters and bird-watchers throughout the state in recent years, coupled with the possible range expansion of the deer tick, *Ixodes scapularis*, into the region, a statewide survey for ticks and tick-borne pathogens was initiated during the summer of 2010. Seven state wildlife management areas were selected for sampling, representing five different eco-regions; the Red River Valley, the Prairie Pothole region, the Turtle Mountains, the Missouri Coteau, and the Badlands. Ticks were collected via dragging (=questing tick population) and small mammal trapping (=feeding tick population). Ticks were identified to species, sex and lifestage. Ticks used for pathogen testing were washed and surface sterilized. DNA was extracted from pools of 3 to 10 con-specific ticks from each site. Multiple targets were used identify the presence of tick-borne microorganisms. All tick DNA was initially screened for the presence of bacterial symbionts using a PCR protocol designed to amplify a 800 bp fragment of the bacteria-wide 16S rRNA gene. *Dermacentor* DNA was screened for *Rickettsia* using a nested PCR designed to amplify a 434 bp fragment of the rickettsial 17 kDa gene. *Ixodes* DNA was screened with multiplex PCR for simultaneous detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* using primer sets designed to specifically the *B. burgdorferi* 23S rRNA and *A. phagocytophilum msp2* genes. As of this writing (May 2010), several questing adult *Ix. scapularis* ticks have been collected from the Red River Valley, confirming the presence of this tick species in eastern North Dakota.

653

RICKETTSIAL DISSEMINATION AND SPECIFIC TICK RESPONSE DURING TYPICAL AND ATYPICAL RICKETTSIAL INFECTION

Piyanate Sunyakumthorn

Louisiana State University, School of Veterinary Medicine, Baton Rouge, LA, United States

For tick-associated spotted fever group *Rickettsia*, ticks serve as vector and also reservoir hosts that can transmit *Rickettsia* via vertical and horizontal transmission; however, persistent transmission is dependent on specificity of the tick/*Rickettsia* relationship. Despite the apparent specificity between tick and *Rickettsia* species, the ability of different *Rickettsia* species to infect distinct tick genera has not been explored in detail. Therefore, the objective of this study is to monitor rickettsial dissemination in *Dermacentor variabilis* during *Rickettsia montanensis* (typical) and *R. amblyommii* (atypical) rickettsial infection. We hypothesize that only typical *Rickettsia* widely disseminates to tick tissues acquired for transmission and specific tick-derived molecules control rickettsial dissemination in ticks. We compare the dissemination of *R. montanensis* and *R. amblyommii* in female *D. variabilis* tissues (salivary gland, gut, and ovary) and characterize the tick tissue-specific response to different rickettsial species. Ticks are exposed to either *R. montanensis* or *R. amblyommii*. A *Rickettsia* species-specific qPCR and IFA are used to assess rickettsial dissemination. Additionally, transcription of selected tick molecules are compared among *Rickettsia*-uninfected and *Rickettsia*-infected tick tissues. The data indicate that tick molecules are differently regulated in a temporal and tissue specific manner. Gene expression profiles of β-thymosin, α-catenin, vATPase, Glutathione S-transferase 2, defensin-like protein, and Factor D-like serine proteinase genes are differently transcribed in ovarian tissues response to *R. amblyommii*, compared to *R. montanensis* infection. Studying the tissue-specific molecular interactions between ticks and rickettsiae will enhance our understanding of the key mechanisms that mediate transmission of *Rickettsia* by ticks, and the epidemiology of tick-borne rickettsial diseases.

654

EVALUATION OF METHYLATED RECOMBINANT OMPB FRAGMENTS AS REAGENTS FOR SERO-DIAGNOSIS OF *RICKETTSIA TYPHI* IN ELISA

Chien-Chung Chao, Zhiwen Zhang, Hua-Wei Chen, Elissa Mutumanje, Wei-Mei Ching

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

Rickettsia typhi, a Gram-negative, obligate, intracellular bacterium, is the causative agent of murine typhus. The bacteria possess a heavily methylated outer membrane protein B (OmpB), which has been shown to be an immunodominant antigen responsible for serological reactions and can elicit protective immune responses. We have previously expressed, purified and refolded two fragments (AN, aa 33-744 and K, aa 745-1353) that encompass the full length of OmpB. As the native OmpB have multiple methylation at various lysine residues, we also performed chemical methylation to artificially create methylated recombinant proteins (Me-AN and Me-K) to closely mimic native OmpB protein. Western blot analysis confirmed that Me-K exhibited increased sensitivity in comparison with K fragment without methylation. In this study, we evaluated the potential usage of these recombinant proteins for detecting anti-*R. typhi* antibody in patient sera either individually or in combination by ELISA. Among the samples we tested, some are only detected by AN fragment but not by K fragment and vice versa. The specificity of the assay was also evaluated by using negatives from the endemic area as well as patient sera confirmed of other diseases. The combination of both methylated AN and un-methylated K fragments showed higher sensitivity than any of the fragment used individually. In general, methylation of each fragment increased the sensitivity in comparison to un-methylated fragment, especially for IgM detection. However, in some cases, the methylation of K fragment did not exhibit better sensitivity than un-methylated K especially for IgG detection. Taken together, the results suggested that the combination of both methylated AN and un-methylated K provides the best sensitivity and specificity to detect antibody against *R. typhi* in patient sera.

655

INFECTION RATES OF COMMON TICKBORNE PATHOGENS IN LONE STAR TICKS (*AMBLYOMMA AMERICANUM*) AND AMERICAN DOG TICKS (*DERMACENTOR VARIABILIS*) FROM KENTUCKY

Charissa Fritzen[1], Junjun Huang[1], Kathleen Westby[1], James D. Freye[2], Brett Dunlap[2], John Dunn[1], Abelardo Moncayo[1]

[1]Tennessee Department of Health, Vector-Borne Diseases Section, Communicable and Environmental Diseases, Nashville, TN, United States, [2]United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, Madison, TN, United States

Ehrlichiosis, Rocky Mountain Spotted Fever (RMSF), and Lyme disease are tickborne diseases reported in Kentucky. Bacterial pathogens causing these diseases are *Ehrlichia chaffeensis, Rickettsia rickettsii,* and *Borrelia burgdorferi*, respectively. However, *Ehrlichia ewingii* has been documented as causing human ehrlichiosis in the United States. Additionally, other spotted fever group rickettsia, *R. amblyommii* and *R. parkeri*, have been associated with human rickettsiosis and could be a cause of human disease diagnosed as RMSF. Lastly, *Borrelia lonestari* has been associated with a Lyme-like illness referred to as southern tick associated rash illness, or STARI. *Amblyomma americanum* is the primary vector for *E. chaffeensis, E. ewingii, R. amblyommii* and *B. lonestari*. *Dermacentor variabilis* is the primary vector for *R. rickettsii* while *Ixodes scapularis* is the main vector

for *B. burgdorferi* in the eastern US. We conducted a survey to describe infection rates of tickborne pathogens in *A. americanum* and *D. variabilis* ticks collected in Kentucky. During 2007-2008, USDA-Wildlife Services collected 288 ticks, 179 *D. variabilis* and 109 *A. americanum*, from six counties in Kentucky. Ticks were removed from domestic and wild animals and were screened individually for *Borrelia spp.*, *E. chaffeensis*, and *E. ewingii* using conventional PCR. A real time PCR was used to screen the ticks for *Rickettsia spp*. followed by a conventional PCR. PCR positive ticks were subsequently tested by restricted fragment length polymorphism assay to determine rickettsial species. Forty-two (14.6%) ticks were PCR positive for a *Rickettsia spp*., 14 (4.86%) were positive for *E. chaffeensis,* and 4 (1.39%) were positive for *E. ewingii* infection. Two (0.69%) ticks, confirmed by sequence analysis, were positive for *Borrelia lonestari*. We described tick infection rates for common tickborne diseases in Kentucky. Physicians should be aware of the common tickborne diseases in their area of practice and include them in the differential diagnosis for patients with a febrile illness.

656

GENOTYPE IDENTIFICATION AND SEQUENCE ANALYSIS OF *ORIENTIA TSUTSUGAMUSHI* ISOLATED FROM SCRUB TYPHUS-INFECTED CHIGGER-MITE COLONIES

Ratree Takhampunya, Ampornpan Kengluecha, Taweesak Monkanna, Brian P. Evans, Jason H. Richardson

Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Molecular characterization and pathogen-vector association studies were performed on twelve Orientia tsutsugamushi (Ot)-infected chigger colonies maintained in our laboratory in Thailand. Analyses were carried out on four Ot-infected Leptotrombidium chiangraiensis, 7 Ot-infected L. imphalum and 1 Ot-infected L. deliense colonies. The full-length 56-KDa type-specific antigen gene of O. tsutsugamushi was amplified and sequenced from 12 infected chigger lines followed by phylogenic analysis. The tree was constructed based on the full-length 56-KDa gene of 12 Ot sequences from infected chiggers along with reference sequences (Karp-type, Gilliam-type, TA716- and TA763-type strains) and scrub typhus sequences from a wide range of geographical regions available in GenBank databases. Moreover, the sequence diversity of O. tsutsugamushi and its associated vector species was also analyzed.

The clonal infection of O. tsutsugamushi was also analyzed from 2 Ot-infected chigger lines, L. chiangraiensis (Lc1) and L. imphalum (Li1). In so doing, we looked at the sequence diversity of the O. tsutsugamushi 56-KDa type-specific antigen gene from 2 infected chiggers and the infected mouse which developed symptoms after the bite of corresponding chiggers. Ten sequences of 56-KDa gene from each sample were selected and analyzed from cloning reactions. Our molecular characterization studies on laboratory colonies demonstrated that O. tsutsugamushi and its associated vector originated from naturally-infected chiggers collected from field rodents.

657

COLLAGENOLYTIC ACTIVITY RELATED TO METALLOPROTEASES (AND SERINE PROTEASES) IN *HYSTEROTHYLACIUM ADUNCUM* (NEMATODA: ANISAKIDAE), A WORLDWIDE FISH PARASITE

David Malagón[1], **F.J. Adroher**[1], Manuel Díaz-López[2], Rocío Benítez[1]

[1]Department of Parasitology, Faculty of Pharmacy, University of Granada, Granada, Spain, [2]Departamento de Biología Aplicada, Escuela Politécnica Superior, University of Almería, Almería, Spain

Proteases have a vital role in both the life cycle of parasites and the parasite-host relationship and are considered important virulence factors. In this study the presence of proteases with collagenolytic activity was investigated in the fish nematode *Hysterothylacium aduncum* during *in vitro* development. Collagenolytic activity was found in all studied developmental stages of the nematode (third- (L3), fourth- (L4) larval stages and adults). In L3, the activity was maximum at pH 6.5 and in the other stages, at 7.0. Pepsin is known to favour *in vitro* development of the worm, but, in this study, collagenolytic activity was shown to be significantly greater when no pepsin was added to the culture medium (at pH 6.5, $p = 0.011$). At pH 7.0 most activity was observed in the immature adult, after the final moult, suggesting that the collagenolytic activity may be involved in the remodelling of the cuticle and in sexual maturity. On the other hand, at pH 6.5, activity may be related to tissue migration by L3 within the host. Using specific inhibitors, it was demonstrated that most of the collagenolytic activity detected in all the developmental stages was due to metalloproteases (40-100%), although serine proteases were also detected in L4 and adults (10-30%).

658

BRONCHIAL WALL REMODELING IS REDUCED BY DEXAMETHASONE TREATMENT DURING LARVAE PULMONARY MIGRATION OF *STRONGYLOIDES VENEZUELENSIS* IN RATS

Cristiane Tefe-Silva, Cristina T. Beneli, Eleuza R. Machado, Marlene T. Ueta, Elaine M. Floriano, Carlos A. Sorgi, Lúcia H. Faccioli, Simone G. Ramos

University of Sao Paulo, Ribeirao Preto, Brazil

Strongyloidiasis is an intestinal parasitosis with an obligatory pulmonary cycle. The immune response is associated with Th2-type activated CD4 T cells, which amplify the cellular response through the secretion of cytokines in an attempt to eliminate the parasite. Although the inflammatory response seems similar to asthma with eosinophilia, elevated serum IgE and airway inflammation, the possible mechanisms of bronchial remodeling during pulmonary migration of larvae has not been established. The aim of this study was to delineate the principal mechanisms involved in bronchial wall remodeling occurring during the passage of *Strongyloides venezuelensis* larvae in rat lungs and to determine the ability of dexamethasone treatment to interfere with this process. Animals were divided into four groups: a control group, (C); a control plus dexamethasone group (CD); an infected group (I); and an infected plus dexamethasone group (ID). The (I) and (ID) groups were inoculated with 9,000 *S. venezuelensis* larvae. The (CD) and (ID) groups received 2 mg/kg of dexamethasone. At 1, 3, 5, 7, 14 and 21 days, the animals were killed. Morphologic and morphometric analyses with routine stains and immunohistochemistry were conducted, and cytokines were evaluated by ELISA. Goblet cell and smooth muscle hyperplasia, collagen deposition and inflammatory infiltrate (primarily composed of eosinophils and mast cells) were seen in thickenings of the bronchial wall, and IL-1β, IL-4 and VEGF levels were elevated throughout the course of infection. Both the morphologic alterations and the immunomodulatory response to the infection were drastically reduced in the dexamethasone-treated animals. In conclusion, our results strongly indicate that airway remodeling occurs during passage of *S. venezuelensis* into the lungs as a consequence of T helper type 2 inflammation. Furthermore, dexamethasone treatment can inhibit this process, acting primarily by suppressing cytokines.

659

PRELIMINARY CHARACTERIZATION OF TWO PROTEIN TARGETS WITH POTENTIAL USE FOR THE DIAGNOSIS OF ANGIOSTRONGYLIASIS

Alessandra L. Morassutti[1], Paulo M. Pinto[1], Alexandre J. da Silva[2], Patricia Wilkins[2], Carlos Graeff-Teixeira[1]

[1]Faculdade de Biociências da PUCRS, Porto Alegre, Brazil, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States

The nematode species *Angiostrongylus cantonensis* and *A. costaricensis* are agents of human eosinophilic meningitis and

abdominal angiostrongyliasis, respectively. Both infections have been reported worldwide, especially eosinophilic meningitis, which has also been reported in the United States. Parasitological diagnosis of angiostrongyliasis is rarely possible, since larvae are retained in human tissues due to a severe inflammatory reaction. Instead, both serological and real-time PCR tests have been employed for diagnosis. To improve serological diagnosis for angiostrongyliasis, excretory-secretory (ES) antigens were purified and identified. The ES was prepared by culturing worms in RPMI medium with antibiotics at 37ºC in 5% CO2; fresh medium was replaced every 24 hours. The supernatant resulting from centrifugation at 15000 x g for 10min was precipitated with TCA and the proteins were solubilized into SDS-PAGE sample buffer. Samples were in-gel rehydrated and second dimension separation was performed in SDS-PAGE gels (12.5% acrylamide). The proteins were stained or transferred onto nitrocellulose membrane, incubated for 1 hr in 5% skim-milk at room temperature and then incubated for 2h with a 1:200 pooled serum samples from patients with a confirmed histological diagnosis of angiostrongyliasis. The spots that reacted with the pooled sera were manually excised from 2-D gels, digested with trypsin and applied to a liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis using the ESI-Q-TOF equipment. The peptides obtained from two different spots matched with MTM-3 protein of *Caenorhabditis briggsae* and LEC-5 protein of *C. elegans*. Further studies to verify the specificity of these targets for diagnosis of angiostrongyliasis are under way.

660

TAX/*HBZ* MRNA RATIO AND REGULATORY T-CELL EXPANSION *IN VIVO* ARE HIGHLY ASSOCIATED IN HTLV-1/ *STRONGYLOIDES* CO-INFECTION

Giovanni Lopez[1], Carlos Rodríguez[1], Nicolás Barrós[1], César Sánchez[1], Martín Montes[1], Elsa González[1], Daniel Clark[2], Kristien Verdonck[1], Eduardo Gotuzzo[1]

[1]Instituto de Medicina Tropical "Alexander von Humboldt", Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Laboratorios de Investigación y Desarrollo (LID), Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru

Strongyloides stercoralis infection might promote the clonal expansion of HTLV-1 infected cells. HTLV-1 in turn leads to an increase of regulatory T cells (Treg), which down regulates the immune response to the parasites. We have shown that the proportion of Treg is higher in patients with strongyloidiasis co-infected with HTLV-1 than in those with only *Strongyloides stercoralis* (PLos. Neglected Tropical Diseases. 2009; 3(6): e456). We hypothesized that the expression patterns of TAX and HBZ, two key proteins important for viral activity, determine the clonal expansion of Treg. We compared viral *tax* and *hbz* mRNA levels and the proportion of Treg in two groups of patients: (1) HTLV-1 asymptomatic carriers with no history of Strongyloidiasis [AC] (n=4); and (2) patients with a history of HTLV-1/ *Strongyloides* co-infection [SS] (n=8). Peripheral blood mononuclear cells were isolated to measure HTLV-1 proviral load, *tax* and *hbz* mRNA levels by qPCR, and the proportion of Treg by flow cytometry. Statistical analysis was based on non-parametric tests. Between both groups, there were differences in the proportion of infected Treg [AC: 6.31, SS: 10.44, p =0.035]. The proportion of Treg positively correlated with the HTLV-1 proviral load [Spearman r =0.687, p=0.01] and higher with the *tax/hbz* mRNA ratio [Spearman r=0.780, p=0.002], whereas it correlated negatively with the *hbz* mRNA [Spearman r = -0.615, p=0.013]. We concluded that the two groups of patients studied have a distinctive pattern for Treg; the correlations of HTLV-1 proviral load, *tax/hbz* mRNA ratio with the proportion of Treg suggest that provirus *tax* mRNA and *Hbz* mRNA expression during co-infection *in vivo* is associated with an extensive proliferation of infected clones with Treg phenotype, might be induced when *tax* expression predominates over *Hbz* expression in a context of high viral activity.

661

ANGIOSTRONGYLUS CANTONENSIS AND DETECT IN SNAIL INTERMEDIATE HOST

Angiostrongyliasis, caused by infection with *Angiostrongylus cantonensis*, is a potentially fatal food-borne disease. Outbreaks have become increasing common in China due to the spread of efficient intermediate host snails. *A. cantonensis* was discovered in the pulmonary arteries and hearts of domestic rats in Guangzhou (Canton), China, by Chen in 1933. Human acquire infections by ingestion of raw or undercooked snails or slugs, paratenic hosts such as prawns, or contaminated vegetables that contaminated with the infectious third-stage larvae of the worm. During 1997 to 2008, nine outbreaks of the disease have been reported in the mainland of China and three in Taiwan. The biggest outbreak in the capital Beijing in 2006 demonstrated that angiostrongyliasis had moved beyond its traditional endemic areas located in the southeastern coastal regions of China. *P. canaliculata*, which has high compatibility of *A. cantonensis*, is believed to be one of the closely associated snail intermediate hosts with angiostrongyliasis in China.Three methods, e.g. tissue homogenate, enzyme digestion and lung-microscopy are major detection methods in detecting larva of *A. cantonensis* from the snails. But the first two methods are time-consuming, due to their difficulty to digest the muscle of the mollusks by enzymes. Though the third method is fast, all those methods required skilled staff and are lack of specificity in large scale detection. Newly developed PCR-based assays are able to overcome abovementioned shortcomings with its greater sensitivity, especially in the setting of low burden of parasite infection. We have established a multiplex PCR assay to detect the infection and provided an alternative method with a higher sensitive rate in detection of *A. cantonensis* in *P. canaliculata*. Despite those advantages of developed techniques, the high cost of reagents, equipment, and quality assurance hindered the application of PCR-based assay in detection of *A. cantonensis* infection. Additionally, a small sampling around 100 mg from an individual snail whose weight can reach to 70 g, can not accurate tell positive or negative. Individual detection by multiplex PCR would be a heavy workload and high cost. Pooling field specimens could reduce the number of assay and thus increase the efficiency in detecting and screening pathogen infections by polymerase chain reaction (PCR)-based assay. We investigated a pooling strategy on diagnosis of *A. cantonensis* in *P. canaliculata*. Two settings of specimens were prepared, divided into portions and detected by multiplex PCR. Specimens A was 0.4490 g positive lung tissue of 28 larval nodes from 4 snails mixed with 1.310 g negative lung tissue from 6 snails and divided into 32 portions. Specimens B was 0.5448 g positive lung tissue with 26 larval nodes from 2 snails mixed with 1.092 g negative from 7 snails and divided into 48 portions. Samples were detected by multiplex PCR. Repeated sampling was performed and sample size-accumulated positive rate curves were drawn. According to the sample size-accumulated positive rate curves, the appropriate sample size of the two specimens were 18 and 15, respectively, which is 0.36~0.58 to the total sample size. These test characteristics and the relevant factors to the sample size would need to be determined in much larger studies and more appropriately in field populations. The result indicates the number of larval node is not the most and only factor to the sample size. And it implies the feasibility to detect *A. cantonensis* in *P. canaliculata* by pooling strategy. In conclusion, by using the pooling strategy to detect the infection of *A. cantonensis* in *P. canaliculata,* we are not only able to carry out detection work in large sample, but also reduce the amount of detection samples.

662

GEOHELMINTHS AND HIV AMONG PREGNANT WOMEN FROM COASTAL KENYA: THE ASSOCIATION WITH MATERNAL HEALTH

Ernest Midega

Case Western Reserve University, Mombasa, Kenya

Both HIV and geohelminth infections are associated with maternal morbidity and impaired fetal development. Geohelminth infections in pregnancy have been associated with maternal anemia and impaired nutritional status. An overlapping distribution of the two pathogens becomes important if there is a synergistic effect of concomitant infections.Our study is aimed at describing geohelminth incidence among expectant mothers in a Sub-Saharan site, coastal Kenya to identify factors associated with variability in distribution, as well as to describe the association of cross-infection with maternal morbidity. This information will provide an evidence base to guide antenatal health care policy, particularly as it relates to the management of co-infections in HIV in expectant mothers. 1000 expectant women were recruited in their second trimester through two district hospitals in coastal Kenya, representing one urban and one rural site. Data collected at antenatal clinic includes HIV status, socio-economic status, blood and stool samples. The role of co-infection will be investigated through its association with maternal morbidity (anemia and nutritional status). Preliminary analysis has described a number of features of infection that suggest need for targeted guidelines for screening and treatment of geohelminth infection in pregnancy. The rural sample was characterized by a higher and more varied worm burden (risk of being infected rural R.R=3.3 vs. urban R.R=0.109). The association between HIV status and worm infection was not in the expected direction with P =2.1. This will be discussed. Both HIV and worm infection was related to the Hb levels of the mothers, as well as their nutritional status. The risk of low Hb or weight being associated with co-infection was greater single infections with R.R of =1.2.

663

MORPHOLOGICAL CHANGES OF *ASCARIS* SPP. OVA DURING THEIR DEVELOPMENT OUT OF THE HUMAN HOST

Ligia M. Cruz, Michael Allanson, Ricardo Izurieta

University of South Florida, Tampa, FL, United States

Ascaris lumbricoides is of particular importance to public health as it causes a great burden of disease in children in developing countries. Information on the infective stage and the pathological damage caused by the parasite is abundant and widely available in the literature; while information about early embryonic development and its life cycle out of the human host is limited. The objective of this study was to register and describe the morphological changes within the parasite ova during incubation *in vitro* at 28 C°.*A. suum* were used as a model for *A. lumbricoides.* Five milliliters of 0.1N H_2SO_4 was prepared in a 50ml centrifuge tube (4000 ova/ml) and placed in an incubator at 28 C° in the dark, for 21 days. Every day, subsamples of approximately 100 *Ascaris suum* ova were taken from the incubation solution for microscopic evaluation. Development, morphological changes and viability of the first 40 ova observed were registered in a log sheet and documented with photos. Twelve stages of development were identified within the ova: 1-Cell, 2-Cell, 3-Cell, 4-Cell, Early Morula, Late Morula, Blastula, Gastrula, Pre-larvae 1, Pre-larvae 2, Larvae 1, and Larvae 2. Each stage was observed for at least three continuous days. By the end of the first week most ova observed were in Late Morula stage (72.5%); on day 14 of incubation, 90% had developed to Larvae-1 stage and by day 16, 62.5% had developed to Larvae-2 stage. No difference was found between viability recorded from day 5 to 20 of incubation, and viability reported after three weeks of incubation (Z test for proportions, 99% CI). In conclusion, ***A. suum*** ova went through clearly identified morphological changes at different speed of development. At the end of incubation, 21 days, 100% of ova observed were in Larvae 2 stage. Two new additional stages of development were identified: Prelarvae-1(larvae coiled creating no more than one concentric ring) and Prelarvae-2 (larvae coiled creating at least one and a half concentric ring). Viability of ***Ascaris*** spp. ova may be established at earlier stages of incubation.

664

AGRICULTURAL PRACTICES ARE RELATED TO HUMAN HELMINTH INFECTION IN SUB-SAHARAN AFRICA

Naomi Hauser[1], Innocent B. Rwego[1], Derek Meyer[2], Elizabeth Canfield[3], Tony L. Goldberg[4], J. Byaruhanga[5], A. Nyamwija[5], Thomas R. Gillespie[1]

[1]Rollins School of Public Health, Emory University, Atlanta, GA, United States, [2]University of Illinois College of Medicine, Chicago, IL, United States, [3]Department of Environmental Studies, Emory University, Atlanta, GA, United States, [4]University of Wisconsin School of Veterinary Medicine, Madison, WI, United States, [5]Makere University's Biological Field Station, Kibale National Park, Uganda

Sub-Saharan Africa is a region of high species diversity and frequent gastrointestinal (GI) helminth infection. To better understand the relationship between behavior and infection, occupational and farming practices were examined relative to parasitic nematode infection in Kabarole District in western Uganda, a region typical of developing rural economies characterized by very close human-livestock associations, making it ideal for a study of zoonoses. One hundred eighty-four individuals from nine villages were included in the analysis. Subjects were surveyed and screened for GI helminthes via fecal flotation and sedimentation methods. Logistic regression and correlation analyses were used to determine associations between villages, agricultural practices, and helminth infection. Infection with any parasite was found to be associated with tending cattle (p=0.093) and pigs (p=0.089); *Trichuris* sp. was associated with doing fieldwork less than daily (p=0.0068), tending goats (p=0.055), and being an agricultural worker (p=0.015) or student (p= 0.086); *Oesophagostomum* sp. was associated with tending pigs (p=0.078); and *Trichostrongylus* sp. was associated with tending pigs ever (p=0.028), tending pigs (p=0.0035) and cattle (p=0.096) daily, and being an agricultural worker (p=0.091). The village of Kamakune I showed the highest prevalence of infection overall (68%) and Kamakune II showed the lowest (14%). Among the thirty people from Nyaruzigati, significant positive associations were found between the number of pigs at a household and *Ascaris* sp. (p=0.0028) and *Trichuris* sp. (p=0.033). Results of this study suggest that, even in small numbers, pigs are highly associated with human infection with multiple GI helminths. Other farming practices are similarly associated with helminth infections to lesser degrees. These results could prove important for restructuring the agricultural framework in rural sub-Saharan Africa to significantly decrease the human risk of GI helminth infection and associated morbidity and mortality.

665

CHILDREN PREVALENCE AND RISK FACTOR OF SOIL TRANSMITTED INFECTION IN KINSHASA (DRCONGO)

Solange E. Umesumbu, Thierry L. Bobanga, Alain N. Mandoko, Mimie M. Zanga, Ange L. Landela, E. Metelo, O. Fataki, Celestin N. Nsibu

Universite de Kinshasa, Kinshasa, The Democratic Republic of the Congo

Soil Transmitted Helminths (STH) infestation is prevalent in underdeveloped countries and may affect children's growth. The overall burden of disease is estimated to Ancylostoma sp *A.lumbricoides* and *T. trichiura* and 39 million DALYS. The objective of this study was to determine the prevalence and risk factors of intestinal STH in children of preschool and school age. We conducted a cross sectional study with a standardized sampling proportion in Kinshasa. It was divided into 5 strata with selected households were visited, and which stool samples were obtained for qualitative STH analysis. Questionnaire data on various demographic,

housing and lifestyle variables were available. The Pearson chi-square was used for comparison of frequencies at a significance level of 5%. Logistic regression identified different risk factors. 1160 stool samples were collected and examined. The prevalence of any STH infection was 17.4% and 38.5% respectively for preschool age children (0-5years) and those of school age (6-15years) with a very highly significant difference (p <0, 0001), with an average of 28.8%. *Ascaris lumbricoides* and *Trichuris trichiura* were the common STH with respectively 22,4% and 13,7% the common STH with. Age below 5 years (OR = 0.32, CI = 0.24-0.42, P <0.000), Community-Directed Treatment with Ivermectin area (CDTI) (OR = 0.54, CI = 0.38-0.76, p <0.000) and washing hands after defecation (OR = 0.73, CI = 0.54 to 0.98, P <0.039) were associated with reduced risk of STH infection; the low level of maternal education (OR = 1.45, CI = 1.10 -1.92, P <0.008) and not washing food before consumption (OR = 1, CI = 1.09-1.95,P <0.011) were associated with increased risk of STH infection. Approximately one fifth preschoolers and half of those of school age have been infected by different species of STH. We found a reduced risk of STH infection in relation with hygiene practices and safe supply of water. The national de-worming approach must be changed including of school age.

666

BILE SALT STIMULATED LIPASE GENOTYPES IN GHANAIAN COUPLES DISCORDANT FOR HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INFECTION

Yvonne Affram[1], Martijn Martijn[2], Georgios Pollakis[2], Kwamena W. Sagoe[1], Julius A. Mingle[1], William K. Ampofo[3], William A. Paxton[2], Barnor Jacob[3]

[1]University Of Ghana Medical School, College of Health Sciences, University of Ghana, Accra, Ghana, [2]Experimental Virology Unit, Academic Medical Centre (Amc), University of Amsterdam., Amsterdam, The Netherlands, [3]Virology Department; Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Accra, Ghana

Some individuals remain HIV-1 seronegative despite multiple sexual exposures to HIV-1 virus. This study analyzed the possible role of bile salt stimulated lipase (BSSL) genotypes in the lack of HIV-1 transmission in Ghanaian HIV-1 serologically discordant couples (SDCs). BSSL is a Lewis X-carrying glycoprotein secreted by the pancreas and present in human milk, the testes, adrenals and blood plasma of humans. BSSL has been postulated to have variant capacity to bind Dendritic cell-specific ICAM-3 grabbing non-integrin (DC-SIGN) and potentially block viral transmission across a mucosal surface. A total of 32 couples were enrolled in the study. These comprised of 12 SDCs and 20 serologically concordant couples (SCC). Five milliliters of blood was taken from couples. HIV-1 antibody testing was done using Abbott HIV-1/2 Determine assay and confirmed with Innolia HIV-1/2 assay. HIV-1 negative serostatus of discordant negative partners was confirmed by polymerase chain reaction (PCR) and BSSL genotypes of all couples were also identified by PCR. HIV antibody testing with PCR confirmation revealed 8 SDC and 24 SCC. BSSL genotypes were grouped into high high (HH), high low (HL) and low low (LL) genotypes based on the number of repeats (ranged from 6 to 19 repeats; 16 repeats or more was denoted as high (H) and less than 16 repeats was low (L)). Each patient had 2 types of the repeats. Fifty five percent of SDCs had HL genotype found to be associated with strong binding of BSSL to DC-SIGN, 20% had HH genotype and 25% had LL genotype both of which are associated with weak binding of BSSL to DC-SIGN. On the other hand, 40% of SCCs had HL genotype, 45% had LL genotype and 15% had HH genotype. In conclusion, SDCs could be more protected against HIV-1 transmission from DC-SIGN to CD_4 cells than SCCs.

667

DRUG RESISTANCE MONITORING IN HIV-1 INFECTED PATIENTS ON ART'S AT KOFORIDUA IN GHANA

Jacob Barnor[1], Jim Brandful[1], William Ampofo[1], Alexander Nyarko[1], **Michael Alale**[1], Evelyn Bonney[1], Kofi Bonney[1], Samson Badu Ofori[2], Nobuo Ohta[3], Koichi Ishikawa[3], Shoji Yamaoka3[3]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]HIV/AIDS Clinic, Government Regional Hospital, Koforidua, Koforidua, Ghana, [3]Molecular Virology Department, Tokyo Medical and Dental University, Tokyo, Japan

Since the first case of HIV was reported in Ghana in 1986, the number of cases and prevalence of the disease has increased at astronomical rates. Pragmatic interventions led to a gradual decline in the prevalence rates (currently, 1.8%) in the last couple of years. These interventions, including antiretroviral therapy (ART), were directed towards reducing new infections and improving the quality of life of infected persons. The objective of this study was to identify mutations related to drug resistance and to monitor plasma HIV-1 viral load as a marker of treatment success. Patients on ART were recruited with their consent. Blood samples drawn from the patients were analyzed for CD4/CD8 counts. Viral RNA was extracted from the plasma for viral load quantification and nucleotide sequencing done for drug related mutations. Majority of patients were within 21-50 years old with an average age of 38 and 42 in females and males respectively. 70.2 %(n=424) of patients were females. The prevailing strain of HIV is the CRFO_2AG. ART suppressed HIV in 86 %(177) of patients while the remaining 14 %(29) showed high viral loads ranging between 10^3-10^6, 6 of which were confirmed as having drug resistant mutations by the Stanford software. In conclusion, currently, ARTs designed specifically against HIV-1 B strains had shown to be effective against the CRFO_2AG strain in Ghana. However, the emergence of drug resistant mutants could create a paradigm shift in this success story if not controlled.

668

LOW APOPTOSIS AND G2 PHASE ARREST-INDUCING EFFECTS OF VPR MUTATED FRAGMENTS ON JURKAT CELLS

Diallo Mamadou[1], Zheng Yu-huang[2], He Yan[2], Zhou Hua-ying[2], Liu Meng[1], Chen Xia[1], Chen Zi[1]

[1]Department of Infectious Diseases, Laboratory of AIDS Research, Xiangya Second Hospital, Central South University, Changsha, China, [2]Xiangya Second Hospital, Central South University, Changsha, China

Vpr (viral protein R) is a vital HIV-1 accessory protein with multiple functions in the viral life cycle, the pathogenesis and in the induction of apoptosis and cell G2 arrest. Recent studies have shown that mutation of certain amino acid sequences in the C-terminal domain might influence the course of disease progression and attenuate its apoptosis-inducing capacity on infected cells. The present study was designed to transfect Jurkat cells with several HIV-1 vpr fragments carrying specific mutation sites, and to observe their ability of inducing distinctively apoptosis and G2 arrest. 14 vpr variant fragments were chosen from Chinese HIV-1 infected individuals. After PCR amplification, the products were purified and double digested with Hind III and BamHI. The pcDNA3.1 (+) eukaryotic expression plasmid was used for the ligation and transduction experiments. The recombinant plasmids were transiently transfected into Jurkat cells with liposomes; blank cells and empty vector cells established as control. mRNA expression of target genes was detected by RT-PCR, the DNA content, the percentages of apoptosis and the cell G2 arrest monitored by flow cytometry. Cells transfected with vpr fragments presenting 70V, 85P, 86G or 94G mutations displayed reduced percentages of apoptosis and G2 arrest when compared to the wild consensus genes. In conclusion, we found that although the HIV vpr could induce apoptosis and G2 arrest, but certain mutations such like 70V, 85P, 86G or 94G could drastically reduce this ability hence rising up a great interest for further research on gene therapy.

669

THE INCIDENCE OF OPPORTUNISTIC AND OTHER INFECTIONS IN HIV-1 INFECTED CAMBODIAN CHILDREN IN CORRELATION WITH CD4 CELL PERCENTAGE

Julia Vujcikova[1], **Vladimir Krcmery**[1], Andrea Shahum[1], Peter Kisac[2], Juraj Benca[1], Irad Beldjebel[1], Jaroslava Sokolova[2], Silvia Seckova[1], Veronika Sladeckova[1]

[1]St. Maximilian Kolbe HIV Tropical Programme Phnom Penh, Cambodia and St. Elizabeth University College, Bratislava, Slovakia, [2]St. Elizabeth University College, Bratislava, Slovakia

Infectious complications of HIV infection are important indicators of disease progression. In pre- HAART era opportunistic infections (OIs) and other infections were the critical cause of morbidity and potential mortality of children with HIV infection. In HAART era, OIs continue to be the presenting symptom of HIV infection among children whose HIV- exposure status is not known, in whom drug resistance causes clinical failure of treatment, because of poor adherence or suboptimal care. Multiple drug interactions may decrease treatment efficacy also. In our study we report the occurrence of OIs at the St. Max Kolbe Clinic in Phnom Penh, Cambodia, within 5 years. Between October 2003 and October 2008, 91 HIV- infected Cambodian children were enrolled into retrospective study. Our aim was to estimate the incidence of 12 targeted OIs and other infections occurring with association with CD4 cell percentages in a country with limited resources. In case of 66 children on first line of HAART we compared the occurrence of OIs and other infections and the duration of HAART use. For each of events, we calculated the incidence rate, 95% confidence interval per 100 person-years. The median of age of children at the presentation was 6.75 years. Average CD4 cell percentage at the presentation was 10.6% (median 8%, IQR 1.67-16.85), 67% of children had severe immunosuppression (CD4 < 15%). Severe form of malnutrition was diagnosed in case of 23% of children. The most common first time infections in the group with CD4 < 15% were pneumonia, pulmonary TB, fungal skin infections (dermatophyte infections), oropharyngeal candidiasis. The frequency of OIs and other infections generally showed statistically significant decreasing trends with increasing CD4%. The incidence of pneumonia, otitis media and dermatophyte infections remained still high in contrast to candidiasis, which correlated with low CD4%. Similarly correlated the occurrence of herpes zoster, but still kept to be present in the group with CD4>25%. We did not observe infections as PCP, DMAC, CMV, toxoplasmosis or cryptococcosis. The high incidence of OIs and other infections in the group of children with low CD4% is comparable with other studies. Our diagnostic facilities were limited. We found HAART effective for HIV infected children in resource- limited setting despite the initiation of the treatment in the advanced stage of the disease.

670

NEVIRAPINE-USE AND EVALUATION OF AN HIV PMTCT PROGRAM AT KILIMANJARO CHRISTAIN MEDICAL CENTRE (KCMC)

Anastasia Grivoyannis[1], Josephine Maleo[2], Olola Oneko[2]

[1]Weill Cornell Medical College, New York, NY, United States, [2]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania

Implementation of a Prevention of Mother to Child Transmission (PMTCT) program: designed to identify and treat pregnant women at high risk for transmitting HIV to their offspring by vertical transmission was evaluated in the Obstetrics & Gynecology Department at Kilimanjaro Christian Medical Centre (KCMC) in Moshi, Tanzania from 2006-2007. The study population was drawn from women giving birth at KCMC which serves ~17,000 people (of this 9,600 are women of childbearing age). Data from 3,170 women admitted to the Labor Ward from July 2006 to July 2007 were abstracted including: maternal HIV status at time of delivery, extent of mother's use of ARVs throughout pregnancy, method of delivery, infant feeding choice (breast milk vs. formula), estimated blood loss, maternal age & parity, and newborn statistics (i.e. gestational age, birthweight, APGAR score). Of the 3,170 deliveries, 174 (5.5%) women were HIV+ at the time of delivery, 2,435 (76.8%) were HIV negative. Of the HIV+ women, 133 (76%) received nevirapine before delivery, 3 were undocumented, 1 refused therapy, and 37 did not receive nevirapine because they were admitted after the second stage of labor, during which time nevirapine administration is not indicated. If the mother's HIV status was unknown, as was the case for the remaining 561 (17.7%) women, precautions for prevention of HIV transmission during delivery were not followed. Of the 561 women, 307 were subsequently tested and 29 of these were found to be HIV+. Guidelines were successful in screening 82% of the population at risk but failed to help those 37 women who did not arrive to Labor & Delivery in time, and 29 who tested HIV+ after delivery. Also, there are 254 women, for whom HIV status remains unknown. In order to ensure the success of PMTCT program implementation, the department must identify methods to expand HIV testing so that high risk deliveries can be better identified and treated obeying PMTCT protocol.

671

THE INTERDICTION PROJECT: AN INNOVATIVE PROGRAM FOR HIV+ PERSONS PRESENTING WITH A NEW STD

Samuel Frimpong, M. Maximillion Wilson

Duval County Health Department, Jacksonville, FL, United States

STD/HIV field investigations reveal that some persons with HIV infection have unprotected sex, multiple anonymous sex partners, and don't disclose their HIV positive status. Recurrent STDs are common among these persons, making HIV transmission more likely. The Interdiction Project is a clinic-based, individual-level HIV intervention that combines linkage for treatment adherence/risk reduction education and testing with ongoing monitoring of patient care and epidemiological data systems. This project targets HIV positive persons who present with a new STD. This presentation describes processes for establishing a program to reduce the spread of HIV and repeat STD infections among HIV positive persons. The program integrates use of existing data systems and personnel (medical providers, STD field staff, and HIV health educators). Strategies for overcoming client identification/retention and institutional barriers are reviewed. Finally, this presentation will describe initial outcomes and how this approach helps early diagnosis of HIV and notification among clients' sex partners. Seventy-one clients were referred by medical providers to project staff for an initial HIV knowledge and risk assessment and intensive health education. Data was collected from medical charts and STD/HIV records. Client's knowledge and subsequent STD infection was tracked to determine effectiveness of the health education component. Project screenings indicate patterns of high STD morbidity (especially syphilis), high numbers anonymous sex partners, and only 52% condom use at last sexual intercourse. Initial post-test findings reveal improvement of HIV transmission/treatment knowledge, improved condom negotiation skills, and 96% intent to use condoms with all sex partners. In conclusion, the combination of HIV risk reduction behavior intervention and epidemiologic contact investigation in a clinic may help reduce unprotected sex and the spread of HIV by known previous HIV positive persons.This project may show innovative use of existing resources to curb the spread of HIV/STDs.

672

MOBILITY IS A TRIGGERING FACTOR OF HIV/AIDS EPIDEMIC IN THE REGION OF KAYES, MALI

Modibo Keita[1], Yaya I. Coulibaly[2], Amadou Maiga[3], Brehima Bagayoko[1], Brehima Dicko[4], Hamidou A. Toure[5], Sitanfoune Veronique Coulibaly[6], Soumaila Keita[6], Oumar Guindo[7], Souleymane Ag Aboubacrine[8], Mahamdou M. Keita[9], Abdoulaye M. Traore[6], Dramane Sogoba[10], Abdel K. Traore[6], Sahare Fongoro[6], Sounkalo Dao[6]

[1]Hospital Fouseyni Daou of Kayes and CNAM, Bamako, Mali, [2]MRTC, Bamako, Mali, [3]Hospital Fouseyni Daou of Kayes, Kayes, Mali, [4]Reference Health Center of the District, Yelimane, Kayes, Mali, [5]Hospital Fouseyni Daou of Kayes, Bamako, Mali, [6]Hospital and University Center of Point G, Bamako, Mali, [7]Reference Health Center of the District of Commune IV, Bamako, Mali, [8]Ministry of Health Sectorial Cell for AIDS Control, Bamako, Mali, [9]Center for Vaccine Development/CNAM, Bamako, Mali, [10]SEREFO Research Project on HIV/TB, Bamako, Mali

The overall HIV prevalence rate is 0.7% in the region of Kayes -one of the lowest of the country. It represents the highest immigration area of Mali, West Africa. Due to these characteristics, we assessed the impact of the mobility of the population on the HIV/AIDS transmission pattern in the region. We defined by mobility any displacement of a resident of Kayes outside the country. We undertook a prospective study from January 2007 to December 2007 at the Regional Hospital Fousseyni DAOU of Kayes. A total of 109 HIV positive patients aged 14 years and above who have been found infected have been included. We compared patients having mobility history to those who never left the country according to vulnerability factors, attitudes, behaviors and practice towards HIV infection. Overall, 36.7% (40/109) of the patients had a mobility history. The frequency of that history was higher within the men 80.8% (25/31) as compared to the women 19.2% (15/78) (Fisher Exact test, $p<10^{-3}$). The notion of sexual intercourse during the mobility period has been reported by 67.5% of the 40 patients categorized as mobile. West Africa was the most frequent destination for these mobile subjects (57.5% of the cases). Mobile patients were more tolerant towards people living with HIV than those not having traveled. This attitude was illustrated by the fact they were significantly more susceptible to accept to share a meal with subjects infected with HIV even if they were not infected by the virus (OR=3.52; 95% CI= 1.48 - 8.37) or to work with them (OR=2.56; 95% CI= 1.082 - 6.075). Despite that positive attitude, mobile patients were 3 times more susceptible than the ones who never went abroad to have more than a sexual partner (OR=3.21; 95% CI= 1.37-7.57), to frequent a sex professional (OR=40.8; 95% CI= (5.12-325.3) and to have occasional partners (OR=31.12; 95% CI= 1.72-561.6). According to these data, the mobility in the region of Kayes, despite some positive attitudes towards social acceptation of people living with HIV, is an important factor of HIV expansion in the region of Kayes.

673

GENDER DIFFERENCES IN HEALTH LITERACY ABOUT TUBERCULOSIS (TB) AMONGST SOUTH AFRICAN HIGH SCHOOL STUDENTS

Myra Taylor

University of KwaZulu-Natal, Durban, South Africa

Health literacy, including modes of transmission, TB/HIV co-infection, prevention, signs/ symptoms and treatment, has a critical role in TB control through improved health-seeking behaviour, early diagnosis and adherence to TB treatment, and ultimately compliance with the NTB Programme recommendations. The objective of this study was to investigate health literacy about Tuberculosis amongst KwaZulu-Natal high school students. In a cross sectional study of 10 randomly selected KwaZulu-Natal urban/rural schools, students completed an anonymous semi-structured questionnaire investigating their knowledge, beliefs and attitudes, social support, self-efficacy, cues to action, intentions, and barriers to health seeking behaviour and treatment adherence, about TB using the Integrated Behaviour Change theoretical model. Of 1138 students, 98.0% isiZulu speaking (47.5% male, mean age 17.08 (SD 1.64) and 52.5% female, mean age 16.47 (SD (1.56), 36.5%, 32.4% and 31.1% were in grades 9, 10 and 11 respectively. Of these students 5.9% had previously received TB treatment. Although 69.2% of students considered TB to be a disease that usually affects the lungs, 54.7% females vs 44.3% males confirmed that TB can infect many part of the body (p=0.007). Significantly more females than males knew coughing >3 weeks to be a symptom of TB, that TB was not transmitted through using the same toilet as someone infected (41.9% vs 32.3%, p<0.005), perceived TB as treatable (45.7% vs 38.7%, p=0.006), knew that treatment takes 6 months (35.4% vs 25.8%), would encourage a TB patient to go to the clinic monthly (49.1% vs 41.6%, p=0.02), would remind to take TB tablets (48.1% vs 40.7%, p=0.005) and as a cue to action, knew someone cured of TB (30.2% vs 19.6%, p<0.005). However, fewer males (16.2% vs 20.1% believed that people with TB often get HIV). In conclusion, tuberculosis health literacy amongst KwaZulu-Natal high school students needs attention with a special focus on males to improve their health-seeking behaviour.

674

TREATMENT DEFAULT IS LOW AMONG PATIENTS INITIATING HAART AT THE KORLE-BU TEACHING HOSPITAL IN ACCRA, GHANA

April K. Wilhelm[1], Gabrielle Paci[1], Awewura Kwara[1], Margaret Lartey[2]

[1]Alpert Medical School of Brown University, Providence, RI, United States, [2]University of Ghana Medical School, Accra, Ghana

The expansion of highly active antiretroviral therapy (HAART) in the developing world has improved access to treatment and prognosis for HIV patients. An estimated 13,000 HIV-infected Ghanaians received HAART in 2007, of which Korle-Bu Teaching Hospital (KBTH) was a major provider. All KBTH HIV patients complete mandatory pre-treatment adherence counseling; however, it remains unclear how to further improve treatment outcomes in this setting. The objective of this study was to identify sub-groups of patients at-risk for treatment default in order to improve treatment outcomes among high-risk groups. We conducted a cross-sectional retrospective chart review of 290 HIV-infected patients who initiated HAART between January 1, 2008, and June 31, 2008. Demographics, clinical presentation, laboratory parameters, and treatment outcome data were collected from medical records. Chi-square and t-tests were used to compare demographic and clinical characteristics from patients continuing HAART and those who defaulted therapy. Of the 290 patients who initiated HAART, 242 (84%) continued on HAART, 41 (14%) defaulted, and 7 (2%) died during the 18-month study period. The mean±SD age was 38.7±9.4, 184 (64%) were female and 188 (66%) completed less than a secondary school education. The mean±SD baseline CD4 cell count was 183±144 cells/µl and body weight was 56±11 kg. Age, gender, educational level, marital status, presence or absence of opportunistic infection, BMI, baseline CD4 cell count, WHO disease stage 3 or 4, HAART initiation while pregnant, any incidence of poor adherence, and time to initiating HAART from clinic enrollment were not associated with treatment default ($P > 0.05$). A majority of patients initiating HAART in an urban Ghanaian clinic remained in care through one-year of follow-up. Patients who defaulted therapy were indistinguishable demographically and clinically from those who remained in care. Standardized pre-treatment adherence counseling sessions may have influenced the favorable outcomes.

675

IMMUNE RECONSTITUTION SYNDROME WITH HANSEN'S DISEASE IN A PATIENT WITH AIDS

Subramanian Swaminathan[1], Ooriapadikal Abraham[2], Susanne Abraham[2]

[1]Wayne State/Detroit Medical Center, Troy, MI, United States, [2]Christian Medical College, Vellore, India

A 35 year old man was diagnosed to have HIV infection, with a past history of cervical tuberculous lymphadenitis and esophageal candidiasis. His CD4 lymphocyte count was 50 cells/µl and HIV viral load 266370 copies/ml. Therapy was started with anti retroviral drugs with stavudine, lamivudine and nevirapine. Fifteen days after starting therapy, examination revealed mildly edematous and erythematous plaques on his trunk and extremities, and non tender enlargement of the common peroneal nerves. Skin smears from one of the plaques revealed borderline tuberculoid leprosy with type I reaction. CD4 lymphocyte count at 2 months of anti retroviral therapy (ART) was 112 cells/ µl and viral load was undetectable (<400 copies/ml). Therapy was started for Hansen's disease with chloroquine along with a tapering dose of prednisone. The skin lesions improved markedly with therapy, and became smear negative on follow up. He completed 2 years of therapy and has remained well without relapses on further follow up for 5 years. Our patient manifested with features of Hansen's disease as an immune reconstitution syndrome after initiation of ART for AIDS with prompt response to therapy. New skin lesions after starting ART should trigger performance of skin smears for Hansen's disease in endemic areas.

676

PNEUMOCYSTIS JIROVECII IN SUB-SAHARAN AFRICA: LOW PREVALENCE OF LUNG COLONIZATION IN UGANDAN AIDS PATIENTS WITH NON-PNEUMOCYSTIS PNEUMONIA

Steve M. Taylor[1], Steven R. Meshnick[1], William Worodria[2], Alfred Andama[2], J. Lucian Davis[3], Adithya Cattamanchi[3], Saskia den Boon[2], Samuel Yoo[2], Laurence Huang[3]

[1]University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC, United States, [2]Makerere University-University of California, San Francisco Research Collaboration, Kampala, Uganda, [3]University of California, San Francisco, San Francisco, CA, United States

Pneumocystis pneumonia (PcP) is a common opportunistic infection in patients with AIDS in the developed world, but the burden of PcP in sub-Saharan Africa in unknown. *Pneumocystis jirovecii* colonization can provide insight into the epidemiology and biology of the fungus. We assessed the prevalence of *P. jirovecii* colonization in respiratory specimens from consecutive HIV-positive patients with cough ≥ 2 weeks who were admitted to Mulago Hospital in Kampala, had negative sputum acid-fast bacillus smears, and underwent bronchoscopy; all samples were Diff-Quik (modified Giemsa) stain-negative, and were tested with a nested PCR assay targeting *P. jirovecii* mitochondrial rRNA. 124 patients were enrolled from September 2007 to July 2008. The median CD4 cell count was 88 cells/mm3 (IQR 22 - 196), and for 31 (25%) patients HIV was previously undiagnosed. Of the 93 patients with known HIV infection, 77 (83%) reported taking either TMP/SMX (n=75) or dapsone (n=2) to prevent PcP. Ultimate clinical diagnoses were bacterial pneumonia in 68 (55%), pulmonary tuberculosis in 37 (30%), and other or unknown diagnoses in 19 (15%). The prevalence of *P. jirovecii* colonization was 6% (7/124). In 93 patients with known HIV infection, 5 (5%) were colonized, all of whom reported taking TMP/SMX; among 29 patients with previously-undiagnosed HIV, 2 (7%) were colonized. The median CD4 count was lower in colonized (58) than non-colonized patients (91; p=0.47). During followup, 5 of 7 colonized patients died (71%), compared with 29 of 117 non-colonized patients (25%; p<0.01); controlling for CD4 count, clinical diagnosis, ARV and prophylactic antibiotic receipt, and age, *P. jirovecii* colonization was independently associated with death (OR 16; 95% C.I. 1.42 - 177). Sequencing of the mitochondrial DNA target suggested multiple *P. jirovecii* strains in study patients. In contrast to reports from the developed world, the prevalence of *P. jirovecii* colonization is low in hospitalized HIV-positive patients in Kampala. Its strong association with death during followup merits further inquiry.

677

BEST PRACTICES IMPLEMENTED AT A LOCAL ART CENTER IN GUJARAT, INDIA

Katya Calvo[1], Ravi Durvasula[1], Pratab Shah[1], Arti Trivedi[2]

[1]University of New Mexico, Albuquerque, NM, United States, [2]Pandit Deendayal Upadhyay Medical College, Rajkot, India

In resource limited settings, the percentage of HIV patients on anti-retroviral therapy (ART) that are lost to follow-up (LFU) ranges between 8-21%. In India, the National AIDS Control Organization (NACO) has 217 clinics nationwide, with an average LFU of 7%. In the state of Gujarat, a local ART center in Rajkot has a LFU percentage of 2.4%, while maintaining a record of more than 95% adherence in 92 percent of patients on ART. The objective of this study was to observe and record clinic-based best practices implemented that decrease LFU and increase adherence. Observation and recording of day-to-day clinical practices was utilized. Best practices implemented at ART clinic include: aggressive pre-appointment outreach including phone calls and text messaging, rapid re-scheduling for missed appointments, multiple sessions with counselors for education on HIV and ART therapy during a single clinic visit, questionnaire given to patient assess level of understanding after counseling, session with pharmacist at every visit, providing allowance for meals for patients on ART, Community Care Centers, and Linked Centers. In conclusion, the implementation of several clinic-based best practices has decreased the LFU rate to 2.4% in an ART center in a resource limited country

678

MALARIA PIGMENTS: A SIGNATURE OF IMPAIRMENT OF PHAGOCYTOSIS IN HIV AND MALARIA CO-INFECTION

Alex Ogwal

Infectious Diseases Institute, Kampala, Uganda

Phagocytosis by monocytes and neutrophils has long been recognized to play a crucial role in the defense of the host against opportunistic infections including malaria. We hypothesized that HIV-infection might impair phagocytosis by peripheral monocytes and neutrophils predisposing infected patients to frequent bacterial and fungal infections. To test this hypothesis, we assessed the proportion of phagocytes containing malaria pigments as a phagocytosis outcome in HIV-infected and uninfected patients. Giemsa stained peripheral blood smears were microscopically examined for phagocytes containing malaria pigments in 101 patients, consisting of 50 patients presenting with HIV-1 and malaria co-infection; and 51 HIV-negative patients presenting with malaria infection alone. The proportion of phagocytes containing malaria pigments were higher in HIV-negative patients (69.8%) than in HIV-infected patients (30.2%) (p< 0.0001). HIV-infected patients were four times more likely to have impaired phagocytosis (Odds ratios (OR) = 4.4, 95% CI=1.9-10.3) than HIV-negative patients. There was no significant difference in malaria parasitemia between the two groups. (p< 0.46). In conclusion, HIV infection may impair phagocytosis. The non significant difference in parasitemia could be due to the usage of cotrimoxazole by HIV infected patients. Functional improvement of phagocytosis may lead to better disease outcome in HIV-infected patients.

679

BIOMARKERS OF MORBIDITY ASSOCIATED TO HTLV-1 INFECTION

Edgar M. Carvalho[1], Paulo Oliveira[1], Marina Caskey[2], Andre Muniz[1], Silvane Santos[1], Glória Orge[1], Matheus T. Santos[1], Davi Tanajura[1], Marshall Glesby[2], Neviton Castro[1]

[1]Federal University of Bahia, Salvador, Brazil, [2]Cornell University Medical College, New York, NY, United States

The human T cell lymphotropic vírus type 1 (HTLV-1) infection is a neglected disease because morbidity associated to this virus infection is considered to be low. In fact HTLV-1 associated mielopathy or tropical spastic paraparesis (HAM/TSP), the main disease associated to HTLV-1, only occurs in less than 2% of the cases. The objective of this study is to demonstrate that a high percentage of HTLV-1 infected individuals have clinical and neurological manifestations and to determine biomarkers of expression of neurologic disease in HTLV-1. In addition to clinical, dentistry and neurological examination, determination of cytokines TNF-α, IFN-γ and IL-17 were performed in supernates of unstimulated mononuclear cell cultures. A cross sectional study comparing manifestations in 115 HTLV-1 carriers and 115 seronegative blood bank donors showed that dry mouth, periodontitis, arthritis, foot numbness, weakness, urinary manifestations and erectile dysfunction (ED) were significantly more frequent in HTLV-1 than in controls. A cross sectional study was also performed in 105 males divided in 3 groups: 1) HAM/TSP; 2) HTLV-1 infected subjects with neurological complains but who do not fulfill the criteria for HAM/TSP and 3) asymptomatic HTLV-1 carriers. ED and or overactive bladder (OB) was observed in all patients with HAM/TSP, in 67% of patients in group 2 and in 35% of patients of group 3. Moderate and severe ED was observed in a large percentage of these individuals and there was no association between ED and age. OB was highly associated with ED (P<.001). While there was no difference between IFN-γ and TNF-α levels and proviral load between patients with OB and HAM/TSP, these markers were significantly increased in individuals with OB in comparison to asymptomatic HTLV-1 carriers. These data indicate that a large percentage of HTLV-1 infected individuals who do not fulfill the criteria for HAM/TSP have already evidence of ED and OB. Additionally proviral load and increasing in pro-inflammatory cytokines are biological markers of neurological damage in HTLV-1 infection.

680

DISSEMINATED LEISHMANIASIS WITH INTESTINAL LESIONS IN AN HIV PATIENT

Sadia Zahid, Shadab Ahmed, Wondwoosen Gebre, Kaleem Rizvon, Rekha Nagwekar, Gurpreet Bambra, Tabassum Yasmin, Janice Verley, Hesham M. Taha

Nassau University Medical Center, East Meadow, NY, United States

Leishmaniasis is a protozoal disease transmitted by sandfly vectors and is endemic in South America, Asia, Africa, southern Europe, and Mediterranean countries. 45 years old male immigrant from Guatemala was admitted with ataxia and pancytopenia. The patient had HIV/AIDS with CD4 count 60/mm3 and HIV-1RNA 338cps/ml on ARV therapy. On examination, he weighed 54kg, was cachectic with marked hepato-splenomegaly and no lymphadenopathy. CT revealed the liver measuring 17 x 14 x 23 cm and the spleen measuring 25 x 21 x 11 cm. He had WBC of 1100/mm3, Hb 6.6g/dL, Hct 19.5, and platelets 99000/mm3. He underwent EGD and colonoscopy. Biopsy of the small bowel, gastric antrum, and rectum showed predominant histiocytes with amastigotes in lamina propria and mucosa consistent with *Leishmania*. These slides were sent to Centers for Disease Control and Prevention, Atlanta where diagnosis was confirmed. The Indirect Fluorescent Antibody titre for *L. donovani* was ≥ 1:256. Bone marrow aspirate, sent to Wadsworth Labs, NYSDOH actually grew *Leishmania* identified as species *donovani.* Stains and serology for *Toxoplasma*, *Histoplamsa*, and *Cytomegalovirus* were negative. Treatment was given with Liposomal Amphotericin B (Ambisome®) at 4mg/kg for 7 days followed by once weekly dosing for 5 weeks. After completing one cycle of the treatment, he had repeat biopsies which showed elimination of the organisms from most of the gut except parts of small intestine. Also there was significant decrease in his hepato-splenomegaly. Patient is undergoing 2nd cycle of treatment with Ambisome® at 5mg/kg. Leishmaniasis is rare in the United States but has been reported in areas bordering Mexico such as rural southern Texas. The most common forms are cutaneous leishmaniasis, causing skin sores; and visceral leishmaniasis, which affects spleen, liver, and bone marrow. Disseminated leishmaniasis in HIV/AIDS with such extensive GI tract involvement especially involving the stomach, small & large intestine and rectum; besides liver, spleen, and bone marrow, as seen in our patient is rare. It is considered an opportunistic pathogen in immunosuppressed patients with a high relapse rate after treatment, thus necessitating perhaps, secondary prophylaxis. There is no definite guideline available for treatment failure or relapse. We recommend a subsequent cycle of therapy and maintenance dosing as secondary prophylaxis.

681

SEROPREVALENCE OF *LEISHMANIA INFANTUM* IN DOGS FROM KOREA

Se Eun Choe, Seung Won Kang, Chang Hee Kweon, Suk Chan Jung

National Veterinary Research and Quarantine Service, Anyang, Republic of Korea

It has been reported that clinically infected dogs were the main reservoir hosts of visceral leishmaniasis (VL) caused by *Leishmania*. Leishmaniasis has never been reported in Korea. Many techniques have been used for diagnosis of VL, among which latex agglutination test (LAT) is known to be simple and applicable to all species. The objective of this study was to assess the prevalence of *L. infantum* antibodies in dogs from Korea using LAT. To achieve this, LAT was standardized with 8 positive control samples obtained from Italy and the test result was compared with that of commercial ELISA. The Kappa value (κ) was used to evaluate the level of agreement between LAT and ELISA. Strength of agreement based on κ was judged according to the following guideline: <1.45= poor, 0.45-0.75= good, >0.75= excellent. The level of agreement between LAT and commercial ELISA for diagnosis of VL was found to be 0.73 (serum dilution rate 1:32) and 1(serum dilution rate 1:64). A total of 332 serum samples collected from dogs in Korea were tested by the LAT using a 1: 64 titer as positive cut-off, and all sera were negative for *L. infantum* antibodies. In this study, we report for the first time the result of serological survey of *L. infantum* in dogs in Korea. Also, the LAT standardized in this study yielded a satisfactory agreement with ELISA, indicating it can be recommended as a rapid, field applicable and reliable test for diagnosis of VL.

682

DIHYDROQUINOLINES WITH *IN VITRO* AND *IN VIVO* ACTIVITY AGAINST AFRICAN TRYPANOSOMES

Carolyn Reid[1], Shanshan He[1], Donald A. Patrick[2], Premalatha Kokku[1], Jean Fotie[1], Tanja Wenzler[3], Reto Brun[3], Richard R. Tidwell[2], **Karl Werbovetz**[1]

[1]The Ohio State University, Columbus, OH, United States, [2]University of North Carolina, Chapel Hill, NC, United States, [3]Swiss Tropical and Public Health Institute, Basel, Switzerland

Dihydroquinolines showed selective *in vitro* activity against *Trypanosoma brucei rhodesiense* STIB900, and the lead compound OSU-40 (1-benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate) displayed efficacy in the *T. b. brucei* STIB795 murine trypanosomiasis model (as reported previously). These compounds are believed to act as prodrugs which are converted to active dihydroquinolin-6-ols. The dihydroquinolin-6-ols may exert their trypanocidal effects through redox cycling in the parasite. Subsequent studies sought to further define the antitrypanosomal structure-activity relationship of this series of molecules and to

examine the stability and antitrypanosomal activity of dihydroquinolin-6-ol hydrochloride salts. Placement of substituents at the 3-, 4- and 8-positions of the 6-acetoxydihydroquinoline core dramatically reduced antitrypanosomal activity compared to OSU-40. The addition of a phenyl ring at the 7-position also abolished activity against *T. brucei*. However, activity was maintained when a small substituent was placed at the 7-position. We also found that a prodrug approach is not required; dihydroquinolin-6-ol hydrochloride salts are stable crystalline materials that display nanomolar *in vitro* antitrypanosomal activity. Both 6-acetoxydihydroquinoline prodrugs and dihydroquinolin-6-ol hydrochloride salts produced cures in the *T. b. rhodesiense* STIB900 murine trypanosomiasis model. OSU-75 (1-(2-methoxybenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate) and OSU-95 (1-(2-methoxybenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol hydrochloride) cured infected mice when these compounds were administered i.p. for 4 days at 50 mg/kg/day starting the day after infection.

683

DEVELOPMENT OF PK-PD MODELS TO PREDICT THE THERAPEUTIC DOSE AND CNS DISPOSITION OF SCYX-7158 IN THE TREATMENT OF STAGE 2 HUMAN AFRICAN TRYPANOSOMIASIS

Stephen Wring[1], Eric Gaukel[1], Bakela Nare[1], Robert Jacobs[1], Cy Bacchi[2], Beth Beaudet[1], Tana Bowling[1], Daitao Chen[1], Yvonne Freund[3], Matthew Jenks[1], Luke Mercer[1], Andy Noe[1], Matt Orr[1], Robin Parham[1], Jacob Plattner[3], Ryan Randolph[1], Cindy Rewerts[1], Jessica Sligar[1], Nigel Yarlett[2], Robert Don[4]

[1]Scynexis Inc., Research Triangle Park, NC, United States, [2]Pace University, New York, NY, United States, [3]Anacor Pharmaceuticals Inc., Palo Alto, CA, United States, [4]DNDi, Geneva, Switzerland

SCYX-7158 is a potent trypanocidal oxaborole-6-carboxamide that DNDi is currently progressing through formal pre-clinical safety studies with the goal of becoming the first oral treatment for Stage 2 (neurological or CNS) Human African Trypanosomiasis (HAT). SCYX-7158 achieves 100% cures in a murine Stage 2 model of HAT after 7 daily oral 25mg/kg doses. Efficacy correlates with SCYX-7158 exposure (concentration and time) in brain tissue. This work presents PK and tissue distribution data in rodents, dogs and non-human primates, with *in vitro* time-kill data, to develop predictive models of (i) SCYX-7158 concentrations in brain and CSF from plasma concentrations and (ii) efficacious dose in rodents. The importance of PK and *in vitro* DMPK data in building the PK-PD models will be discussed. SCYX-7158 is highly permeable in an *in vitro* MDCK-MDR1 model of the blood-brain barrier (Papp >400nm/s) and is not a substrate for the P-gp efflux transporter (absorption quotient <0.1), suggesting it should readily enter the CNS. Binding to plasma proteins was concentration dependent, and with binding to brain tissue proved most important for influencing CNS disposition. The unbound fraction (fu) of SCYX-7158 in mouse plasma at the MIC (~0.6µg/mL) was 0.3% rising to 3.2% at plasma concentrations equivalent to Cmax at steady-state (~15µg/mL, 25mg/kg doses). Binding to brain tissue was independent of concentration (fu(brain) ~5%). Plasma and brain unbound fractions were determined for 3 additional oxaboroles that have demonstrated efficacy in either Stage 1 (hemolymphatic) but not Stage 2, or both Stage 1 and 2 murine HAT models. Including SCYX-7158, the log D range was 3.8-4.6 corresponding to fu(plasma) 3.3-0.35%, and fu(brain) 17.7-5.4% (at 2µM). These data supported the hypothesis that CNS disposition and hence efficacy in the Stage 2 model is driven by the balance of binding to plasma proteins and brain tissue. We are currently extending this work to develop allometric scaling parameters to model CNS exposure and predict an efficacious dose for clinical studies.

684

SEVERE ORGAN AND TISSUE ABNORMALITIES IN ANKOLE CATTLE EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA BRUCEI BRUCEI*

Ann Nanteza[1], Zachariah Nsadha[1], Sam Eyanu[1], Monica Namayanja[1], Rosemary Flores[2], Elisabeth Knapp[2], Roger Prichard[3], Vidadi Yusibov[2], George W. Lubega[1]

[1]Department for Veterinary Parasitology and Microbiology, Makerere University, Kampala, Uganda, [2]Fraunhofer USA Inc., Center for Molecular Biotechnology, Newark, DE, United States, [3]Institute of Parasitology, McGill University, Montreal, QC, Canada

In Sub-Saharan Africa, trypanosome infection causes serious diseases in humans (sleeping sickness) and in animals (nagana). The acute disease in cattle is believed to be caused by *Trypanosoma congolence* and *T. vivax*, whereas *T. b. brucei* is considered to be a mild pathogen. However, conclusive evidence that *T. b. brucei* is not a significant pathogen in cattle is obscured as it often occurs in mixed infections with *T. congolense* and *T. vivax*. To this end, we undertook controlled indoor infection studies in Ankole Longhorn breed calves with a *T. b. brucei* strain, which was isolated from naturally infected Ugandan cattle. All infectious doses tested were lethal to the Ankole Longhorn breed calves. We monitored disease progression using conventional parameters such as parasitemia and packed cell volume. We performed extensive post-mortem examinations on the diseased cattle throughout the progressing stages of the disease to examine tissue-invasiveness of this pathogenic *T. b. brucei* strain. Detailed histological analysis revealed accumulation of trypanosomes in different organs. We observed organ abnormalities and severe lesions in sacrificed animals even though the parasites were absent from the bloodstream during the entire chronic phase of the disease. We suggest that the inflammatory and degenerative tissue changes observed might, at least partially, be due to mononuclear cell infiltration. Our findings substantiate that *T. b. brucei* differs from *T. congolense* and *T. vivax*, which are known to be confined to the vascular system. Our data suggest that the role of *T. b. brucei* as a pathogen might have been underestimated in the past.

685

DISCOVERY AND OPTIMIZATION OF A SERIES OF BORON-CONTAINING SMALL MOLECULES AS POTENTIAL DRUG CANDIDATES FOR THE TREATMENT OF HUMAN AFRICAN TRYPANOSOMIASIS

Matthew Orr[1], Eric Gaukel[1], Matthew Jenks[1], Bakela Nare[1], Cindy Rewerts[1], Stephen Wring[1], Charles Ding[2], Yvonne Freund[2], Jacob Plattner[2], Cyrus Bacchi[3], Nigel Yarlett[3], Robert Don[4], Robert Jacobs[1]

[1]SCYNEXIS, Inc., Durham, NC, United States, [2]Anacor Pharmaceuticals, Inc., Palo Alto, CA, United States, [3]Pace University, New York, NY, United States, [4]Drugs for Neglected Disease Initiative, Geneva, Switzerland

Human African Trypanosomiasis (HAT) represents a significant public health problem in sub-Saharan Africa affecting hundreds of thousands of individuals. An urgent need exists for the discovery and development of new, safe, and effective drugs to treat HAT, as existing therapies suffer from poor safety profiles, difficult treatment regimens, limited effectiveness, and a high cost of goods. From a collaborative effort between SCYNEXIS, Anacor Pharmaceuticals, Pace University, and DNDi, we report the discovery and lead optimization of a novel class of boron-containing small molecules. These compounds inhibit *in vitro* growth of *T. brucei* with sub-micromolar IC50's, show no cytotoxicity to mammalian cells, and exhibit good physiochemical and pharmacokinetic properties. Development of a structure-activity relationship (SAR) profile for this chemical series and efforts to improve biological and pharmacokinetic profiles through chemical modifications will be described.

686

IDENTIFICATION OF POTENTIAL DRUG TARGETS IN PROTOZOAN PARASITES USING COMPARATIVE GENOMICS

Barbara Nerima[1], Pascal Maeser[2]

[1]National Livestock Resources Research Institute, Tororo, Uganda, [2]Swiss Tropical and Public Health Institute, Basel, Switzerland

Protozoan parasites are unicellular organisms responsible for many diseases like Malaria, Trypanosomiasis, Chagas disease, Leshimanioses, etc. Management of such diseases relies heavily on accurate diagnosis and effective chemotherapy. However, for many of the diseases chemotherapy relies on a few drugs that are ineffective, highly toxic and expensive. There is therefore need to identify new drug targets for which drugs can be developed. With the availability of fully sequenced genomes, potential drug targets can be identified by comparative genomics. The advantage with this approach is that a drug`s target is known in advance thus providing a rational base for drug combinations. Parasites have lost many metabolic reactions or pathways in the course of evolution. However, they have also retained certain reactions that are missing in the host and therefore can be targeted by drugs. In this work we reconstructed and compared core metabolism of free-living organisms and obligate parasites using the available genome information and metabolic pathway databases. Several enzymes were found to be specific to parasites and hence potential drug targets. For example salvage of methionine in *Trypanosoma brucei* is catalysed by methionine synthase (EC2.1.1.14) and homocysteine methyltransferase (EC2.1.1.10), both of which are absent in the human host. We investigated the importance of such parasite-specific enzymes by reverse genetics and found them to be essential to the parasite at Physiological concentrations of methionine and hence potential drug targets.

687

DEVELOPMENT OF A MEDIUM THROUGHPUT SYSTEM THAT USES LYMPH NODE *EX VIVO* EXPLANT CULTURES TO IDENTIFY COMPOUNDS AGAINST CUTANEOUS LEISHMANIASIS

Alex G. Peniche[1], Elvia Y. Osorio[1], Peter C. Melby[2], Bruno L. Travi[1]

[1]University of Texas Health Science Center at San Antonio, San Antonio, TX, United States, [2]University of Texas Health Science Center at San Antonio/South Texas Veterans Health Care System, San Antonio, TX, United States

Cutaneous leishmaniasis affect >10 million people in endemic regions, worldwide. New drugs are needed because current therapies are toxic, expensive, and their efficacy is hindered by parasite resistance. Golden hamsters and Balb/c mice are susceptible to *Leishmania Viannia spp*. and *L. major*, respectively, and provide good models for testing anti-Leishmania drug candidates. We developed a model system to screen for anti-Leishmania compounds that utilizes the intracellular amastigote form of the parasite, including other cell populations involved in the host's immune response. The system uses *Leishmania* transfected with an episomal vector containing the luciferase reporter gene, which facilitates parasite quantification compared with traditional microscopy. The animals were inoculated intradermally in the snout and ears with stationary phase infective promastigotes selected by incubation with hamster Complement (*L. panamensis*) or concentration of metacyclic forms using peanut agglutinin (*L. major*). The cervical lymph nodes were used as sources of lymphocytes and infected macrophages to test compounds in the *ex vivo* system. The evaluation of parasite burden by means of luminometry showed that at 28 days p.i. the parasite load was adequate (>100 photons/sec for 1.2-1.5x104 cells) for evaluating the leishmanicidal efficacy of drugs in a medium throughput format. Using an established *in vitro* therapeutic index >5, a varying proportion of 54 lead compounds that were previously identified as active against *L. donovani* showed to have activity against *L. major* (87%) and *L. panamensis* (35%). Among them, Disulfiram, which inhibits acetaldehyde dehydrogenase in humans, has shown promising results as topical treatment for both species of cutaneous leishmanias (>90 fold decrease in lesion parasite burden) and different therapeutical regimes are currently under evaluation in animal models.

688

DISCOVERY OF CYSTEINE PROTEASE INHIBITORS WITH ANTI-*TRYPANOSOMA CRUZI* ACTIVITY

Momar Ndao[1], Deborah Nicoll-Griffith[2], Christian Beaulieu[2], Cameron Black[2], Elise Isabel[2], Fabio Vasquez Carmago[1], Frédéric Massé[2], Christophe Mellon[2], Nathalie Méthot[2], Doris Lee[2], Hyeram Park[2], Milli Nath Chowdhury[1]

[1]McGill University, Montreal, QC, Canada, [2]Merck Frosst Research Centre, Kirkland, QC, Canada

Chagas disease which is estimated to affect some 16 million people in South America is caused by the parasite *Trypanosoma cruzi*. There are currently no FDA approved therapies available to treat the infection. The cysteine protease cruzipain is involved in all stages of the development of the parasite *T. cruzi*. This parasite is responsible for the propagation of Chagas disease. An *in vivo* POC study in mice demonstrated the efficacy of the basic cruzipain inhibitor Cz008 to significantly reduce the number of parasites in mice infected with the Brazilian strain of *T. cruzi*. However the basic nature of Cz008 is likely associated with lysosomotropism in which the accumulation of the compound in lysosomes could induce a loss of selectivity over off-target cathepsins such as Cat F, L and S. This prompted us to develop the non-basic inhibitor of cruzipain Cz007 for *in vivo* testing. A second study in *T. cruzi*-infected mice was conducted and showed that Cz007 was equipotent to Cz008, demonstrating that a basic inhibitor is not required for *in vivo* efficacy. The inverse dose response observed for Cz007 may be attributed to inhibition of off-target cathepsins at higher doses of this poorly selective inhibitor. A major breakthrough in intrinsic selectivity of these non-basic compounds was obtained with the replacement of the fluoroleucine in P2 by valine to afford a potent and selective inhibitor. Further SAR around P-1 and P-3 portions led to the identification of Cz009 which has suitable potency, selectivity and pharmacokinetics for further development.

689

DEVELOPMENT OF A REAL-TIME POLYMERASE CHAIN REACTION ASSAY FOR IDENTIFICATION OF THE CAUSAL AGENTS OF LEISHMANIASIS IN PERU

Jorge Nunez[1], Maxy De los Santos[1], Pablo Tsukayama[1], Valeria Soberon[1], Braulio Valencia[2], Allejandro Llanos-Cuentas[2], David J. Bacon[1], Paul C. Graf[1]

[1]Naval Medical Research Center Detachment, Lima, Peru, [2]Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru

In Peru, several species of *Leishmania* (*Viannia*) and *Leishmania* (*Leishmania*) are responsible for cutaneous and mucocutaneous leishmaniasis. The gold standard for species identification is multilocus enzyme electrophoresis (MLEE), a time-consuming method that also requires successful culture of the parasites from the leishmaniasis lesion on the patient. Based on sequence polymorphisms in the mannose phosphate isomerase (MPI) and 6-phosphogluconate dehydrogenase (6PGD) genes, used for MLEE, we have developed a new real-time polymerase chain reaction (RT-PCR) that combines fluorescence resonance energy transfer (FRET) and melting curve analysis. This assay allows discrimination among closely related species, specifically *L.* (*V.*) *braziliensis* from *L.* (*V.*) *peruviana* and *L.* (*V.*) *guyanensis* from *L.* (*V.*) *panamensis*, directly from clinical samples. One hundred seventeen biopsies and fourteen lancet scrapings were tested in our assay. Samples were also assayed by conventional *Leishmania* diagnostic tests including intra-dermal reaction (Montenegro test), microscopy and parasite culture. More than 90% of the cases were diagnosed as *L.* (*V.*) *peruviana*, *L.* (*V.*) *braziliensis*, and *L.* (*V.*) *guyanensis*,

the three most common species present in Peru. Four biopsy samples from Ecuador were also tested and diagnosed as *L.* (*V.*) *panamensis*. The sensitivity of the molecular assay was of 85% and 70% for biopsies and lancet scrapings, respectively, as compared to 20% to 70% for the traditional diagnostic methods. The RT-PCR assay is a sensitive and rapid alternative that could be incorporated as an additional diagnostic method of leishmaniasis in reference centers in Peru.

690

HAND-HELD EXO-CRYSTALLIZATION THERMOTHERAPY AS A PROMISING ALTERNATIVE FOR AMERICAN CUTANEOUS LEISHMANIASIS

Braulio M. Valencia[1], David A. Miller[2], Richard Witzig[3], Alejandro Llanos-Cuentas[1]

[1]Institute of Tropical Medicine Alexander von Humboldt, Lima, Peru, [2]University of Chicago, Department of Internal Medicine, Chicago, IL, United States, [3]Infectious Diseases Section, Tulane School of Medicine, New Orleans, LA, United States

American Cutaneous leishmaniasis (CL) is characterized by variable cure rates and reduced therapeutic alternatives. Pentavalent antimonials (Sb5+) are associated with several side effects and clinical resistance is now more frequently reported. Hand-held Exo-Crystallization thermotherapy (HECT-CL) is based in heat released due to sodium acetate crystallization reaching control and maintained temperatures of 51 +/- 1ºC for at least 5 minutes. Patients with confirmed CL (scraping, culture or PCR) with/ without prior therapy were treated with 5-7 days of HECT-CL alone or in combination with topical Imiquimod (IMQ). HECT-CL was applied daily during 3 minutes continuously or divides 2 or 3 times depending of tolerability. These patients received HECT-CL as a compassionate treatment due to pregnancy, exclusive breastfeeding, cardiac contraindications, or inability to receive the only second line treatment recognize in our country (Amphotericin B). Patients were continuously evaluated during the 5-7 days of treatment to identify second-degree burns or super-infection. Close follow-up was performed during 6 months to identify early failure and start a new-therapeutic regimen. 9 patients were included; 6 were male, mean age was 23.6 years (SD: 18) and 8 of them received previous treatment. Only one patient was treatment naïve and received HECT-CL due to exclusive breastfeeding. All patients received Sb5+ like previous treatment and 2 of them were children who received 2 courses of Sb5+ and required Amphotericin B like a second-line alternative. 8 presented the recurring form of Leishmaniasis recivida cutis (LRC, small nodules over or close to the prior atrophic scar) and the breastfeeding patient presented 2 nodular lesions. All patients received HECT-CL with a duration illness (or reactivation) of less than 4 weeks. 4 patients cured (HECT-CL+IMQ), one presented reactivation during the third month of follow-up (HECT-CL+IMQ) and the remaining 4 are actually in follow-up without signs of relapse (2 with HECT-CL+IMQ and 2 with HECT-CL alone). In conclusion, HECT-CL is a safe alternative for recurrent and new cases of CL. Its use can be extended to special cases of CL during pregnancy, breastfeeding, people with cardiac contraindications and children. It seems to be useful for treatment of early lesions without increase risks of failure.

691

TUBULIN-BASED VACCINE CANDIDATES TO COMBAT AFRICAN ANIMAL TRYPANOSOMIASIS

Elisabeth Knapp[1], Lauren Goldschmidt[1], Rosemary Flores[1], Thomas Kraemer[1], Monica Namayanja[2], Ann Nanteza[2], Jessica Chichester[1], Tatiana Golovina[1], Roger Prichard[3], George W. Lubega[2], Douglas Holtzman[4], Vidadi Yusibov[1]

[1]Fraunhofer USA Inc., Center for Molecular Biotechnology, Newark, DE, United States, [2]Department for Veterinary Parasitology and Microbiology, Makerere University, Kampala, Uganda, [3]Institute of Parasitology, McGill University, Montreal, QC, Canada, [4]The Bill and Melinda Gates Foundation, Seattle, WA, United States

African trypanosomiasis is fatal to humans and animals if left untreated. The disease poses a serious threat to public health and causes enormous economic losses in sub-Saharan Africa. Despite decades of efforts, no effective vaccine has been developed against this disease, because the trypanosome continuously changes the dominant variable surface glycoprotein (VSG) antigens that cover nearly the entire surface of the parasite, making it inappropriate for vaccine development. To overcome this obstacle, we identified non-variable antigens of the parasite that can generate protective immunity. Tubulin, one such candidate, was shown to confer protection in mice when animals were challenged with homologous or heterologous strains of *Trypanosoma*. We have engineered regions of α and β-tubulin of *Trypanosoma brucei* as fusions with the coat protein of a plant virus, *Alfalfa mosaic virus* (AlMV) and produced them as virus-like particles. Plant-produced recombinant AlMV particles displaying target peptides from α or β tubulin stimulated protective immune responses in animals. Current research is dedicated towards understanding the mode of protection.

692

EFFECTS OF IMMUNOSUPPRESSION IN THE EXACERBATION OF PARASITISM BY *TRYPANOSOMA CRUZI* IN MICE WITH ACUTE CHAGASIC INFECTION

Maritza Alarcon, Claudia Ruiz-Agelvis, Ana Lugo-Yarbuh, Elio Moreno, Sonia Araujo

Universidad de los Andes, Merida, Bolivarian Republic of Venezuela

Chagas disease is caused by *Trypanosoma cruzi,* described by Carlos Chagas in Brazil, 1909. This disease has a wide distribution in Latin America with high levels of prevalence and severity of clinical pictures. One of the lines of research into this disease is based on the histopathology. In this study, 12 mice male NMRI were injected intraperitoneally with 2x104 bloodstreams of M/HOM/BRA/53/Y *T. cruzi* strain. At day 5 of infection, the mice were immunosuppressed with 0.05 mL of Endoxan, receiving a second dose two days later. The patent parasitemia increased in infected mice immunosuppressed, between 5 and 15 days post-infection, compared with the mice not immunosuppressed and with significant differences ($P<0.05$). At 15 days pi, mice were sacrificed, and the heart and skeletal muscle were removed for evaluate *T. cruzi* infection. The tissue were fixed in formalin to 10%, included in paraffin and staining with Hematoxylin and Eosin. The histopathological study revealed nests of amastigotes, inflammatory infiltrate with mononuclear and polymorphonuclear cells, destruction of skeletal muscle fibres and cardiac tissue with myositis and myocarditis. The results demonstrated an exacerbation of the parasitism in infected mice, as result of the immunosuppressive chemical agent activity during the course of infection by *T. cruzi.* It might be related with the reduction of the activity on the immune system of infected mice immunosuppressed compared with control mice. These findings can be extrapolated to human cases with Chagas´disease either medical treatment immunosuppressive or with other clinical conditions.

693

MATERNAL INFECTION BY *TRYPANOSOMA CRUZI* INDUCES AN CELLULAR IMMUNE RESPONSE WITH CYTOKINES PRODUCTION IN FETUSES

Maritza E. Alarcon, Mary C. Perez-Aguilar, Loredana Goncalves, Sonia Araujo, Elio A. Moreno

Universidad de los Andes, Merida, Bolivarian Republic of Venezuela

To detect the cytokines IFN-γ, IL-4 and IL-10 expressed by CD4 + T cells in tissues of fetal mice with acute chagasic infection. Examined the fetuses of NMRI mice infected with 22x103 trypomastigotes metacyclic the strain M/HOM/BRA/53/Y of *T. cruzi* and pregnant during the acute phase of infection, for the detection and localization of inflammatory infiltrates, nest parasites, remains antigenic and cytokines, we used hematoxylin-eosin techniques, peroxidase-anti-peroxidase and immunofluorescence. The study immunohistochemistry revealed the presence of abundant inflammatory infiltrate and antigenic deposit with amastigotes nests in fetal skeletal muscle. The detection of IFN-γ, IL-4 and IL-10 was carried out in the placenta, heart and skeletal muscle fetal using CD4+ and CD4- cells. In these fetal tissue cytokines IL-10 and IFN-γ were detected in CD4+ populations whereas in CD4- cells only IFN-γ was detected. Fetus is capable of generating an immune response own front to antigens transmitted by her mother, which induces the secretion of cytokines that act in synergy with the maternal antibodies confer a state of protection against infection and transmission of the parasite depends on factors specific to each mother, which may modify its ability to control such transmission to placental or systemic level.

694

SEVEN YEARS OF DATA COLLECTION AND ANALYSIS FOR THE LEISHMANIA DIAGNOSTIC LABORATORY AT WALTER REED ARMY INSTITUTE OF RESEARCH

Juan Mendez, Ioana E. Brasov, Li Y. Diao, John Tally, Max Grogl, Peter J. Weina

Walter Reed Army Institute of Research, Silver Spring, MD, United States

Leishmaniasis is a disease complex caused by 42+ species of protozoan parasites belonging to the genus *Leishmania*. Successful culturing of *Leishmania* parasites requires several techniques to insure proper identification of the species involved in the disease manifestation. An accurate and verifiable final diagnosis is the most important tool in finding the correct treatment for the affected individuals. Finding the proper diagnosis requires a series of laboratory techniques such as culturing and expanding the parasites and later identifying the species of *Leishmania* involved via molecular techniques. Our diagnostic laboratory is the only known certified laboratory by the *College of American Pathologist Laboratory,* approved by the *Clinical Laboratory Improvement Program* for high complexity testing of *Leishmania* disease, under the Department of Defense (DoD). This laboratory has seen a significant number of cases over the last 7 years with our involvement in the Middle East and elsewhere that *Leishmania* is endemic. Understanding that it is very important to discover, develop, implement and validate all possible methodologies to expand the knowledge in dealing with this neglected disease, we have reviewed seven years of data collected from submissions to this laboratory not only from DoD but from civilian sources as well. This data tabulation and interpretation provides to any interested party and most importantly to laboratory personnel who may encounter the need to assist in the diagnosis of the disease, a clear view of the past and present problems associated with diagnosing the disease, monitoring the incidence of exposure and infectivity in mapping different population groups, and discovering patterns of sample submission. This valuable information will hopefully help us to predict future problems and help us learn how to avoid them.

695

INFECTION AND PROLIFERATION DYNAMICS OF *TRYPANOSOMA CRUZI* IN HUMAN MACROPHAGES

Alexander Lankowski

Boston University School of Medicine, Boston, MA, United States

Significant geographic variation exists in the pathogenicity and virulence of different strains of *Trypanosoma cruzi*, the etiologic agent of Chagas disease. In particular, strains recently isolated from Bolivia are associated with a higher incidence of cardiac and gastrointestinal tract complications than previously characterized strains from Brazil and Peru. Macrophages play an important role in the immune response and pathogenesis of *T. cruzi* in humans. Not only are these cells important as innate mediators of parasite killing, but they are also capable of being infected by *T. cruzi* trypomastigotes and serving as vehicles for parasite proliferation. In order to compare the macrophage infection dynamics of different *T. cruzi* strains, an *in vitro* assay has been established which uses parasite strains induced to stably express β-galactosidase, allowing the measurement of relative parasite numbers via colorimetric reaction. Macrophages were isolated from 8 healthy volunteers and infected with β-galactosidase-expressing clones of either the strain "Bolivia" (strain DH29, clone L24) or "Tulahuen" (clone C4 - originally isolated from Brazil). Parasite numbers were then monitored by β-galactosidase activity for up to 72 hours. In 8/8 macrophage samples, the Tulahuen strain displayed both greater efficiency of initial infection and higher parasite density at all subsequent time points than did the Bolivia strain. These observations verify the usefulness of this assay as a method for comparing the virulence of different *T. cruzi* strains, and may also lend support to the hypothesis that the severity of Chagas disease pathology associated with certain strains may be more a result of an excessive immune response than of direct parasite-mediated toxicity.

696

EVALUATION OF THE ANTIBODY RESPONSE DIRECTED AGAINST TSETSE SALIVA ANTIGENS IN HUMANS

Emilie T. Dama

CIRDES, Bobo-Dioulasso, Burkina Faso

Saliva from blood sucking arthropods contains a rich array of pharmacologically active compounds whose primary function is to prevent the hemostatic mechanisms of the host. Furthermore, recent studies have shown that many of these saliva molecules are immunonogenic and elicit an antibody response. Such antibodies directed against saliva antigens may thus serve as marker of exposure to the bite by hematophagous vectors. The objective of this work was to assess if the IgG response directed against *Glossina* saliva was representative of the human-tsetse contact. For this purpose saliva was collected from *Glossina palpalis gambiensis* flies reared at CIRDES, and reactivity of human plasma was evaluated by an indirect ELISA. The study sample was composed of 301 plasma, from two active HAT foci in Guinea (Forécariah and Dubreka), two historical HAT foci in South-West Burkina Faso (Batié and Loropéni) and from volunteers living in Bobo-Dioulasso (a tsetse free area). The highest anti-saliva responses were observed in the HAT foci of Guinea, whereas responses were significantly lower in subjects living in Bobo-Dioulasso ($p<0.0001$) and in the Loropeni area ($p<0.0001$). High responses were also observed in Batié indicating that this population is still highly exposed to tsetse bites thus suggesting that the risk of re-emergence of HAT is important in this area notably in the context of the return of repatriates or seasonal workers from endemic areas from Côte d'Ivoire. Furthermore significant associations were also observed between the anti-saliva response and activities favoring human contact with tsetse flies (watering at backwater; $p=0.005$). Finally follow up in time of study subjects in endemic area suggest that the anti-saliva response is a dynamic process. As a whole, our results suggest that evaluation of the anti-saliva response may be a good alternative or complementary epidemiological tool to classical entomological methods to target most exposed populations and to evaluate efficiency of tsetse elimination programs.

697

DEVELOPING A MOUSE MODEL TO DETERMINE THE EFFECT OF SAND FLY SALIVA ON THE VISCERALIZATION OF *LEISHMANIA CHAGASI*

Melody Schmid[1], Claudio Meneses[2], Dia-Eldin Elnaiem[2], Gregory Lanzaro[1]

[1]University of California, Davis, Davis, CA, United States, [2]Vector Molecular Biology Unit, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Visceral Leishmaniasis (VL) is a vector borne disease that affects some of the world's poorest populations. In the New World VL is caused by the parasite *Leishmania chagasi*, which is transmitted by the sand fly *Lutzomyia longipalpis*. Sand fly saliva is known to have anti-hemostatic effects and immunomodulatory activities, which likely account for its ability to enhance cutaneous infections in BALB/c mice. These observations led us to hypothesize that sand fly saliva promotes the visceralization of *L. chagasi*. Indeed, preliminary data obtained using hamster hosts showed that the saliva of different populations of *Lu. longipalpis* have different effects on the visceralization of *L. chagasi*. However, the hamster model is limited because of the unavailability of immunological reagents, and the current BALB/c mouse model for cutaneous infection is unsuitable because it clears the visceral infection. The objective of this study is to develop a natural mouse model for VL that can be used to determine the effect of sand fly saliva on the visceralization of *L. chagasi*. We infected eight strains of mice via intraperitoneal injections of *L. chagasi* obtained from hamsters. Three immunodeficient strains (NuNu, NIHIII, and SCID Beige) showed signs of visceral disease up to sixteen weeks following injection, with typical parasite loads of 103 parasites per 100 host cells in the liver and spleen. Parasites isolated from the mouse spleens were then injected into naïve mice intradermally with sand fly saliva. The presence of parasites in the spleens of these mice supports the optimization of these strains as mouse models of VL. If sand fly salivary components prove to play an important role in visceralization they could be promising targets for a transmission blocking vaccine that could be integrated with current control methods.

698

UNBALANCED RATIO OF 28S AND 18S RIBOSOMAL RNAS IN *LEISHMANIA* AMASTIGOTES RECOVERED FROM BALB/C MICE LESIONS

María del Carmen Orozco-Fernández, Patricia Reyes-Uribe, Renzo Perales, Jorge Arevalo

Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Perú

Ribosome synthesis requires the coordinate gene expression of both ribosomal proteins and rRNA to satisfy living organism demands. *Leishmania* is an intracellular parasite that infects mammalian macrophages, including human beings. Whenever an infected sandfly vector feed on the skin, flagellated promastigotes invade the host cells and differentiate into non flagellated amastigotes. Complex changes should take place during promastigotes to amastigote conversion. Relative proportions of 18S rRNA and the 28S rRNA levels were measured in *Leishmania amazonensis*, These rRNAs were obtained either form cultured promastigotes or intracellular amastigotes obtained from ear dermis lesions in Balb/c mice. RNA from parasites in the log growth phase (day 3) and the early stationary phase (day 5) were obtained and subjected to reverse transcription with *Leishmania* specific primers. The ratio between 18S rRNA and 28S rRNA relative concentrations were measured by quantitative Real Time PCR. In promastigotes both logarithmic and stationary phase, like it was expected, the ratio between large and small rRNA was almost 1 (1.08±0.02). Noteworthy, when similar procedure was followed for amastigotes rRNA obtained from parasites present in lesions, the 28S rRNA was 8 fold times more abundant respect 18S rRNA. This observation was consistently observed in three different experiments. Functional ribosomes imply equimolar concentrations of large and small ribosomal sub units. Our results indicate that this is true for the promatigote stage but it is not the case for the amastigote stage. In this case, only a minor proportion of ribosomal subunits will be assembled like whole ribosomes. This non coordinated synthesis between 18S and 28b subunits, should lead to alterations of protein synthesis machinery, possibly encompassing parasite adaptation mechanisms to survive within the intracellular host environment.

699

EXPERIMENTAL VISCERAL LEISHMANIASIS IN ALYMPHOPLASIA (*ALY/ALY*) MICE

Saruda Tiwananthagorn[1], Kazuya Iwabuchi[2], Manabu Ato[3], Tatsuya Sakurai[1], Yuzaburo Oku[1], Ken Katakura[1]

[1]Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Hokkaido, Japan, [2]Division of Immunobiology, Research Section of Pathophysiology, Institute for Genetic Medicine, Hokkaido University, Hokkaido, Japan, [3]Department of Immunology, National Institute of Infectious Diseases, Tokyo, Japan

Visceral leishmaniasis, caused by *Leishmania donovani* or *L. infantum* (*chagasi*), is one of the neglected tropical diseases causing significant health problems in humans and dogs worldwide. Relapses are frequent in immunocompromised patients and dogs in which parasites persist in the body even after treatment. Experimental infection of mice with *L. donovani* results in the development of organ-specific immunity in liver and spleen. However, a role of lymph node in parasite persistence and immune response has not been fully understood. We employed alymphoplasia *(aly/aly)* mice that lack lymph nodes and Peyer's patches, and demonstrate structural abnormalities of spleen and thymus due to a point mutation of NF-κB inducing kinase (NIK) as a model in this study. Intravenous inoculation of *aly/aly* and *aly/+* (control) mice with 5 x 107 *L. donovani* promastigotes was conducted and parasite burdens, liver histology and cytokine/chemokine responses were analyzed. The parasite burden was less in *aly/aly* mice in the early phase (4 weeks post-infection; WPI). However, the parasites remained in the liver of *aly/aly* mice at 12-WPI, when the most of parasites were removed in the *aly/+* mice. Impairment of granuloma formation and retention of infected cells in the liver were also demonstrated. Accordingly, higher parasite DNA was detected in the spleen, bone marrow, and peripheral blood of *aly/aly* mice at 12-WPI. In addition, RT-PCR/qPCR analysis revealed that 2- to 6-folds lower mRNA levels of chemokines and cytokines, including IP-10, MCP-1, RANTES, IFN-γ, TNF-α, GM-CSF and iNOS, were detected in the liver of *aly/aly* mice compared with the control mice at 4-WPI. These cytokines seemed to be necessary for the development of hepatic granuloma to resolve infection. Interestingly, mRNA level of FoxP3 in the liver of *aly/aly* mice was higher than that of *aly/+* throughout the course of infection. Defects of NF-κB pathway and/or the lymph nodes may elucidate the rather paradoxical responses (resistant in the early and susceptible in the late phase) to *L. donovani* infection in *aly/aly* mice.

700

PROPHYLACTIC EFFICACY OF TCVAC2 AGAINST *TRYPANOSOMA CRUZI* IN MICE

Shivali Gupta, Nisha Jain Garg

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

Trypanosoma cruzi is the etiologic agent of Chagas disease that is a major health problem in Latin America and an emerging infectious disease in the US. Previously, we have screened *T. cruzi* sequence database by a computational/bioinformatics approach, and identified antigens that exhibited the characteristics of good vaccine candidates. In this study, we have tested the protective efficacy of a multi-component heterologous prime/boost vaccine (TcVac2) constituted of the selected candidates in

a murine model of *T. cruzi* infection. ELISA and Flow Cytometry were employed to measure humoral and cellular responses. H&E and Masson's trichrome staining were used to evaluate pathologic changes in vaccinated and/or challenged mice. C57BL/6 mice vaccinated with TcVac2 elicited a strong antigen-specific antibody response dominated by IgG2b/IgG1 isotypes and moderate T cell proliferation. Upon challenge infection, TcVac2-vaccinated mice expanded the IgG2b/IgG1 antibody response and elicited a strong CD8+ T cell response associated with type 1 cytokines (IFN-γ & TNF-α) that resulted in control of acute parasite burden. In chronic phase, antibody response persisted in vaccinated mice; however, splenic activation of CD8+ T cells and IFN-γ/TNF-α cytokines subsided, and IL-4/IL-10 cytokines became dominant. The tissue parasitism, inflammation, and associated cell necrosis in skeletal and heart muscles of TcVac2-vaccinated chronic mice was undetectable. In comparison, control mice elicited mixed type1/type 2 responses to *T. cruzi* infection that persisted during the chronic phase, and contributed to parasite persistence and immunopathology in chagasic hearts. We conclude that TcVac2 immunization of mice elicited a strong antibody response and balanced type 1/type 2 T cell responses that were efficacious in controlling the acute and chronic tissue parasite burden and chronic immunopathology in chagasic hearts. Demonstrative experiments with similar vaccine formulation in dogs are being conducted and will be presented.

701

PERSISTENCE OF PARASITES IN SCARS CAUSED BY PAST HISTORY OF *LEISHMANIA MAJOR* INFECTION

Rabiaa-Manel Sghaier, Fouad Benhnini, Amor Zaatour, Nabil Bel-Haj-Hamida, Hanène Attia, Ghada Mkannez, Aymen Bali, Fatma Z. Guerfali, Afif Ben-Salah, Koussay Dellagi, **Dhafer Laouini**

Institute Pasteur of Tunis, Tunis-Belvedere, Tunisia

A vaccine to prevent leishmaniasis has been a goal for nearly a century based on the knowledge that a cured infection protects the individual from re-infection. It is generally believed that after spontaneous or chemotherapy induced healing of leishmaniasis, sterile cure is never achieved and that few residual living parasites will remain sequestered within some host cells that offer them a safe shelter. This statement is supported by data from experimental leishmaniasis in mice of susceptible or resistant phenotype, in which, live parasites could be recovered from lesions even after healing, and in which disease reactivation can be obtained by immune manipulation even after apparent complete cure. Whether maintenance of a long-term immune effector memory in humans will also require persistence of live parasites is presently unknown but stresses the importance of addressing the issue in zoonotic cutaneous leishmaniasis (ZCL) in the perspective of vaccine development. Our aim was to address the issue of *Leishmania major* parasite persistence vs. sterile healing in ZCL by analyzing biopsies of scars from healed volunteers. Skin-punch scars' biopsies (n=49) have been obtained from volunteers who had a past history of ZCL and who gave their written consent. The specimens were taken under sterile conditions and local anesthesia using a sterile single use puncher. Each specimen was divided into three parts: (*i*) the first sample was processed for quantitative real time PCR, (*ii*) the second was cultured *in vitro* on enriched medium and (*iii*) the third was inoculated into the footpad of susceptible BALB/c mice. We will present herein the preliminary data obtained on the persistence of live parasites on these collected scars' biopsies.

702

GENE EXPRESSION PROFILING REVEALS CONTRASTED EFFECTS OF *LEISHMANIA SPP* ON HUMAN MACROPHAGE TRANSCRIPTOME AND IDENTIFIES PARASITE SPECIFIC SIGNATURES

Fatma Z. Guerfali[1], Dhafer Laouini[1], Lamia Guizani-Tabbane[1], Florence Ottones[2], Khadija Ben-Aîssa[1], Jacques Marti[2], Koussay Dellagi[1]

[1]Institut Pasteur de Tunis, Tunis, Tunisia, [2]Institute of Human Genetics, Montpellier, France

Zoonotic cutaneous leishmaniasis caused by Leishmania (L) major is presented primarily as localized self healing cutaneous sores with a broad range of clinical variations. In addition, L. infantum is responsible in the Mediterranean basin for visceral (VL) or sporadic cutaneous leishmaniasis (SCL). Macrophages are the main target of these parasites and their shelter; they participate to shape the host immune response in an attempt to ultimately kill the parasites. As such, they could be operationally used as sensors to screen for functional diversity between parasites. To identify inter and intra-species' differences, we performed a gene expression profiling in human macrophages infected with one of the four selected *Leishmania* parasite strains: two *L. major* strains expressing contrasted levels of virulence (high vs. low virulence) according to their experimental pathogenicity and two *L. infantum* strains expressing contrasted tropisms (visceral vs. cutaneous), using Serial Analysis of Gene Expression technology (SAGE). Using various analysis tools, we were able to discriminate between the human and parasite transcripts. A set of about two hundred human genes showed statistically significant differential expression of genes in macrophages infected with either high- or low-virulent *L. major* strains and viscerotopic or dermotropic *L. infantum* strains. These genes, belonging to different functional families, are likely to be involved in the control of parasite multiplication and may play a dominant role in determining the clinical expression of disease. Further studying of these selected genes may help better understanding the physiopathology of the disease and improving anti-Leishmania drugs' screening.

703

COMPARISON OF GENE EXPRESSION PATTERNS AMONG *LEISHMANIA BRAZILIENSIS* CLINICAL ISOLATES DIFFERING IN SUSCEPTIBILITY TO PENTAVALENT ANTIMONY

Vanessa K. Adaui[1], Kathy Schnorbusch[2], Mirko Zimic[3], Andrés Gutiérrez[3], Saskia Decuypere[2], Manu Vanaerschot[2], Simonne De Doncker[2], Ilse Maes[2], Alejandro Llanos-Cuentas[1], François Chappuis[4], Jorge Arévalo[1], Jean-Claude Dujardin[2]

[1]Institute of Tropical Medicine Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Institute of Tropical Medicine Antwerp, Antwerp, Belgium, [3]Laboratorios de Investigación y Desarrollo, Universidad Peruana Cayetano Heredia, Lima, Peru, [4]Hôpitaux Universitaires de Genève, Geneva, Switzerland

The clinical value of the antileishmanial treatment pentavalent antimonials (SbV) is threatened by the emergence of drug resistance. The evaluation of drug susceptibility of *Leishmania* parasites still depends on *in vitro* biological assays, which are labour-intensive and time-consuming. Molecular markers are urgently needed to simplify the monitoring of SbV-resistance. This paper evaluates the potential of gene expression profiling to characterize *L. braziliensis* clinical isolates differing in SbV susceptibility. Twenty-one isolates were analyzed during *in vitro* promastigote growth for differential expression of 13 genes involved in SbV metabolism, oxidative stress or housekeeping functions. Our study revealed homogeneous expression profiles for most examined genes among the phenotypically different isolates. Two genes, *ODC* (encoding ornithine decarboxylase) and *TRYR* (encoding trypanothione reductase), showed a significantly higher expression rate in the group of SbV-resistant compared to the group

of SbV-sensitive parasites (P<0.01). However, both markers have a low sensitivity, and thus only explain a small part of the drug resistance within present sample. Our results might be explained by (i) the occurrence of a pleomorphic molecular mechanism leading to SbV resistance and/or (ii) the definition of the *in vitro* SbV-susceptibility phenotypes here compared. Further exploration should also consider analysis of the amastigote stages.

705

MICROENCAPSULATION OF TRANSGENIC *BACILLUS SUBTILIS* WITHIN CHITOSAN-COATED ALGINATE MICROSPHERES

Adam Forshaw

University of New Mexico School of Medicine, Albuquerque, NM, United States

Leishmaniasis is a global health concern with an estimated 12 million people infected and 367 million at risk. Visceral leishmaniasis (VL) is the most devastating form of the disease with mortality approaching 100% if left untreated. This disease is caused by *Leishmania donovani*, a protozoan kinetoplastid flagellate, and is transmitted predominantly by the sandfly, *Phlebotomus argentipes*. We have previously identified *Bacillus subtilis* as a commensal microbe within the gut of *P. argentipes*, and are developing this microbe for paratransgenic control of *L. donovani* transmission. By supplementing sand fly larvae with modified B. subtilis, we have demonstrated the transstadial delivery of this microbe to the emergent sand fly. Field application of the paratransgenic strategy for control of VL would require measures that would minimize gene spread into the environment. This work introduces the concept of second generation paratransgenics in which advanced material engineering at the micro-scale level is used for targeted release of the modified microbes to specific sites of pathogen residence within the arthropod itself. To this end, we have encapsulated *B. subtilis* within a chitosan-coated alginate (CCA) micro-particle using a modified aerosolization-coacervation process. We have demonstrated regulated release of *B. subtilis* from these particles specifically at neutral pH, and will show the stability of the CCA particles under a variety of soil conditions. We plan to transstadially deliver these muco-adhesive, pH-gated CCA particles to *P. argentipes*, and expect the particles to only release their microbial payload following the first blood meal and therefore biological pH change (from acidic to neutral) in the sand fly gut. This novel approach for delivery of modified microbes for paratransgenic control should alleviate concerns relating to containment of modified microbes, issues related to environmental spread of recombinant bacteria and potential horizontal gene transfer of foreign DNA to environmental microbiota.

706

IN VITRO EVALUATION OF THE EXPRESSION OF DYSTROPHIN IN CARDIOMYOCYTES STIMULATED WITH SERUM OF MICE EXPERIMENTALLY-INFECTED WITH *TRYPANOSOMA CRUZI*

Lygia M. Malvestio, Cibele M. Prado, Erica C. Campos, Mara Rubia Celes, Valdecir Blefari, João Santana da Silva, Marcos Antonio Rossi

University of Sao Paulo, Ribeirão Preto, Brazil

Dystrophin is implicated in the maintenance of the cell shape, mechanical resistance and signal transduction to cardiomyocytes. Results from our laboratory have been showing decreased expression of the dystrophin glycoprotein complex (DGC), especially dystrophin, in experimentally-induced *Trypanosoma cruzi* myocarditis, both in the acute and chronic phase of the disease. This study tests the hypothesis that serum of mice experimentally-infected with *T. cruzi* affects the expression of dystrophin in cultured newborn cardiomyocytes. Cultured newborn cardiomyocytes were stimulated 5 days after their first spontaneous beating with serum of mice infected with *T. cruzi* for 24 hours. Serum was obtained from male C57Bl/6 mice infected with *T. cruzi* in the peak (12 days post infection) of cardiac tissue inflammation. Imunofluorescence (IF) staining and Western blotting (WB) were performed for evaluation of the expression of dystrophin, phalloidin, troponin, TNF-α, calpain, iNOS, and NF-κB. The IF for phalloidin and troponin confirmed the presence in cardiomyocytes. The immunostaining for dystrophin in control cells was localized around the nucleus and subsarcolemal regions. The cardiomyocytes stimulated with serum of *T. cruzi* infected mice showed decreased expression of dystrophin. This decrease was confirmed by WB analysis. The expression of TNF-α, calpain, iNOS, and NF- κB was increased in the cells. In conclusion, our results lend support to the hypothesis that serum of *T. cruzi* infected mice directly affects expression dystrophin. It can be hypothesized that TNF-α and iNOS could activate NF-κB and contribute to dystrophin disruption damage through activation of intracellular proteases, such as calpain in the present study.

707

EARLY DYSTROPHIN DISRUPTION IN THE PATHOGENESIS OF EXPERIMENTAL CHRONIC CHAGAS CARDIOMYOPATHY

Cibele M. Prado[1], Mara Rubia Nunes Celes[1], Lygia M. Malvestio[1], Erica C. Campos[1], Linda A. Jelicks[2], Herbert T. Tanowitz[2], Marcos A. Rossi[1]

[1]Faculty of Medicine of Ribeirao Preto, Ribeirao Preto, Brazil, [2]Albert Einstein College of Medicine, New York, NY, United States

The most intriguing aspect of chronic Chagas cardiomyopathy (CCC) is that it takes a long time to develop after the initial infection by the protozoan *Trypanosoma cruzi*. Chagas disease is characterized by three phases: acute, latent, and chronic, the heart as the most severely and frequently involved organ. Similarly to CCC, cardiac complications due to cardiomyopathy appear later in life in Duchenne muscular dystrophy due to an absence of or defect in dystrophin. In this study we tested the hypothesis that dystrophin expression could be decreased in the beginning of *T. cruzi*-infected mice preceding the late development of cardiomyopathy. Male CD1 mice were infected with 5×104 trypomastigotes of the Brazil strain of *T. cruzi*. Mice were killed 30 and 100 days post infection (dpi) and the intensity of inflammation, fibrosis and dystrophin expression were evaluated. Echocardiography, magnetic resonance and positron emission tomography were evaluated from days 15-100pi. At 30dpi there was an intense and diffuse lymphomononuclear myocarditis, disruption of myofibers, and multiple intracellular parasite nests. The inflammation subsided significantly and parasites were not detected at 100dpi. Dystrophin immunolabeling was focally reduced or completely lost in cardiac myocytes at 30dpi, this reduction maintained up to 100dpi. Ejection fraction was significantly reduced at 60-100dpi. The RV was markedly dilated from 30-100dpi and the LV wall thickness was increased at 100dpi. Infected mice displayed greater uptake of glucose from days 15-100pi. In conclusion, a late cardiomyopathy developed in mice chronically infected with *T. cruzi* could be associated with dystrophin loss.

708

POPULATION PHARMACOKINETICS (PK) OF PYRONARIDINE IN ADULT PATIENTS WITH UNCOMPLICATED ACUTE *PLASMODIUM FALCIPARUM* OR *PLASMODIUM VIVAX* MALARIA

Thitima Wattanavijitkul[1], Stephan Duparc[2], Isabelle Borghini-Fuhrer[2], Chang-Sik Shin[3], Lawrence Fleckenstein[1]

[1]College of Pharmacy, The University of Iowa, Iowa City, IA, United States, [2]Medicines for Malaria Venture, Geneva, Switzerland, [3]Shin Poong Pharmaceuticals, Seoul, Republic of Korea

A novel pyronaridine/artesunate (PA) combination is being developed for the treatment of malaria. The purpose of this study is to determine population PK of pyronaridine (PYR) in adult patients from Africa and South East Asia participating in Phase II and Phase III clinical trials who received treatment with PA once-a-day for 3 days. A total of 699 blood concentrations collected from 321 adult patients, aged 15-60 years, with uncomplicated *falciparum* and vivax malaria were included in

this analysis. Blood PYR concentrations were natural log-transformed. Two- and three-compartment models were fitted to the data using NONMEM. The influence of covariates (age, sex, weight, height, body mass index, lean body weight (LBW), red blood cell indices, parasite count, liver function tests and geographic regions) on PK parameters was tested. Bootstrap analysis and visual predictive check (VPC) were done to evaluate the model. A two-compartment model with first order absorption and elimination best described the data. Inter-subject variability (ISV) of absorption rate constant (Ka), oral clearance (CL/F), and apparent central compartment volume (V2/F) were described using an exponential error model. The ISV of peripheral compartment volume (V3/F) and intercompartmental clearance (Q/F) could not be estimated. A log error model best described residual variability. Only LBW was found to be a significant predictor of V2/F. Typical model parameter estimates (%ISV) were Ka 29.3 1/d (109%), CL/F 1180 L/d (50%), V2/F 8540 L (82%), V3/F 13200 L and Q 1720 L/d. The estimated elimination half-life was 18 days. The final model provided estimates within the 95% confidence intervals obtained by 1000 bootstrap runs. VPC showed that the final model adequately captured the majority of the data. In conclusion, a 2-compartment model was well-fitted to pyronaridine data. LBW was an important covariate of V2/F in adult patients. The parameter estimates were plausible. The final model was robust and sufficiently captured the overall PYR PK.

709

PUNICALIN AND PUNICALAGIN FAILS CEREBRAL MALARIA?

Deepak X. Bhattacharya

Oddisi Research Laboratory, Bhubaneswar, Orissa, India

In *AJTMH* 2003, 2004 & Multi Lateral Initiative on Malaria Yaounde-05, introduced Indo medicinal fruit called *Dalimba* (P granatum) having therapeutic & prophylactic efficacy against drug resistant malaria *Pf -Pv*, alongwith active moieties, efficacy spectrum, scavenger/anti-oxidative/anti-inflammatory, anti-viral, adjuvant, & K+ as driver candidate (as reported previously). Is a CAM, a invention on global basis termed OMARIA-Orissa Malaria Research Indigenous Attempt (BBC & Eco.Times, India, 24/25-10-2000). *In vitro* results presented from authentic source (as reported previously). Decadal mono-station continuous large scale use (Koraput-Orissa-India) indicates that >15000 individuals & whole families who all had consumed OMARIA seem to block transmission for years end, no signs of resistance, non ever developed cerebral malaria. Why? OMARIA contains 1 small, stable, (A-i) Ellagic acid rich in H+, clinically indicates hepatogen, pylori & nephron toxic symptoms at sustained/bolus doses, & 2 unstable, large, hydrolytic Ellagitanins (B-ii) Punicalagin & (B-iii) Punicalin/folin of low H+, rich in OH, wholly non toxic (C-i) K+. Which Group inflicts thus ? Or A + B + C = synergistic action ? In Indian natural sources, group B is > group-A, non confounding. K+ binds exclusively to group B, which has longer bio-availability (Sreeram-04; Soh-08). Homeopaths use diluted Acetic acid; Ellagic Acid (0.25~5%) as internal medicine to treat tertian malaria *Pf-Pv*. Initially all case report relief, then rebound with hepatic, digestive, bowl problems, long use complication = drug failure. Very same cases report feel good factor & eventual 'clinical clear status' with OMARIA. Tertian cases treated with fruits & herbs rich in group A (Chestnut bark) & non from group B is ineffective & also non gametocidal. Group A do not deliver prophylaxis nor as safe. Drug dose therapeutic response of Group-A is even not equal to Artimisinin. Group B-ii & B-iii → slow onset, long acting, potent, therapeutic, prophylactic, pregnancy safe & Gametocidal even at sub-clinical doses. K+ (C-i) thwarts neuro-cerebral morbidity.

710

ACTIVITY OF 8-AMINOQUINOLINE (8AQ) ANTIMALARIAL DRUG CANDIDATES AGAINST BLOOD STAGE *PLASMODIUM FALCIPARUM*

Yarrow Rothstein[1], Jacob Johnson[1], Aruna Sampath[1], William Ellis[1], Dhammika Nanayakkara[2], Ikhlas Khan[2], Larry Walker[2], Alan Magill[1], Colin K. Ohrt[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS, United States

8-aminoquinolines (8AQ) may prove critical for malaria elimination efforts since they target hypnozoites and *Plasmodium falciparum* (Pf) gametocytes. It is unclear if 8AQs could have a role in targeting the remaining component of the transmission reservoir - asymptomatic blood stage parasitemia. The key toxicity in this class will soon be addressed with animal models predicting hemolytic toxicity in G6PD-deficiency. The Walter Reed Army Institute of Research chemical information system contains data on 1803 8AQs. Of these, 106 have been tested *in vitro* against Pf, with 33 having IC50's < 200 ng/ml. Of 1457 compounds assessed in a single dose mouse *P. bergheii* blood stage model, 195 had curative activity. Ten out of ten assessed as single agents in a 3-day Aotus monkey *P. falciparum* treatment protocol had curative activity. We plan to evaluate the *in vitro* efficacy of all available 8AQs against *P. falciparum in vitro*, to confirm their lack of cross-resistance with standard antimalarial drugs, and to determine if efficacy can be separated from hemolytic toxicity. The existing data and new data relevant to *P. falciparum* blood stage efficacy and therapeutic index will be presented.

711

PHARMACOKINETICS (PK) AND QTC CHANGES AFTER COADMINISTRATION OF TAFENOQUINE (TQ) AND CHLOROQUINE (CQ) IN HEALTHY VOLUNTEERS

Ann K. Miller[1], Khadeeja Mohamed[2], Li Ye[3], Emma Harrell[4], Sharon Baptiste-Brown[1], Jörg-Peter Kleim[2], Colin Ohrt[5], Stephan Duparc[6], Andrew Beelen[7], Alison Webster[2], Sandy Griffith[8]

[1]GlaxoSmithKline Pharmaceuticals, King of Prussia, PA, United States, [2]GlaxoSmithKline Pharmaceuticals, Uxbridge, Middlesex, United Kingdom, [3] GlaxoSmithKline Pharmaceuticals, Collegeville, PA, United States, [4]GlaxoSmithKline Pharmaceuticals, Harlow, Essex, United Kingdom, [5]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [6]Medicines for Malaria Venture, Geneva, Switzerland, [7]Miriad Pharmaceuticals, Salt Lake City, UT, United States, [8]GlaxoSmithKline Pharmaceuticals, Research Triangle Park, NC, United States

Tafenoquine (TQ) is an 8-aminoquinoline in development for the treatment and radical cure of *Plasmodium vivax* malaria. TQ will be coadministered (not coformulated) with chloroquine (CQ). The PK and safety of TQ, CQ and its desethyl metabolite (DECQ) were evaluated when given concomitantly compared to TQ or CQ given alone in healthy adults. Due to the long half-lives of both TQ and CQ, a double-blind, parallel group design with 20 subjects/group was used: CQ alone (600mg on D1 and D2+300mg on D3); TQ alone (450mg on D2 and D3); and CQ plus TQ at the same doses and times. Frequent blood samples for PK were taken on D2 and D3 with additional samples taken out to D56. 12-Lead ECGs were collected in triplicate on D-1; pre-dose, 2 and 12 h post dose on D1-3; and daily on D4-7. Plasma TQ, CQ and DECQ concentrations were determined using an HPLC-MS/MS method. PK parameters were determined using non-compartmental methods. Only a short term significant effect on TQ PK was seen on D2 when taken with CQ (Cmax and AUC(0-24) increased 38% and 24%, respectively) with no significant effects seen for Cmax and AUC(0-24) on D3, AUC(0-∞) and t1/2. TQ had no significant effect on CQ and DECQ PK. No subjects had a QTcF >480msec or a change from baseline ≥ 60msec. QTcF intervals increased when treated with CQ alone but there was no trend for increased QTcF intervals in those treated

with TQ alone nor a trend for increased QTcF intervals in the CQ/TQ arm beyond those seen with CQ alone. On D2 and D3, maximum increases in mean change from baseline QTcF interval seen in the CQ/TQ arm were 5msec compared to CQ and 33msec compared to TQ. Corresponding increases from baseline QTcF intervals were seen with increases in CQ and DECQ plasma concentrations. No correlation was seen between TQ concentrations and change from baseline QTcF intervals. Mild elevations in methemoglobin occurred, maximum mean change from baseline was on D14 (6% in CQ/TQ, 4% in TQ, and <1% in CQ). Safety and tolerability for CQ/TQ were generally similar to TQ alone. Overall, no significant PK or QTcF interaction between TQ and CQ was seen in this study.

712

DEVELOPMENT OF A NOVEL CHEMICAL SERIES WITH ACTIVITY AGAINST BOTH BLOOD- AND LIVER-STAGES OF *PLASMODIUM FALCIPARUM*

Clare E. Gutteridge[1], Michael C. Baxter[1], Stephen M. Hughes[1], Brett W. Sadowski[1], Leighton G. Rodrigo[1], Matthew B. Smith[1], Michael T. O'Neil[2], William F. McCalmont[2], Lucia Gerena[2], Gettayacamin Montip[3]

[1]United States Naval Academy, Annapolis, MD, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Using criteria including properties disclosed in the literature, synthesizability, "drugability" and potential for toxicity, a lead with an *in vitro* IC50 of 75ng/mL against blood-stage *Plasmodium falciparum* was selected for development. By synthesizing a variety of analogs of this lead, including those bearing additional substituents in three distinct regions of the molecule, we have gained an understanding of structure-activity relationships for the compound series and have prepared analogs with increased *in vitro* efficacy against blood-stage *P. falciparum*. Additionally we have established that the series has promising activity *in vitro* against liver-stage *P. falciparum*. Since *in vivo* efficacy testing in *P. berghei*-infected mice failed to show efficacy, we have focused our recent structural modification to those expected to enhance the pharmaceutical properties of the series.

713

SUSCEPTIBILITY OF NORMAL AND GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENT HUMAN ERYTHROCYTES TO PRIMAQUINE ENANTIOMERS AND POTENTIAL HEMOTOXIC METABOLITES

Babu L. Tekwani[1], Narayan D. Chaurasiya[1], Rajnish Sahu[1], Vijendar R. Adelli[1], N.P. Dhammika Nanayakkara[1], Aruna Sampath[2], Colin Ohrt[2], Larry A. Walker[1]

[1]University of Mississippi, University, MS, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States

Primaquine (PQ) is the drug of choice for radical cure of relapsing malaria. However, its therapeutic utility has been limited due to hemolytic toxicity, particularly in populations with glucose-6-phosphate dehydrogenase deficiency [G6PD(-)]. Reactive hydroxylated metabolites generated through cytochrome P450 (CYP)-linked metabolism are primarily responsible for hemolytic toxicity of PQ. Multiple CYP isoenzmes variably contribute to generation of hemotoxic metabolites. Previous reports have indicated enantioselective pharmacologic, pharmacokinetic and toxicologic profile of PQ. The normal and G6PD (-) human erythrocytes were exposed *in vitro* to purified enantiomers of PQ, 5-hydroxyprimaquine (5-HPQ) and 6-methoxy-8-hydroxylaminoquinoline (MAQ), the potential hemotoxic metabolites of PQ. Methemoglobin accumulation, real-time kinetic measurement of oxidative stress and depletion of intraerythrocytic reduced glutathione (GSH) were monitored as multiple biochemical end points for evaluation of hemolytic response. PQ enantiomers, which have earlier shown significantly different toxicity profile in laboratory animals, did not show significant difference in Hemotoxicity *in vitro*. 5-HPQ and MAQ produced robust increase in methemoglobin and oxidative stress both in normal and G6PD(-) erythrocytes. However, the metabolites generated concomitant depletion of GSH only in G6PD(-) erythrocytes, which may be responsible for selective susceptibility of G6PD(-) individuals to hemolytic response during treatment with PQ. Depletion of GSH may be monitored as a marker for susceptibility of individuals to PQ toxicity.

714

NOVEL AMINOINDOLE INHIBITORS OF *PLASMODIUM FALCIPARUM*: *IN VIVO* EFFICACY AND PRELIMINARY SAFETY ASSESSMENT

Robert H. Barker[1], Sameer Urgaonkar[2], Mikaela Levine[1], Cassandra Celatka[1], Renato Skerlj[1], Joseph F. Cortese[2], Hanlan Liu[1], Jose E. Guerrero-Bravo[3], Keila N. Krespo-Llado[3], Adelfa E. Serrano[3], Jin-wen Lin[4], Chris J. Janse[4], Shaheed M. Khan[4], Manoj Duraisingh[5], Bradley Coleman[5], Inigo Angulo-Barturen[6], Maria B. Jimenez-Diaz[6], Sergio Wittlin[7], Petros Papastogiannidis[7], Dyann F. Wirth[5], Jeffrey D. Klinger[1], Mark Bree[1], Roger Wiegand[2], Ian Bathurst[8], Edmund Sybertz[1]

[1]Genzyme Corporation, Waltham, MA, United States, [2]Broad Institute, Cambridge, MA, United States, [3]University of Pueto Rico, San Juan, Puerto Rico, [4]Leiden University Medical Center, Leiden, The Netherlands, [5]Harvard School of Public Health, Boston, MA, United States, [6]GlaxoSmithKline, Madrid, Spain, [7]Swiss Tropical Institute, Basel, Switzerland, [8]Medicines for Malaria Venture, Geneva, Switzerland

The aminoindole Genz-668764 is an analog of Genz-644442 which was originally identified as a hit in a high throughput live-dead screen of the Broad small molecule library against *Plasmodium falciparum*. Genz-668764 is a single enantiomer with IC50 values of 65 and 28 nM against *P. falciparum* strains Dd2 and 3D7 respectively. Similar potencies were seen against *P. knowlesi in vitro*, suggesting that the aminoindoles might be active against blood stages of *P. vivax*. Pharmacokinetic studies showed clearance values of 58, 46 and 13 ml/min/kg and half-life times of 7.30, 2.58 and 4.26 hr in mouse, rat and monkey respectively. Bioavailability was 33% in rats and 21% in monkeys. *In vivo* efficacy studies showed that when dosed 4 days twice/day, the ED50 against the *P. berghei* N-clone was 32 mg/kg/day; dosing at 200 mg/kg/day cured 3/5 mice. When tested at Swiss Tropical Institute against the ANKA strain, the ED50 was 19 mg/kg/day, and 2/5 mice were cured at 100 mg/kg/day b.i.d. The ED50 of Genz-6687864 *in vivo* against *P. falciparum* (3D7) in NOD-*scid* IL-2Rγnull mice engrafted with human erythrocytes was 40 mg/kg/day, while against ANKA strain *P. berghei* in the same model was 26 mg/kg/day. Preliminary 7-day rat safety studies showed a NOAEL of 200 mg/kg/day; primary findings were reticulocytopenia and failure to gain weight at the same rate as controls when dosed at 300 mg/kg/day; however, these findings were reversed 7 days after cessation of dosing, indicating that the effects were transient. Taken together, Genz-668764 appears to be a promising candidate for preclinical development.

715

CENTRAL NERVOUS SYSTEM (CNS) EXPOSURE OF NEXT GENERATION QUINOLINE METHANOLS IS REDUCED RELATIVE TO MEFLOQUINE AFTER INTRAVENOUS (IV) DOSING IN MICE

Geoffrey S. Dow[1], Erin Milner[1], Diana Caridha[1], Sean Gardner[1], Charlotte Lanteri[1], Michael Kozar[1], Ann Mannila[2], William McCalmont[1], Victor Melendez[1], Jay Moon[1], Kevin Read[3], Suzanne Norval[3], Norma Roncal[1], David Shackleford[2], Jason Sousa[1], Jessica Stouten[2], Karen White[2], Qiang Zeng[1], Susan Charman[2]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Centre for Drug Candidate Optimisation, Monash University, Melbourne, Australia, [3]University of Dundee, Dundee, United Kingdom

The clinical utility of mefloquine has been eroded through its association with adverse CNS events. These effects are dose-related. Mefloquine accumulates in the CNS. Next generation quinoline methanols (NGQMs) that do not accumulate in the CNS to the same extent may be useful antimalarials. The objective of this study was to determine whether reduction in CNS exposure relative to mefloquine was feasible amongst NGQMS with a wide range of physiochemical properties and antimalarial activity. Approximately thirty 4-position modified quinoline methanols were synthesized. The plasma and brain levels of mefloquine and these novel quinoline methanols were determined using LCMS/MS at 5 min, 1, 6 and 24 h after an IV administration (5 mg/kg) to male FVB mice. Fraction unbound in brain tissue homogenate was assessed for mefloquine and these novel quinoline methanols using equilibrium dialysis and this was then used to obtain brain unbound concentration from the measured brain total concentration. A five-fold reduction in whole and unbound brain concentrations relative to mefloquine was established as the minimum benchmark required for success. The maximum brain (whole/free) and 5 min plasma concentrations of mefloquine were 1807/4.9 ng/g and 1281 ng/ml respectively. Maximum whole brain concentrations of NGQMs ranged from 23 - 21546 ng/g. The corresponding free brain concentrations were 0.5 to 267 ng/g. Maximum brain concentrations correlated significantly with molecular weight, LogD, polar surface area, hydrogen bond donors and acceptors, albeit weakly ($r2 < 0.34$). The compound with the lowest free brain concentrations exhibited reasonable *in vitro* antimalarial activity (IC90s of 70-250 ng/ml) and is a reasonable early lead. In conclusion, reduction of CNS levels may be feasible in a next generation of antimalarial quinoline methanols. We are currently attempting to improve the potency of early lead compounds to determine if this is also feasible at projected clinically efficacious doses.

716

NOVEL ACRIDONES AS BROAD-SPECTRUM ANTIMALARIALS

Jane X. Kelly[1], Yuexin Li[2], Isaac Forquer[2], Victor Melendez[3], Mark Hickman[3], Michael O'Neil[3], Roland Cooper[4], Erin Harris[3], Brandon Pybus[3], Jason Sousa[3], Qigui Li[3], Martin Smilkstein[5], Rolf Winter[2], Dave Hinrichs[2], Mike Riscoe[2]

[1]Portland State University, Portland, OR, United States, [2]Portland Veterans Affairs Medical Center, Portland, OR, United States, [3]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [4]Old Dominion University, Norfolk, VA, United States, [5]Oregon Translational Research and Drug Development Institute, Portland, OR, 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 *in vitro* efficacy against the blood stage malaria. Our newly optimized derivatives were tested in the following systems: (1) Prevention of *in vitro P. berghei* sporozoite-induced development in human hepatocytes; (2) Prevention of *in vivo P. yoelii* sporozoite-induced blood stage infection in mice; (3) Inhibition of *in vitro P. falciparum* blood stage growth; (4) Efficacy in blood stage rodent malaria models; and (5) Inhibition of *in vitro P. falciparum* gametocyte growth. Details of the design, chemistry, biological activities, and preliminary studies of safety, metabolism and mechanism of action will be presented.

717

A HUMANIZED MOUSE MODEL TO TEST HEMOLYTIC TOXICITY OF 8-AMINOQUINOLINES

Rosemary Rochford[1], Caroline Othoro[1], Aruna Sampath[2], Marino Mauro[1], Babu Tekwani[3], Colin Ohrt[2], Larry Walker[3]

[1]State University of New York - Upstate Medical University, Syracuse, NY, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]University of Mississippi, Oxford, MS, United States

The goal of our research has been to develop a cost-effective, high-throughput animal model to test the hemolytic capacity of novel 8 aminoquinolines (8AQ) in the background of glucose 6-phosphate dehydrogenase(G6PD) deficiency. To achieve this goal, we first developed and validated a human (hu)RBC-SCID mouse model by giving NOD-SCID mice daily transfusions of huRBC from G6PD deficient donors for 14 days. At day 14, engraftment of huRBC was determined by flow cytometry to assess the percentage of human RBC. Levels of engraftment ranged from 55-85% with a mean engraftment of 75% huRBC. Mice were then treated with primaquine (PQ) at varying doses i.p. 2x per day for 7 days. The percentage of huRBC and mouse reticulocytes were determined by flow cytometry every day for 7 days. Treatment with PQ i.p. resulted in loss of huRBC and increase in mouse reticulocytes in a dose dependent manner. We next tested whether PQ given orally (p.o.) would also result in loss of huRBC. We tested PQ at 25 mg/kg/day and 12.5 mg/kg/day and found that the 25 mg/kg/day po induced a similar loss of huRBC compared to the 12.5 mg/kg/day PQ given i.p. As a control, we tested whether chloroquine (CQ) would induce loss of hu-RBC in this model. Chloroquine treatment was comparable to PBS control suggesting that the loss of G6PD deficient hu-RBC in this model is specific to PQ. These data suggest that we can reproducibly induce loss of G6PD deficient huRBC engrafted into NOD-SCID mice following treatment with PQ and that this effect is dose dependent. Further validation with additional hemolytic and non-hemolytic drugs should prove application of this model for screening antimalarial drugs in discovery phase. Importantly, we have developed a new experimental tool to assess G6PD hemolytic toxicity of anti-malaria drugs.

718

ANTIPLASMODIAL ACTIVITY OF SOME MEDICINAL PLANTS USED IN SUDANESE FOLK-MEDICINE

El-Hadi M. Ahmed[1], Bakri Y. Nour[2], Yousif G. Mohammed[1], Hassan S. Khalid[3]

[1]University of Gezira, Department of Chemistry and Pharmacognosy, Faculty of Pharmacy, Wad Medani, Sudan, [2]Blue Nile Research and Training Institute/University of Gezira, Wad Medani, Sudan, [3]Medicinaland Aromatic Plants Research Institute, National Council for Research, Khartoum, Sudan

Ten plants indigenous to Sudan and of common use in Sudanese folk-medicine, were examined *in vitro* for antimalarial activity against schizonts maturation of *Plasmodium falciparum*, the major human malaria parasite. All plant samples displayed various antiplasmodial activity. Three plant extracts caused 100% inhibition of the parasite growth at concentrations of plant material 500 mg/ml. The two most active extracts that produced 100% inhibition of the parasite growth at concentration of plant material 50 µg/ml were obtained from the seeds of *Nigella sativa* and the whole plant of *Aristolochia bracteolata*. The ten plants were phytochemically screened for their active constituents. The two most active plants showed the presence of sterols, alkaloids and tannins.

719

ANTI-PLASMODIAL AND IMMUNOMODULATORY ACTIVITY OF MEDICINAL PLANTS USED IN BURKINA FASO AGAINST MALARIA

Denise P. Ilboudo[1], Silvia Parapini[1], Nicoletta Basilico[1], Mario Dell'Agli[1], Paolo Coghi[2], Richard Sawadogo[3], Karou Damintoti[3], Jacques Simpore[3], Jean-Baptiste Nikiema[3], Diego Monti[2], Enrica Bosisio[1], **Donatella Taramelli**[1]

[1]Università di Milano, Milan, Italy, [2]ISTM-CNR, Milan, Italy, [3]University of Ouagadougou, Milan, Italy

Plasmodium falciparum malaria is the most common cause of death in Burkina Faso. The persistence of drug resistance parasites makes the disease difficult to control. Moreover, the recent reports of emergence of resistance to artemisinin derivatives, which are the most effective antimalarial presently available, confirm that new drugs are greatly needed. The reliability of indigenous herbal drugs may be helpful. In Burkina Faso, the decoctions of *Canthium henriquesianum* Schum, *Gardenia sokotensis* Hutch. and *Vernonia colorata* Willd. are used to treat malaria. The study objective was to evaluate the antiplasmodial properties of these plant extracts and to check the relevance of their use. The plants aerial parts were soxhlet-extracted with water and different solvents and then screened for antiplasmodial activity through the pLDH method on *P. falciparum* sensitive (D10) and resistant (W2) strains. The aqueous extract from *C. henriquesianum* was the most active with IC50 of 80,02± 26,83 and 66.8±21.6 µg/ml on D10 and W2, respectively. The ethyl acetate extract was even more potent with IC50 24.0±7.4 µg/ml. No toxicity was observed against mammalian cells, suggesting a good therapeutic index. The decoction of *C. henriquesianum* contains hydrolysable tannins, flavonoids, saponins and no alkaloids. Extracts of *C. henriquesianum* also induced a dose-dependent inhibition of the production of IL-1β by human monocytes, thus confirming its traditional use as antipyretic. Attempts to identify the active principle for antiplasmodial and anti-inflammatory activities are ongoing.

720

SEASONAL VARIATION AND CLINICAL PROTECTION OF ANTIBODIES TO A PANEL OF PRE-ERYTHROCYTICS AND ERYTHROCYTICS MALARIA VACCINE ANTIGENS IN CHILDREN BELOW FIVE YEARS LIVING IN MALARIA HYPER ENDEMIC AREA OF BURKINA FASO (WEST AFRICA)

Diarra Amidou[1], Ouedraogo André Lin[1], Charrif Mariama[1], Tiono Alfred[1], Soulama Issiaka[1], Ouedraogo Alphonse[1], Yaro Jean Baptiste[1], Ouedraogo Esperance[1], Edith C. Bougouma [1], Sanon Souleymane[1], Amadou T. Konate[1], Gansane Adama[1], Corradin Giampietro[2], Dodoo Daniel[3], Sodiomon B. Sirima [1], Ouedraogo Nebie Issa[1]

[1]CNRFP, Ouagadougou, Burkina Faso, [2]Lausane, Lausane, Switzerland, [3]Noguchi Memorial Institute, Accra, Ghana

The sequencing of ***Plasmodium falciparum*** genome has contributed to the discovery of new antigens which can be used for the development of malaria vaccine candidates. In this study newly pre-erythrocytic (MR48a) and erythrocytic (LR181 & 1574) synthetic peptides and already well known recombinant antigen (LSA1) have been used to i) characterize the seasonal profile of total IgG response and (ii) examine the relationship between natural antibody responses and protection against clinical malaria. We performed two clinical and parasitological cross-sectional surveys, in January 2007 before the low and at the pick of malaria transmission season. From the first cross sectional survey, children were visited biweekly to record clinical malaria cases during one year period. Study includes 380 children under five years from 4 villages of Saponé health district. During cross-sectional surveys, blood films were prepared for parasites check, 5 ml of blood taken and plasma used for total IgG measurement. Mean number of malaria episodes was 1.57 (95%CI: 1.52-1.6) with an incidence of 0.7 episode per child year at risk. Geometric means of 1574 didn't show any difference during both transmission seasons (1574: P = 0.8). However LR181 show high antibodies level (P < 0.000) during low compared to high transmission season. LSA1 and MR48a increase significantly (P < 0.000) during high compared to low transmission season. Correlation to protection was seen only for IgG to LSA1 (P=0.01). In conclusion, antibodies to most of these antigens were affected by the level of malaria transmission season and only recombinant peptide (LSA1) elicited antibodies associated to protection in our study young volunteers. This finding should be taken into account when designing malaria vaccine trial in seasonal transmission settings.

721

ELEVATED EFFECTOR MEMORY CD4+ T CELLS IN CHILDREN WITH SEVERE MALARIAL ANEMIA

Evans Raballah[1], Samuel B. Anyona[1], Collins Ouma[1], Tom Were[1], Prakasha Kempaiah[2], Gregory C. Davenport[2], G. Orinda[3], Michael F. Otieno[3], John M. Vulule[4], Douglas Perkins[2], John M. Ong'echa[1]

[1]University of New Mexico/KEMRI, Kisumu, Kenya, [2]University of New Mexico, Albuquerque, NM, United States, [3]Kenyatta University, Nairobi, Kenya, [4]KEMRI, Kisumu, Kenya

In malaria endemic areas, malaria-induced chronic immune activation may contribute to severe malarial anemia (SMA) pathogenesis. We, therefore, characterized CD4+ T-cell populations in children (n=54; age 12-36 months) presenting with differing severities of malarial anemia at Siaya District Hospital, western Kenya. Complete hematological measures were obtained with a Beckman Coulter Counter®, while Giemsa-stained slides were used to determine parasitemia. Participants were stratified based on hemoglobin (Hb) status as uncomplicated malaria (UM; Hb>11.0 g/dL; n=12), mild malarial anemia (M/MA; Hb>8.0<11.0 g/dL; n=22), and severe malarial anemia (SMA; Hb<6.0 g/dL; n=20)]. Venous blood was isolated and stained with anti-(CD45RA; CD62L; CCR7; CD69 and HLA-DR) antibodies. Cells were then acquired using a four-color FACSCalibur. Proportions of CD4+ T-cell were also analyzed. Children presenting with SMA had the highest proportion of effector memory T-cells (CD45RA-CCR7-CD62L-) [median (IQR) UM, 11.70% (11.00); M/MA, 10.59% (8.21); SMA, 14.80% (6.18); *P*=0.025], with no significant differences across the groups in the proportion of central memory T-cells (CD45RA-CCR7+CD62L+) [median (IQR) UM, 54.95% (17.40); M/MA, 59.92% (16.80); SMA, 56.74% (10.00) *P*=0.788)]. The expression of the early (CD69) and late (HLA-DR) activation markers on CD4 T cells was comparable across the groups, possibly due to continuous antigenic challenge from the chronicity of malaria infections in this region. Taken together, these results suggest that effector memory T-cells may play an important role in modulating the development of pediatric SMA in this holoendemic *P. falciparum* transmission area.

722

ASSOCIATION BETWEEN IMMUNOGLOBULIN GM AND KM GENOTYPES AND PLACENTAL MALARIA IN HIV-1 POSITIVE AND NEGATIVE WOMEN IN WESTERN KENYA

Nnaemeka C. Iriemenam[1], Janardan P. Pandey[2], Aryan M. Namboodiri[2], Keith M. Rocca[2], John Williamson[1], Anna J. Blackstock[1], Ajay Yesupriya[1], Caroline Othoro[3], John Ayisi[3], Anna Maria van Eijk[3], Juliana Oteino[3], Renu B. Lal[1], Richard Steketee[1], Bernard Nahlen[1], Feiko O. ter Kuile[4], Laurence Slutsker[1], Ya Ping Shi[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Medical University of South Carolina, Charleston, SC, United States, [3]Kenya Medical Research Institute, Kisumu, Kenya, [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Immunoglobulin (Ig) GM and KM allotypes, genetic markers of γ and κ chains, respectively, are associated with humoral immune responsiveness. The clinical importance of Ig GM and KM allotypes has been evaluated for

other infectious diseases, but their role in placental malaria (PM) with HIV co-infection during pregnancy has not been investigated. We examined the relationship between Ig GM and KM allotypes and risk of PM in pregnant women with known HIV status, nested in an epidemiological study investigating the relationship between PM and perinatal mother-to-child transmission of HIV-1 in Kisumu, Kenya. DNA samples from 728 pregnant women were genotyped for major GM6 and KM alleles using a restriction-fragment length polymorphism polymerase chain reaction method. The genetic polymorphisms of Ig GM and KM were defined as (1) individual genotype for GM6 (+, +/-, -) and KM (1, 1-3, 3) respectively and (2) combined genotype of the GM6 and KM. Overall, there was no significant effect of individual GM6 and KM genotypes on the risk of PM in HIV-1 negative and positive women respectively. However, the combination of homozygosity for GM6 (+) and KM3 was associated with decreased risk of PM (adjusted OR 0.25, 95% CI, 0.08 to 0.8, $P = 0.019$) in HIV negative women while the combined heterozygosity of GM6 (+/-) and KM1-3 were associated with increased risk of PM in HIV positive women (adjusted OR 2.08, 95% CI, 1.12 to 3.89, $P = 0.021$). In addition, the combination of GM6 (+/-), KM1-3 and KM1 was associated with increased risk of PM only in the subgroup of HIV positive women with viral load <10,000 copies/ml (adjusted OR 2.99, 95% CI, 1.28 to 7.02, $P = 0.011$), suggesting that the viral load has an effect on the relationship between the combined genotypes and susceptibility to PM. These findings suggest that the combination of GM6 (+) and KM3 may protect against PM in HIV negative women, while the HIV positive women with GM6 (+/-) combined with KM1-3 or KM1 may be susceptible to PM.

723

POLYMORPHIC VARIABILITY IN THE TUMOR NECROSIS FACTOR (TNF)-A PROMOTER IS ASSOCIATED WITH INCREASED SUSCEPTIBILITY TO PEDIATRIC SEVERE MALARIAL ANEMIA

Prakasha Kempaiah[1], Collins O. Ouma[2], Gregory C. Davenport[1], Samuel B. Anyona[2], Tom Were[2], Evans Raballah[2], John M. Ong'echa[2], Douglas J. Perkins[1]

[1]University of New Mexico, Albuquerque, NM, United States, [2]University of New Mexico/KEMRI, Kisumu, Kenya

The pleiotropic cytokine tumor necrosis factor (TNF)- α plays a central role in the early stages of intracellular infections by activating the innate immune response. TNF- α has long been considered a candidate susceptibility gene for malaria. Although association of several TNF- α promoter polymorphisms with malaria disease outcome have been reported, their precise role, particularly in modulating severe malarial anemia (SMA) in children residing in holoendemic *Plasmodium falciparum* transmission regions, are largely undefined. The aim of the present study was to investigate the functional associations between TNF- α promoter polymorphisms [(G-238A), (C-308T), (C-376T) and (T-1031C)], and presence of parasitemia and SMA (Hb <5.0 g/dL) manifestation in children (n=736, aged 3-36 mos.). Stratification of parasitemic children (n=578) according to hemoglobin (Hb) levels revealed that the SMA (Hb <5.0g/dL; n=439) (P=0.040) group. Multivariate logistic regression analyses of genotypic variants, controlling for confounding factors, showed that heterozygosity (GA) at the -238 locus was associated with an increased risk of SMA [OR, 1.907,95% CI, 1.067-3.410, P=0.029] and non-significant elevations in circulating TNF- α (P=0.588) relative to wild-type. Additional analyses revealed that the ACCT [-238A/-308C/-376C/-1031T] haplotype was associated with protection against the acquisition of *P. falciparum* parasitemia (OR, 0.516, 95% CI, 0.277-0.961, P=0.037) relative to individuals without this haplotype, and non-significant elevations in circulating TNF- α (P=0.328). Results presented here suggest that variation in the TNF- α promoter conditions susceptibility to malaria and the development of SMA once malaria is acquired. However, the mechanism(s) through which variation in TNF- α promoter conditions susceptibility to malaria outcomes remains to be determined since circulating TNF- α levels were not significantly associated with either genotypes or haplotypes.

724

ERYTHROPOIETIN (EPO) AND ANTI-EPO AUTO-ANTIBODIES IMBALANCE IS ASSOCIATED WITH PROTECTION AND CORRELATES WITH ANAEMIA IN PBANKA INFECTED SEMI-IMMUNE MICE

Gideon K. Helegbe[1], Nguyen T. Huy[1], Tetsuo Yanagi[2], Mohammed N. Shuaibu[3], Mihoko Kikuchi[4], Kenji Hirayama[5]

[1]Institute of Tropical Medicine (NEKKEN), Department of Immunogenetics, Nagasaki University, Nagasaki, Japan, [2]Institute of Tropical Medicine (NEKKEN), Animal Research Center for Tropical Infections, Nagasaki University, Nagasaki, Japan, [3]Institute of Tropical Medicine (NEKKEN), Department of Immunogenetics and Global COE Programme, Nagasaki University, Nagasaki, Japan, [4]Institute of Tropical Medicine (NEKKEN), Department of Immunogenetics and Center for International Collaborative Research, Nagasaki University, Nagasaki, Japan, [5]Institute of Tropical Medicine (NEKKEN), Department of Immunogenetics, Center for International Collaborative Research and Global COE Programme, Nagasaki University, Nagasaki, Japan

Malaria anaemia is still a major public health problem and its pathogenesis still unclear. It has been observed that some individuals develop anaemia at low parasitaemia and are protected and others not due to complications from hyperparasitaemia. A study has shown that treatment of infected mice with exogenous anti-erythropoietin (EPO) auto antibodies (auAb) gives protection, suggesting an important role for anti-EPO auAb in malaria. Thus we hypothesized that elevated levels of anti-EPO auAb is associated with protection in *Plasmodium* infected semi-immune individuals. Semi-immune status was attained in four mice strains (Balb/c, B6, CBA and NZW) by repeated infections with PbANKA, and treatment with chloroquine/pyrimethamine. ELISA was used to measure EPO and anti-EPO auAb, while inflammatory cytokines measurement was done using bead-based multiplex assay kit. Measurement of transferin in the mice sera and anti-EPO auto antibodies in sera of human malaria is underway. High %Hb loss and survival (>40%) was observed in Balb/c in comparism with the other strains (<33% survival). Similar levels of anti-EPO auAb was observed in Balb/c and NZW ($p=0.61$), and were significantly higher than in other strains, $p<0.0001$. Anti-EPO auAb correlated positively with extent of Hb loss ($r2=0.41$; $p=0.0009$). However, anti-EPO auAb/log EPO ratio was significantly associated with Balb/c ($p<0.0001$) which are resistant to infection. Significant elevated levels of IL6 and IFNg ($p<0.0001$), both associated with erythropoiesis suppression were observed in the Balb/c. In conclusion, our data presented here seems to suggest that anti-EPO auAb/EPO imbalance may be an additional contributor in the pathogenesis of malaria anaemia, but of a beneficial role in playing a protective mechanism to severe malaria anaemia in some individuals and detrimental to others, hence implicating host genetic factors.

725

MALARIA INFECTION AND MEASLES VACCINATION EFFICACY - CAUSE FOR CONCERN?

Jeffrey M. Collins[1], Erwan Piriou[2], Kiprotich Chelimo[3], Rosemary Rochford[4], Inna G. Ovsyannikova[5], John Vulule[3], Gregory A. Poland[5], Ann M. Moormann[2]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]University of Massachusetts Medical School, Worcester, MA, United States, [3]Kenyan Medical Research Institute, Kisumu, Kenya, [4]State University of New York - Upstate Medical University, Syracuse, NY, United States, [5]Mayo Clinic College of Medicine, Rochester, MN, United States

Measles Virus (MV) continues to be one of the largest causes of vaccine preventable mortality, due in part to its high transmission rate, requiring greater than 95% seroconversion to induce herd immunity. Although there have been tremendous gains in global vaccination coverage, measles outbreaks continue, especially in areas where malaria is a common

childhood infection. Malaria is known to be immuno-suppressive and/or immune-modulating. Therefore the true influence of concomitant asymptomatic malaria infections on MV vaccine efficacy remains in doubt. In order to shed light on this underlying question, we compared antibody- and cell-mediated immunity to MV vaccine (Edmonton strain) in two prospective cohorts of Kenyan children (2 months until 3 years of age) residing in areas with divergent malaria transmission intensities: holoendemic versus highland, epidemic-prone malaria. Antibody levels were measured using the Luminex microsphere technology. Cell-mediated immunity was measured using IFN-γ ELISPOT assays and a panel of cytokines was measured by Luminex. We found no significant difference in the mean MV-antibody levels following vaccination associated with malaria exposure history. This remained true after controlling for age at vaccination and pre-vaccination antibody levels. There was also no significant difference in MV-specific IFN-γ response *ex vivo* in children from the holoendemic area (20%) compared to the highland children (24%). Highland children were however more likely to secrete higher levels of IL-10 in response to MV, though other cytokine levels did not differ significantly between groups. And yet, IL-10 responses did not correlate with a lack of seroconversion. These results suggest that malaria has a minimal impact on the quality of MV immunity following the first vaccination. Future studies will evaluate factors, such as second MV immunization and prolonged exposure to malaria, which may influence the quality and duration of immunologic memory to the MV vaccine.

726

LONGITUDINAL ANALYSIS OF THE ANTIBODY RESPONSE AGAINST FIVE VAR2CSA DOMAINS AND MULTIPLE STRAIN VARIANTS IN CAMEROONIAN PREGNANT WOMEN LIVING IN A HIGH MALARIA TRANSMISSION AREA

Yeung Y. Lo[1], Marion Avril[2], Rose F. Leke[3], Joseph D. Smith[2], Diane W. Taylor[1]

[1]University of Hawaii, Manoa, Honolulu, HI, United States, [2]Seattle Biomedical Research Institute, Seattle, WA, United States, [3]University of Yaounde I, Yaounde, Cameroon

VAR2CSA, a variant surface antigen on late-stage *Plasmodium falciparum*-infected erythrocytes (IE), is responsible for IE adhesion to chondroitin sulfate A in the intervillous space of the placenta and can lead to placental malaria (PM). PM is associated with babies born with low birth weights, who are at increased risk of malaria early in life. While PM is less prevalent among multigravidae, presumably due to the presence of adhesion blocking antibodies (Ab), it remains unclear to which DBL domain(s) of VAR2CSA protective Ab are directed. The present study aimed to identify correlates of protection (absence of PM) by evaluating sera collected longitudinally from 37 pregnant women living in Ngali II who received ~0.7 infectious bite/night throughout pregnancy. Using a bead-based multiplex assay, Ab levels against recombinant VAR2CSA DBL 1, 3, 4, 5 and 6 were measured for parasite strains 7G8, IT4 and 3D7. Our results demonstrated that all five DBL domains are immunogenic, with robust and dynamic responses observed against DBL3 (7G8) and 5 (7G8, 3D7) as early as 3 months of pregnancy (MoP). Moderate responses were observed to DBL6 - 7G8 but not - IT4. While Ab responses against DBL1 (7G8, IT4, 3D7) were low or absent and generally did not appear until 6 MoP, only minimal responses were detected to DBL4 (7G8, IT4) during pregnancy. Ab levels against DBL3 and 5 increased gradually and steadily throughout pregnancy. Based on 13 women without PM (PM-) and 14 women with PM (PM+), a consistent pattern was observed in that PM- women had higher Ab levels against DBL3 (7G8) and 5 (7G8, 3D7) from 3 to 8 MoP and from 6 to 8 MoP, respectively, than PM+ women. Moreover, Ab of PM- women tended to recognize more domains than PM+ women. In summary, early production and maintenance of Ab against DBL3 and 5 at high levels throughout pregnancy appeared to correlate with the absence of PM at term. A larger repertoire of Ab to multiple domains may also contribute to enhanced protection from developing PM.

727

LONGITUDINAL PATTERNS OF ANTIBODY RESPONSES AND *PLASMODIUM* SPP. INFECTION DURING PREGNANCY

Freya J. Fowkes[1], Rose McGready[2], Mirja Hommel[1], Nadia J. Cross[1], Julie A. Simpson[3], Kurt Lackovic[1], Jack S. Richards[1], Samuel J. Viladpai-nguen[2], Marion Avril[4], Joseph D. Smith[4], David L. Narum[5], Robin F. Anders[6], Francois Nosten[2], James G. Beeson[1]

[1]Walter and Eliza Hall Institute, Parkville, Australia, [2]Shoklo Malaria Research Unit, Mae Sot, Tak, Thailand, [3]Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, University of Melbourne, Melbourne, Australia, [4]Seattle Biomedical Research Institute, Seattle, WA, United States, [5]Malaria Vaccine Development Branch, National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States, [6]La Trobe University, Melbourne, Australia

Pregnant women are more susceptible to, and more severely affected by, malaria and other infectious diseases. In malaria endemic regions pregnant women typically develop high parasite densities, placental infection and associated complications, despite substantial immunity to malaria that may have been acquired prior to pregnancy. This has largely been attributed to both the modulation of maternal immune responses and the sequestration of *Plasmodium falciparum* parasites in the placenta. The contribution of pre-existing immunity, maintenance and boosting of antibody responses throughout pregnancy, and their relation to malaria is unclear. In a nested case-control study of 467 pregnant Karen women (136 malaria cases and 331 non-infected controls), we measured antibody levels at enrolment to blood-stage antigens and pregnancy-specific antigen VAR2CSA and placental-binding isolates CS2 and HCS3. Furthermore, we also determined antibody levels at 2-weekly intervals during pregnancy until delivery in malaria cases and a subset of controls, including over 2000 samples. ELISAs were performed using novel high-throughput technology to facilitate determination of antibody levels in a large number of samples. At enrolment, the sero-prevalence of blood-stage antibodies was higher in cases than controls and the prevalence of antibodies to the pregnancy-specific binding isolates were low. Antibody levels at enrolment were associated with increased odds of parasitaemia during pregnancy. Longitudinal analysis revealed the prevalence of malaria infection (both *P. falciparum* and *P. vivax*) decreased with increasing gestation time as did antibody levels at a similar rate in cases and controls. However, antibody levels did increase with gestation time in those with concurrent *P. falciparum* parasitaemia most likely reflecting boosting of responses with each successive infection. This study provides the most comprehensive analysis, to date, of antibody maintenance, decay and boosting during pregnancy and contributes to our understanding of malaria during pregnancy and immune responses to infectious diseases during pregnancy.

728

NOVEL *PLASMODIUM FALCIPARUM* BLOOD STAGE ANTIGENS: INDUCTION OF HUMORAL RESPONSE AND PROTECTION IN BURKINABE CHILDREN

Andre Lin Ouedraogo[1], Amidou Diarra[1], Mariama Cherrif[1], Alfred Tiono[1], Issiaka Soulama[1], Alphonse Ouedraogo[1], Jean-Baptiste Yaro[1], Esperance Ouedraogo[1], Edith C. Bougouma[1], Souleymane Sanon[1], Amoudou T. Konate[1], Adama Gansane[1], Giampietro Corradin[2], Daniel Dodoo[3], Sodiomon B. Sirima[1], Issa Nebie[1]

[1]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [2]University of Lausanne, Lausanne, Switzerland, [3]Noguchi Memorial Institute for Medical Research, Legon Accra, Ghana

Protective human immunity against clinical malaria involves the acquisition of specific antibodies. The identification of vaccine candidate antigens that elicit the induction of protective humoral response is a valuable step for an effective malaria vaccine development. Here, we determine the

role of specific IgG antibody responses in the protection against clinical malaria in an area with stable and distinct seasonality of transmission. A total of 422 children below 5 years from the Sapone's health district (Burkina Faso) provided 844 plasma samples at low and high transmission seasons. Specific IgG antibody responses against the recombinant CSP construct (control antigen) and 3 synthetic α-helical coiled coil blood stage antigens (LR179A, AS155 and MR198) were measured. A year-round clinical malaria active case detection was conducted and malaria clinical data related to antibody titers for immunological correlates of protection. Geometric means of specific antibody levels to CSP, AS155 and LR179A significantly associated with age and season. In contrast, antibody levels induced by the antigen MR198 were influenced neither by age nor by season. During the entire follow up, the mean number of malaria episode per child was 1.57 with an incidence rate of 0.7 episode per child year at risk. The assessment of the role of the four antigens in eliciting a protective humoral response in children revealed a negative association between the geometric mean antibody level to AS155.4 and clinical malaria cases (p=0.007). In conclusion, our findings suggest that α-helical coiled coil protein motif-based vaccine candidates are immunogenic in children less than five years and AS155.4 antibodies strongly associate with protection against malaria clinical. Therefore, further clinical investigations including IgG subclasses are recommended for more insights into such a protective immunity against the malaria parasite.

729

ACUTE *PLASMODIUM CHABAUDI* INFECTION LEADS TO LOSS OF TRANSITIONAL B CELLS IN THE SPLEEN

Joel Wilmore, Alex Maue, Rosemary Rochford

State University of New York - Upstate Medical University, Syracuse, NY, United States

The spleen is a critical interface between *Plasmodium* infected RBC and B cells. Because *Plasmodium* infection leads to a high antigen load and disruption of splenic architecture, we wanted to determine the effects of *Plasmodium* infection on B cell subset distribution in the spleen during the acute phase of infection. Peripheral B cell subsets in the spleen include both immature B cells (e.g. transitional T1 and T2 cells) and mature marginal zone and follicular B cells. Using a mouse model of the erythrocyte stage of *P. chabaudi* infection in C57BL/6 mice, we examined the alterations in splenic B cell subsets during the acute phase of infection. Mice were injected i.p. with 5x105 parasitized RBCs and after 6 and 12 days post infection (dpi) the spleens were removed and the B cell subsets were analyzed using flow cytometry. At 12 dpi, which is the peak of parasitemia, the spleens had an increase in overall cellularity; however, the total number of B cells was not significantly altered. Interestingly, the two immature T1 (B220+, AA4+, CD23-, IgM+) and T2 (B220+, AA4+, CD23+, IgM+) transitional B cell subsets showed a significant reduction in number at both 6 and 12 dpi. These decreases in transitional B cells was the result of apoptosis in both the T2 and to a greater extent T1 B cell subsets as determined by Annexin V staining. We also observed a decrease in the marginal zone B cell population (CD19+, AA4-, CD21hi, CD23lo) by 12 dpi. Experiments are ongoing to determine if the decline in marginal zone B cells is due to apoptosis of these cells or due to loss of the transitional B cells that differentiate to marginal zone B cells. Our results demonstrate that acute infection with *P. chabaudi* can lead to specific affects on B cell homeostasis in both the mature and immature subsets.

730

FREQUENCY OF *PLASMODIUM FALCIPARUM* INFECTION AMONG UGANDAN CHILDREN AND ITS RELATIONSHIP WITH VARIANTS OF THE CYTOKINE RANTES PROMOTER

Catherine Lwanira, Mark Kaddu Mukasa, Steven Kiwuwa, Allan Ligaajju, Fred Kironde

Makerere University, Kampala, Uganda

Chemokines including Regulated on Activation, Normal T Expressed and Secreted (RANTES) control host immunity to pathogens. We have investigated two highly polymorphic nucleotide positions (-403 and -28) in the RANTES promoter of genomic DNA from Ugandan children and the relation of mutation at these positions to *Plasmodium falciparum* infection. To our knowledge, this is the first study of the relation between diversity of RANTES gene promoter and *P. falciparum* infection. In a cross-sectional study, we examined DNA from 319 Ugandan children (158 infected with *P. falciparum* and 161 healthy controls) aged between 0.5 and 9 years. *P. falciparum* infection was determined by microscopy of blood smears. Using DNA polymerase chain reaction and enzyme digestion, we determined the occurrence of mutation at nucleotides -403 and -28 of RANTES promoter. The profile of variants at nucleotide -403 was as follows: 55.8 % (178/319) heterozygous (mutation at one chromosome), 21.9 % (70/319) homozygous (mutation at both chromosomes), and 22.3% (71/319) wild type. The prevalence of *P. falciparum* infection was significantly higher in children carrying -403 mutation on one chromosome (P=0.001; odds ratio =2.6), or bearing the -403 mutation on both chromosomes (P = 0.014; OR 2.3) than in -403 wild type individuals. The prevalence of mutations at nucleotide -28 was 3.8% (12/319) heterozygous, 0 % homozygous and 96.2% (307/319) wild type. Importantly, eight out of the twelve children (66.7%, 8/12) carrying mutation at -28 also had a mutation at -403 and were 6.2 times more likely to have *P. falciparum* infection than those with wild type alleles, suggesting that dual mutations (at -403 plus -28) lead to higher risk for malaria. There was no significant relationship between the rare -28 nucleotide mutation and *P. falciparum* infection (P= 0.23). Further, there was no significant association between RANTES variants and parasitemia (P= 0.78).

731

EVALUATION OF THE BINDING AND INHIBITORY PROPERTIES OF NOVEL MONOCLONAL ANTIBODIES TO *PLASMODIUM VIVAX* LIGAND DOMAIN

Francis B. Ntumngia[1], Jesse Schloegel[1], Amy McHenry[1], Samantha Jones[1], Christopher L. King[2], Sanjay Singh[3], John H. Adams[1]

[1]University of South Florida, Tampa, FL, United States, [2]Case Western Reserve University, Cleveland, OH, United States, [3]ICGEB, Aruna Asaf Ali Marg, India

The Duffy Binding protein of *Plasmodium vivax* is vital for host erythrocyte invasion and the region II (DBPII) contains critical residues for receptor recognition thereby making the molecule an attractive vaccine candidate against vivax malaria. Although an ideal target, the allelic variation within the DBPII and associated strain specific immunity may be a major challenge for development of a broadly effective vaccine for vivax malaria. To understand the specificity of protective immune responses to DBPII, we have generated a panel of monoclonal antibodies (MABs) to identify and map the various domains of the DBPII that correlate with protection to *P. vivax.* Using rDBPII from different alleles we have assayed the specificity of the MABs by ELISA and inhibition of binding by standard erythrocyte binding assay. Analysis by ELISA determined that some MABs react strongly with epitopes conserved on all rDBPII alleles tested, while other MABs react with allele-specific epitopes. Quantitative and qualitative analysis, with ELISA and *in vitro* erythrocyte-binding inhibition assays respectively, failed to demonstrate a consistent correlation between

the end point titers and functional inhibition. These data are consistent with the differential inhibitory patterns observed with human sera from individuals naturally exposed to *P. vivax*. Identification of conserved epitopes to which inhibitory antibodies bind is critical to optimizing DBP immunogenicity for protection against diverse *P. vivax* strains.

732

DEFINING THE IMMUNOREACTIVE SURFACE OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN REGION II

Jesse L. Schloegel[1], Francis B. Ntumngia[1], Samantha N. Jones[1], Sanjay Singh[2], Christopher L. King[3], Joanne L. Casey[4], Michael Foley[4], John H. Adams[1]

[1]University of South Florida, Tampa, FL, United States, [2]Gennova Biopharmaceuticals, Pune, Maharashtra, India, [3]Case Western University, Cleveland, OH, United States, [4]Department of Biochemistry, Latrobe University, Melbourne, Victoria, Australia

Region II of the Duffy binding protein (*Pv*DBPII) is a high priority candidate for inclusion in a subunit vaccine designed to protect against disease caused by the malaria parasite *Plasmodium vivax*. The *Pv*DBPII coding sequence predicts an approximately 38-kDa antigen, which comprises the erythrocyte-binding domain of the Duffy binding protein (*Pv*DBP). Although the crystal structure of *Pv*DBPII is yet to be published, it is known to consist of a Duffy binding-like (DBL) domain, belonging to a family of structural homologues found in other adhesion molecules of other species of *Plasmodium*. Based on its high degree of homology with the DBL domain of *Plasmodium knowlesi* DBP, for which there is a crystal structure, *Pv*DBPII is thought to be largely alpha-helical and may be assigned into three sub-domains delineated by six disulphide bonds. Using a phage display approach to express these sub-domains individually or in combination in their correctly refolded and disulphide bonded conformations, we are currently mapping the conformation-dependent epitopes of a panel of monoclonal antibodies recognizing the antigen. Finer epitope mapping is being achieved using a random peptide library and a gene fragment library of *Pv*DBPII displayed on phage. Many of the antibodies being studied are capable of inhibiting recombinant *Pv*DBPII expressed on COS cells from binding to Duffy-positive erythrocytes. The studies performed here suggest that the epitopes of binding-inhibitory antibodies map to a different region of *Pv*DBPII compared with those of non-inhibitory antibodies. Ultimately information derived from these studies will contribute to the assessment of this antigen for inclusion in a vaccine designed to protect against disease caused by vivax malaria.

733

A STAT6 SINGLE NUCLEOTIDE POLYMORPHISM IS ASSOCIATED WITH PROTECTION AGAINST CEREBRAL MALARIA IN GHANAIAN CHILDREN

Daniel Amoako-Sakyi[1], Ben Gyan[2], Daniel Dodoo[2], Marita Troye-Blomberg[3]

[1]University of Cape Coast, Cape Coast, Ghana, [2]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [3]Stockholm University, Stockholm, Sweden

The IL-4/Stat6 signalling pathway could be crucial for Th2 mediated immunity and protection against malaria. Although we and others have previously shown associations between some IL-4 polymorphisms and severe malaria, the role of Stat6 and IL-4R-α polymorphisms in malaria pathogenesis is yet to be established. This study investigated the distinctive and interactive association of known polymorphisms of the IL-4 gene (+33C/T, 590C/T, VNTR), IL-4R gene (Arg551Gln) and STAT6 gene (1570C/T) with total IgE production and subsequently, malaria severity in Ghanaian children. PCR-RFLP was used to genotype all polymorphisms in a hospital based cross-sectional study involving 290 malaria cases and controls. Malaria cases were categorized into uncomplicated malaria (UM), severe malarial anaemia (SMA), and cerebral malaria (CM). We found that a single nucleotide polymorphism (SNP) (rs3024974) which causes a C → T change in intron 18 of the stat6 gene is associated with protection from cerebral malaria (OR = 0.361, P = 0.0107). All other polymorphisms studied did not show any association with malaria severity except the IL-4 VNTR polymorphism. Our data did not show any association between rs3024974 and levels of total IgE. Data from this study suggests that rs3024974 is associated with protection against cerebral malaria in Ghanaian children. However, this protection maybe mediated by other factors other than total serum IgE. To the best of our knowledge, this study is the first to suggest a role for the stat6 SNP (rs3024974) in malaria pathogenesis.

734

DETECTION OF LATENT RESERVOIRS OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTIONS BY SHORT AMPLICON PRIMERS AND TERTIARY NESTED PCR IN MACHA, SOUTHERN ZAMBIA

Mwiche Siame, Jodi Chondoka, Aniset Kamanga, Philip Thuma, Sungano Mharakurwa

Malaria Institute at Macha, Choma, Zambia

Malaria endemic countries have scaled-up effective interventions; this has prompted the increased need to monitor asymptomatic infection reservoirs so as to avoid potential resurgences. Using short amplicon primers and an alternative tertiary amplification strategy, the present study reports the detection of widespread sub-microscopic asymptomatic *Plasmodium falciparum* infections. In a 2000 km2 area in Macha, Southern Zambia, 1500 willing residents of all ages were screened for malaria by microscopy, with simultaneous collection of dried finger-prick blood spots on Whatman 3MM filter paper. Chelex extracts from the dried blood spots (DBS) were subjected to regular nested PCR using primers targeting the *P. falciparum* 18S rRNA and DHFR genes. PfDHFR was amplified using regular nested PCR with published primers and a new tertiary PCR strategy using three sets of regular published primers. While the malarial parasite rate was 1.1 % by microscopy, nested PCR showed 47.2 % parasite rate with 18S ribosomal primers, 56.2 % with regular published primers and 86.5% with short amplicon primers. Tertiary nested PCR enhanced the detectable parasite rate to 71.9 % ($p = 0.04$, $n = 90$) compared with the same primers in two rounds of amplification. This study documents the existence of ultra-low asymptomatic *P. falciparum* parasitaemia below detection limit of microscopy and standard nested PCR in an area of apparently depleted malaria prevalence. These methods can be used to significantly enhance detection of latent reservoirs of infection to minimize risk of resurgences.

735

MOLECULAR ANALYSIS OF THE *PLASMODIUM FALCIPARUM* SARCOPLASMIC AND ENDOPLASMIC RETICULUM CALCIUM ATPASE (*PF*ATPASE6 SERCA) GENE ASSOCIATED WITH ARTESUNATE RESISTANCE IN GHANAIAN PATIENTS WITH UNCOMPLICATED MALARIA

Ruth Ayanful[1], Michael D. Wilson[1], Kwadwo A. Koram[1], Brown Charles[2], Bimi Langbong[3]

[1]Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana, [2]College of Health Sciences, University of Ghana, Accra, Ghana, [3]Department of Animal Science and Conservation Biology, University of Ghana, Accra, Ghana

Resistance of *Plasmodium falciparum* to first line antimalarials such as chloroquine and Fansidar® resulted in a national policy change in 2005, to artemisinin combination therapy (ACT), artesunate+amodiaquine (AS+AQ) in Ghana. Certain mutations in the *P. falciparum* ATPase6 SERCA gene (E431K, A623E and S769N) have been reported to be associated with artemisinin derivatives resistance and are known to cluster geographically. The aim of this study was to identify mutations in *Pf*ATPase6 SERCA gene that can be associated with artesunate resistance in Ghana. Archived filter

paper blood blots from patients who experienced AS+AQ clinical failures (treatment failure group) and an equal number of samples selected from responders (susceptible group) were studied. Sixty four Day 0 and 3 post-treatments samples from patients attending 7 hospitals at sentinel sites for monitoring drug resistance were used. PCR identification of *P. falciparum* was performed after which the ATPase6 SERCA gene fragments were amplified and sequenced to identify mutations. Analysis of the sequenced data did not detect any polymorphism at positions 623 and 769 in the treatment groups when compared to the reference *P. falciparum* Dd2 DNA sequenced data in the GeneBank database. However, four parasites (3 in treatment failure and 1 in susceptible group) had the E431K mutation reported in Senegal. Five novel mutations (N569K, E633K, H747Y, K776N and one synonymous at position 460) were observed in the Day 0 samples. Three of the novel mutations were in the susceptible parasites and the other in the treatment failure group. Also, two parasites in the susceptible group were found to be double mutant. Considering the absence of mutations in the post-treatment parasites' genes (n=3), the small sample size, the possibility of re-infection, no definitive conclusion could be made on their association with treatment outcome. Further studies to assess other mutations in *Pf*ATPase6 SERCA gene and other genes should be looked at to know the actual target of artemisinins and verify their usefulness in monitoring ACT resistance.

736

GENETIC DIVERSITY OF THREE VACCINES CANDIDATES ANTIGENS IN *PLASMODIUM FALCIPARUM* ISOLATES FROM RURAL AREA IN SENEGAL

Magatte Ndiaye

University Cheikh Anta Diop, Senegal, Senegal

Genetic variation allows the malaria parasite *Plasmodium falciparum* to overcome chemotherapeutic agents, vaccines and vector control strategies and remain a leading cause of global morbidity and mortality. Several of the intended *P. falciparum* vaccine candidate antigens are highly polymorphic and could render a vaccine ineffective if their antigenic sites were not represented in the vaccine. In this study, we characterize the genetic variability of vaccine candidate antigens as msp3, ama1 and eba-175 in isolates of *P. falciparum* from Senegal. DNA analysis was completed on 111 isolates of *P. falciparum* collected from endemic area in Keur Soce, Senegal from years 2006 to 2007. Genetic diversity was determined in immunological important in merozoite surface protein-3 (MSP-3), apical membrane antigen-1 (AMA-1) and erythrocyte binding antigen (EBA-175). Alleles identified by DNA nested PCR and RFLP were analysed by Genelex 6, Arlequin v3.1 and Epi Info v6.04. The data comparisons were made using the Chi square or Fisher's exact test, and the Student's t-test and ANOVA for normally distributed continuous data with a statistical significance threshold of P< 0.05. The allele's frequencies were estimated based on the GenAlEx AFL. The genetic diversity was calculated by determining the heterozygosity of alleles detected for each antigen in each population. From 111 samples, PCR product where obtain from 70 (63,06%), 89 (80,18%%)and 80 (72,07%) respectively for ama1, msp3 and eba-175. The results showed that the eba-175 gene presented 4 different alleles [eba175F_loop (45,9%), eba175C_loop (31,1%), eba175~400bp (12,6%), eba175~360bp (6,3%)] and the alleles found had frequencies high than 5% in the respective parasite population. Regarding the msp-3 patterns, the analysis revealed the presence of three alleles MSP3_K1 (40,5%), MSP3_3D7 (47,5%) and MSP3~350bp (11,7%). For ama1 patterns, the results showed three different alleles ama-1_K1 (37%), ama-1_HB3 (30,9%), ama-1_3D7(32,1%). In conclusion, characterization of the genetic diversity in *Plasmodium* isolates from Keur Soce (Senegal) showed that *P. falciparum* in these antigens have polymorphisms more similar to Peru than to India.

737

GEOGRAPHIC STRUCTURE OF *PLASMODIUM VIVAX* IN SRI LANKA

Geethika Sharmini A. Gunawardena[1], Nadira D. Karunaweera[1], Marcelo U. Ferreira[2], G.M. Kapilananda[1], Michael Alifrangis[3], Rupika S. Rajakaruna[4], Flemming Konradsen[3], Priyanie H. Amerasinghe[5], Mette L. Schousboe[3], Gawrie N. Galappaththy[6], Rabindra R. Abeyasinghe[6], Daniel L. Hartl[7], Dyann F. Wirth[8]

[1]Department of Parasitology, Faculty of Medicine, University of Colombo, Colombo, Sri Lanka, [2]Institute of Biomedical Sciences, University of São Paulo, Brazil, São Paulo, Brazil, [3]Centre for Medical Parasitology, Department of International Health, Immunology and Microbiology, University of Copenhagen, and Department of Infectious Diseases and Department of Clinical Microbiology, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark, [4]Department of Zoology, University of Peradeniya, Peradeniya, Sri Lanka, [5]International Water Management Institute, New Delhi, India, [6]Anti-Malaria Campaign, Ministry of Health, Colombo, Sri Lanka, [7]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, United States, [8]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA, United States

Understanding the genetic diversity and population structure of *Plasmodium vivax* in Sri Lanka would be helpful in differentiating indigenous from imported parasite isolates within the country. This would enable meaningful surveillance and control strategies for the elimination program currently being conducted in the country. 190 *P. vivax* isolates collected from various locations within Sri Lanka (between 1999 and 2008 were genotyped using 14 highly polymorphic microsatellite markers. All samples were PCR-amplified and the length variations of the PCR products were measured. The single or predominant allele at each locus was considered for computing allele frequencies. The presence of more than one allele at a particular locus was interpreted as a multiple-clone infection. Genetic diversity was determined by calculating heterozygosity (*HE*) and standardized index of association (*ISA*) used to test for multilocus linkage disequilibrium. STRUCTURE software was used to test for clustering of haplotypes according to geographic and temporal origins. The parasite population was highly polymorphic with 189 unique haplotypes. The number of alleles per locus varied between 13 and 47. Almost 66% (n=125) had multiple-clone infections. Mean genetic diversity (*HE*) was 0.8747. Significant multilocus linkage disequilibrium was present (*ISA*=0.0265, *P*<0.001). The population structure revealed temporal variations and partial clustering of *P. vivax* isolates according to geographic locations. Microsatellite typing would serve as an excellent tool for surveillance of *P. vivax* malaria within Sri Lanka enabling effective strategies for control depending on the origin of the parasite.

738

GENETIC MARKERS AND RISK OF MALARIA INFECTIONS: GENETIC-EPIDEMIOLOGY STUDY IN A LOW MALARIA ENDEMIC AREA OF SRI LANKA

Nadira D. Karunaweera[1], Rajika Dewasurendra[1], Deepika Fernando[1], Patcharaporn Sereejaitham[2], Prapat Suriyaphol[2]

[1]Faculty of Medicine, University of Colombo, Colombo, Sri Lanka, [2]Mahidol University, Bangkok, Thailand

Malaria transmission is seasonal and unstable in the dry zone of Sri Lanka and the levels have steadily declined over the past 10 years. This study is part of an immuno-epidemiological study that was conducted in residents of 8 villages in the district of Moneragala, a known malaria endemic area with transmission of *P. vivax* (predominant species >80%) and *P. falciparum*. The original study was a cohort study with active case detection of 1,951 individuals during 1992/93. In year 2006, 1133 of these individuals were traced, blood samples obtained and history of malaria attacks during the past 15 years recorded. DNA was extracted from whole blood and SNPs in selected genes including those related to

immune-response in malaria were investigated. Serum was separated for serological investigations and titers of seven antibodies, AMA1, MSP1, MSP2, NANP, IgE, Pv_AMA1 and Pv_MSP1, were determined by ELISA. Antibody levels were further classified into low and high level. SNP data were analyzed in relation to past history of malaria attacks and serum antibody levels. A total of 169 SNPs were typed in 1107 study subjects. After sample and genotype quality control, 96 SNPs in 1017 study subjects were selected for analysis. Genotype frequencies in 7 SNPs in 7 genes were found to be significantly different between those who have experienced repeated malaria attacks and those with apparent protection ($p<0.05$; Chi-square test). When classified antibody levels into low-high binary trait, we found significant association in 11 SNPs for AMA1; 5 SNPs for MSP1; 9 SNPs for MSP2; 8 SNPs for NANP; 5 SNPs for Pv-MSP1; 11 SNPs for IgE; and none for Pv-AMA1. However, there was no SNP which gave significant association in all tested antibodies. Preliminary evidence is in favour of a genetic basis for susceptibility to or protection against malaria infection in this population, which may or may not have links with the generation and/or maintenance of anti-malarial antibodies, the levels of which appear to be maintained in spite of low malaria transmission levels.

739

THE APICOPLAST GENOME OF *PLASMODIUM VIVAX*

Vishal Saxena[1], Shilpi Garg[1], Dhanpat K. Kochar[2], Ashis Das[1]

[1]Birla Institute of Technology and Science, Pilani, Pilani, Rajasthan, India, [2]Kothari Medical and Research Institute, Bikaner, Rajasthan, India

Plasmodium vivax is responsible for causing greater than 60% of human malaria cases in Southeastern Asia and the Indian subcontinent. The World Health Organization has projected that of the 100 million total cases of malaria in the South East Asia Region, 70% occur in India with more than 50% (35 million) owing to *P. vivax*. The rising severity of the disease and the resistance shown by the parasite towards usual therapeutic regimen has put forth a demand for a novel drug target to combat this disease. Apicoplast, an organelle of prokaryotic origin, and its circular genome are being looked upon as a potential drug target. The Apicoplast genome is known to carry various genes of functional importance. Except for a few reports, this genome has not been detailed from *P. vivax*. Our group for the first time has reported any complete gene (*tufA*) from this genome of *P. vivax* (as reported previously). In the present study we have characterized major genes of the IR-A region and some genes of the IR-B regions of this genome. These include *ssu* and *lsu* ribosomal RNA and *tRNA* genes, *sufB*, *clpC*, genes, *RNA Polymerase B*, *C* and *D* subunit genes and various ribosomal protein genes. The Apicoplast genes were amplified and sequenced from *P. vivax* field samples. A comparative analysis of *P. vivax* Apicoplast genes with alleles from other *Plasmodium* species (especially *P. falciparum*) was performed along with codon usage pattern. About 8 - 13% differences were observed at both nucleotide and amino acid level. Peptides based on *P. vivax* Apicoplast Ef-TuA were used to colocalize the organelle in *P. vivax* infected blood smear slides obtained from the field..

740

HIGH THROUGHPUT GENOMICS SCREENING FOR MALARIA ANTIGEN DISCOVERY

Ping Chen[1], Bennett Myers[1], Elena Curti[2], Joao Aguiar[2], Keith Limbach[2], Noelle B. Patterson[2], Martha Sedegah[2], Thomas L. Richie[2], Joseph T. Bruder[1]

[1]GenVec Inc., Gaithersburg, MD, United States, [2]Naval Medical Research Center, United States Military Malaria Vaccine Program, Silver Spring, MD, United States

Malaria is the most burdensome parasitic disease of man, exacting an estimated toll of 863,000 deaths and 243 million clinical cases per year. It is important to develop a vaccine that can effectively prevent the disease. Up to now, there are few identified malaria antigens, representing less than 0.3% of the 5,300 proteins encoded by the *Plasmodium* parasite, and those have limited efficacy in vaccine clinical development,. Here, we report a new approach employing adeno-array technology for high throughput discovery of pre-erythrocytic *Plasmodium falciparum* antigens using orthologues identified in the *Plasmodium yoelii* mouse model. To obtain molecular information for highly expressed *P.yoelii* pre-erythrocytic antigen genes, we performed bioinformatics data mining using publicly available genomic and proteomic databases. Based on expression abundance data from microarray analysis and protein mass spectrometry analysis by several research groups, we prioritized sporozoite stage and liver stage candidate *P. yoelii* genes with identifiable *P. falciparum* orthologues for amplification and cloning. Amplified *P. yoelii* genes were first cloned into a shuttle plasmid, tested for appropriate insert size using colony PCR and then transferred to the pAdFlex vector, which contains the full length, E1/E3-deleted adenovirus type 5 genome. pAd*Py* adenoplasmids were then purified and transfected into 293 cells seeded in 96 well plates. After several passages, the cell lysates containing high titer Ad*Py*Antigen vectors are utilized to perform the antigen discovery screen and archived for titering and identity analysis. Currently, we are building the Py adenovector array. In the antigen discovery screen, we will infect antigen presenting cells with individual adenovectors from the Py array. The infected APC will be incubated with splenocytes from mice immunized with known protective regimens of Radiation Attenuated Sporozoites (RAS), and antigen-specific CD8+ T cell responses will be measured by the FACS analysis. Novel P. yoelii antigens identified as targets of RAS vaccination induced T cells will be presented.

741

CHARACTERIZATION OF MAL13P1.319, A *PLASMODIUM FALCIPARUM* SPOROZOITE GENE

Renee N. Roberts[1], Michael Kariuki[1], Alexis LaCrue[2], Maggie Schlarman[1], Ruguang Ou[1], Brenda Beerntsen[1]

[1]University of Missouri-Columbia, Columbia, MO, United States, [2]University of South Florida, Tampa, FL, United States

During the life cycle of *Plasmodium falciparum*, the most pathogenic of the human malaria parasites, sporozoites are infectious for both the mosquito salivary gland and vertebrate tissue. Since this dual infectivity of sporozoites is critical to the survival and development of the parasite, the sporozoite presents an effective target for control by vaccines, drug therapy, and/or novel mosquito control methods. In an effort to discover molecules that aid sporozoite invasion of host tissue, a *P. falciparum* sporozoite gene known as *MAL13P1.319* was identified by a search of the annotated *Plasmodium* genome database (PlasmoDB) using specific criteria, i.e., presence of a signal peptide sequence, expressed as a sporozoite protein as shown by mass spectrometry, and predicted to be a surface or secreted protein. The *P. falciparum* MAL13P1.319 protein demonstrates significant protein homology with other *Plasmodium spp*. and appears to be unique to *Plasmodium spp*. Transcription of *MAL13P1.319* during the sporozoite and erythrocytic stages, which was reported in PlasmoDB, was confirmed by RT-PCR and protein expression during the sporozoite and erythrocytic stages was demonstrated by immunofluorescent assays and Western blot analysis. Currently, we have transfected a MAL13P1.319-GFP construct into erythrocytic stage parasites to analyze MAL13P1.319 protein trafficking in various stages. To assess the functional role of *MAL13P1.319*, a gene disruption construct has integrated into the *MAL13P1.319* chromosomal location and a clonal population will be obtained via limiting dilution. We also have initiated a comparative study of the *P. berghei* ortholog of *MAL13P1.319* by analyzing its gene/protein expression and assessing a functional role. Overall, these studies will be used to analyze the role of *MAL13P1.319* in sporozoite biology and, more specifically, to determine if it has a role in host tissue invasion.

742

CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* PROTEIN, PFE0565W

Maggie Schlarman[1], Michael Kariuki[1], Alexis LaCrue[2], Renee Roberts[1], Ruguang Ou[1], Brenda Beerntsen[1]

[1]University of Missouri, Columbia, MO, United States, [2]University of South Florida, Tampa, FL, United States

Malaria is a resurging disease due, in part, to resistance that has developed in *Plasmodium spp.* and mosquitoes against drugs and insecticides, respectively. Because of this disease resurgence, better control methods are in great need. A key stage in the *Plasmodium* life cycle is the sporozoite because it exhibits dual infectivity in both the mosquito vector and vertebrate host and, therefore, is a promising target for discovering effective ways of controlling malaria. The *P. falciparum* gene, *PFE0565w*, was chosen as a candidate for study due to its potential role in the invasion of host tissues. This gene was selected based on data from PlasmoDB, indicating that it is expressed both at the transcriptional and protein levels in sporozoites and likely encodes a putative surface protein. Additional sequence analysis shows that the PFE0565w protein has orthologs in other *Plasmodium* species, but none outside of the genus *Plasmodium*. PFE0565w expresses transcript during both the sporozoite and erythrocytic stages of the parasite life cycle. The PFE0565w protein is expressed on the sporozoite surface, as suggested by confocal microscopy. In contrast, the protein is not expressed during the asexual stages, as demonstrated by both Western blot analysis and confocal microscopy. A GFP-trafficking construct has been made and studies are in progress to track the expression profile of the PFE0565w protein throughout the parasite's life cycle and to confirm protein expression results described above. Furthermore, both gene disruption and deletion constructs have been successfully created for PFE0565w and studies are in progress to assess the function of the protein in parasite development and to determine if it plays a role in host tissue invasion. Lastly, a comparative study between the *P. berghei* ortholog of PFE0565w, PB107985.00.0, is in progress.

743

A 7TM-RECEPTOR FAMILY MEMBER IN *PLASMODIUM* PLAYS A ROLE IN PARASITE VIRULENCE AND INFECTIVITY

Joanne Thompson, Gabriella Lindergard

University of Edinburgh, Edinburgh, United Kingdom

The genome of *Plasmodium* encodes a number of multipass-transmembrane domain proteins that bear the structural hallmarks of integral membrane receptors mediating intercellular and environmental signal transduction. One of these, PFL0765w, is a member of a phylogenetically widespread family of putative G-Protein Coupled Receptors (GPCR) found in a range of mammals, insects, plants and protozoa, including proteins with heterotrimeric Gα-protein binding activity in *Arabidopsis thaliana*. PFL0765w is expressed in gametocytes, maturing trophozoites and in schizonts adjacent to the rhoptry bulb protein, RAP1. To investigate a role in the initiation of signal transduction pathways in *Plasmodium*, we have generated PFL0765w-orthologue gene knockout lines in the rodent malaria model, *P. berghei,* and performed phenotypic analyses throughout the parasite lifecycle in the mouse and mosquito. Knockout parasites are significantly less virulent than wildtype parasites and show a marked reduction in infectivity.

744

MITOCHONDRIAL GENETIC VARIATION AND EVOLUTIONARY HISTORY IN ASIAN *PLASMODIUM VIVAX*

Miao Miao[1], Zhaoqing Yang[2], Yaming Huang[3], Ananias Alberto Escalante[4], Liwang Cui[1]

[1]Pennsylvania State University, State College, PA, United States, [2]Parasitology Department, Kunming Medical College, Kunming, Yunnan, China, [3]Malaria Department, Guangxi Centers for Disease Control and Prevention, Nanning, Guangxi, China, [4]School of Life Sciences, Arizona State University, Tempe, AZ, United States

The genetic diversity and population structure of *Plasmodium vivax* were investigated using mitochondrial (mt) genome sequences of parasite samples from China, Myanmar and Korea. Among 95 samples, 32 different haplotypes were defined by 29 polymorphic sites. Overall haplotype diversity and nucleotide diversity were 0.88 and 0.15, respectively. Coding sequence diversity was large with an average of one SNP every 193 bp. A total of 17 single nucleotide polymorphisms (SNPs) were detected in the coding region, resulting in 10 mutated codons. Minimum spanning network analysis of the resulting data, combined with those from previously published populations, revealed that the *P. vivax* population from Myanmar and temperate-zone *P. vivax* parasites from China branch might share the same ancestor with the population from India. This study provides further resolution of the population structure and evolution of *P. vivax*, especially in temperate-zone endemic areas.

745

REDUCTION OF MALARIA TRANSMISSION BY MASS TREATMENT: A COMPARISON OF OPERATIONAL STRATEGIES

Lucy C. Okell[1], Jamie Griffin[1], Immo Kleinschmidt[2], Azra C. Ghani[1]

[1]Imperial College London, London, United Kingdom, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

Mass treatment as a means to reducing malaria transmission was largely discontinued after the first Global Malaria Eradication Programme but is currently being reconsidered by several regional control programmes. In the past it has shown great variation in impact in different settings. We used a dynamic transmission model to explore both the short and long term impact of possible mass treatment strategies. Our results confirm that the expected effect of a single round of mass treatment would be temporary, assuming that 100% identification and cure of cases is highly unlikely. In a scenario of high transmission with moderately seasonal vector populations and initial mean slide prevalence of 60%, slide prevalence is predicted to return to its original level within 1 year. Where slide prevalence is initially at a mean of 5% this is estimated to take ~2 years. Using gametocytocidal drugs could give some advantage, based on artemisinin and primaquine impact on infectiousness observed in clinical trials, however the reduction in transmission was limited by the reservoir of infection in those not participating in the intervention. Screening for infection and treating only test-positives would reduce numbers of treatment courses required by 70-95% in low to moderate transmission settings, but was estimated to achieve only 60-75% of the cumulative impact on transmission, mainly due to lack of prophylaxis in test-negatives. Annual mass treatment could achieve a 20-60% reduction in mean slide prevalence if sustained but was not predicted to eliminate malaria unless initial transmission levels were very low. Intense, fortnightly mass treatment for a period of 2-3 months has a limited probability of achieving local elimination in low-to-moderate transmission settings, however it would be endangered by repeated non-participation of individuals and by immigration. Increasing vector control would delay and reduce the reinvasion of the population by the parasite. The transmission reduction achieved by mass treatment needs to be carefully weighed against the enhanced risk of drug resistance.

746

EFFECTS OF ANAEMIA ON THE EMERGENCE, CLEARANCE AND SEX RATIOS OF *PLASMODIUM FALCIPARUM* GAMETOCYTES IN MALARIOUS CHILDREN

Akintunde Sowunmi, Titilope M. Okuboyejo, Grace O. Gbotosho, Christian T. Happi, Obaro S. Michael, Onikepe A. Folarin

Department of Pharmacology and Therapeutics and Institute for Medical Research and Training, University of Ibadan, Ibadan, Nigeria

Anemia, in *falciparum* malaria, is associated with an increased risk of gametocyte carriage but its effects on transmission have been little evaluated in malarious children. The effects of anemia, defined as a haematocrit < 25%, on the emergence, clearance, population structure, inbreeding rates, and the temporal changes in *Plasmodium falciparum* gametocyte sex ratios were evaluated in 802 children with acute infections treated with artemisinin-based combination therapies (ACTs). Gametocyte sex was determined morphologically, and sex ratio was defined as the proportion of gametocytes that are male. Pre-treatment gametocyte carriage in all children was 8.5% (68/802) and was similar in children with or without anemia (9.4% v 8.3%). Following treatment, the emergence of gametocytes seven days after treatment began was significantly more frequent in anaemic children (7/106 v 10/696, P = 0.002), but gametocyte clearance was similar (2.1 d v 2.4 d). Pre-treatment sex ratio (0.36, 95%CI 0.1- 0.65 v 0.25, 95% CI 0.15-0.35, P = 0.5) was similar but estimated inbreeding rates (the proportion of a mother's daughters that is fertilized by her sons) was lower (0.28 v 0.50) in anaemic children. Pre-treatment sex ratio became more female-biased in non-anaemic children following treatment but in anaemic children, it became male-biased. Sex ratio 3 d after treatment began was significantly lower and more female-biased in non-anaemic children (P = 0.027). Anemia significantly increases gametocyte emergence and may significantly alter the sex ratio after treatment with ACTs. These findings may have implications for malaria control efforts in endemic settings.

747

INCREASED AND SUSTAINED RESOURCE MOBILIZATION PREVENTION USING COMMUNITY HEALTH WORKERS (CHW'S) AS KEY INTERVENTION AGENTS IN THE DISTRIBUTION OF INSECTICIDE TREATED NETS (ITNS)

Kafula Silumbe[1], Cecilia N. Katebe[2]

[1]MACEPA/PATH, Lusaka, Zambia, [2]National Malaria Control Center, Ministry of Health, Lusaka, Zambia

In support of Zambia's national malaria control efforts, there was substantial national and external donor investment beginning in 2004 and increasing through 2007. However, for the past 24 months, there have been markedly reduced disbursements from Global Fund resources for malaria. We examined potential consequences of this growth followed by reduction in resources using ITN procurement and distribution systems as a marker for change. We examined national and donor financing for ITN procurement between 2005 and 2009 and examined ITN distribution and coverage and changes over time across the 72 districts in the 9 provinces of Zambia. Donor financing specifically applied to ITN procurement and distribution increased annually between 2004 through 2007 due largely to substantial investments from Global Fund, World Bank, MACEPA, and United States Agency for International Development&PMI. In 2008 and 2009, GF resources for ITNs decreased dramatically; World Bank resources for ITNs were about 5 million dollars overall, ITN distribution varied substantially on an annual basis (from 1,560 000 distributed in 2005, 1,399,000, 3,453.414 in 2007, 964553 in 2008 and 1,396,347 distributed in 2009). With an estimated 3-year life span of ITNs, if current financing for ITNs is maintained at the 2008/9 level, coverage rates ie actual use of ITNs will drop nationally by 15% from 48% to 33% by 2012. Similarly, assuming stable usage rates in households, only 4% of children under-5 years of age will be using ITNs in 2012. In conclusion, malaria control in Zambia, like many other African countries, is currently heavily reliant on stable financing and procurement and delivery to scale up ITN coverage and maintain that prevention. Recent declines in available resources may have dramatically altered the recent progress in Zambia.

748

IMPACT OF ARTEMETHER-LUMEFANTRINE ON MALARIA TRANSMISSION AND UNDER FIVE MORTALITY IN TWO RURAL DISTRICTS OF TANZANIA

Abdunoor M. Kabanywanyi[1], Mathew M. Alexander[2], Aggrey Malila[2], Raymond Schlienger[3], Christian Lengeler[4], Blaise Genton[4]

[1]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [2]Ifakara Health Institute, Ifakara, United Republic of Tanzania, [3]Novartis Pharma AG, Basel, Switzerland, [4]Swiss Tropical and Public Health Institute, Basel, Switzerland

Deployment of artemisinin-based combination therapy (ACT) with other malaria control measures is key to reduce malaria transmission and overall under five mortality. To date, there is scanty evidence about the contribution of ACTs such as artemether-lumefantrine (AL) in malaria endemic Africa. As part of the ALIVE [Artemether-Lumefantrine In Vulnerable patients: Exploring health Impact] project, we assessed the impact of the introduction of AL as first line treatment for uncomplicated malaria on parasite prevalence, anaemia and under five mortality. Parasite and anaemia prevalence were obtained by repeated cross-sectional surveys conducted in two rural districts (Kilombero and Ulanga) in Tanzania during the sulfadoxine-pyrimethamine (SP) era (2005 & 2006), and after AL introduction beginning in 2008 i.e. 18 months after AL introduction. Mortality rates were obtained using a Demographic Surveillance System (DSS) that covers a dynamic expanding population of about 90,000 in the same districts. Mortality rates of children under five were compared to rates obtained 2 years during and post the SP era (2007 & 2008).

A total of 5903 persons were assessed in 2005, 6324 in 2006, 4557 in 2008 and 7454 in 2009. Asymptomatic parasite prevalence in the whole population was 11.4% in 2005, 13.6% in 2006, 11.0% in 2008 and 4.6% in 2009. Gametocyte carriage rates were 0.3% in 2005, 0.2% in 2006, 1.4% in 2008 and 0.4% in 2009. Prevalence of anaemia in children under five was 17.8% in 2005, 9.7% in 2006, 10.1% in 2008 and 10.9% in 2009. Population coverage with insecticide-treated bednets was 35%, 36%, 44% and 47% respectively. Under five mortality rate per 1000 person-years was 27.0 in 2005, 23.1 in 2006, 21.3 in 2007 and 18 in 2008. After 3 years of AL implementation, there was a considerable decline in parasite prevalence but no change in anaemia prevalence. On average gametocyte carriage rate has remained < 1% throughout the period. Mortality in children <5 years decreased, but trend was consistent with pre- and post-AL introduction.

749

COVERAGE OF INSECTICIDE TREATED NETS AND INTERMITTENT PREVENTIVE THERAPY FOR THE CONTROL OF MALARIA IN PREGNANCY IN SUB-SAHARAN AFRICA: MAPPING PROGRESS

Anna M. van Eijk[1], Jenny Hill[1], Victor A. Alegana[2], Viola Kirui[2], Peter W. Gething[3], Feiko O. Ter Kuile[1], Robert W. Snow[2]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Malaria Public Health and Epidemiology Group, Centre for Geographic Medicine, KEMRI, University of Oxford, Wellcome Trust Collaborative Programme, Nairobi, Kenya, [3]Spatial Ecology and Epidemiology Group, Tinbergen Building, Department of Zoology, University of Oxford, Oxford, United Kingdom

Over the past 10 years, policies for intermittent preventive treatment (IPTp) with sulfadoxine-pyrimethamine (SP) and use of insecticide treated nets (ITNs) for the control of malaria in pregnant women have been almost

universally adopted in sub-Saharan African countries. Both ITNs and IPTp are delivered through antenatal clinics alongside other antenatal care packages. We assessed progress in adoption and implementation of the ITN and IPTp strategies in Africa using information obtained from national surveys (DHS, MICS, MIS, and other). This was linked with estimates of malaria transmission maps obtained through Malaria Atlas Project. ITN use among women 15-49 years was used as proxy measure for ITN use among pregnant women because of the greater availability and the excellent correlation (Pearson 0.94). Results are presented at the subnational level (admin1). ITN policies for pregnant women could be identified for 45 of 47 malarious countries in sub-Saharan Africa; the median year of adoption was 2002 (range 1998-2007). Data from 32 surveys between 2004 and 2009 showed that the median reported ITN use among women aged 15-49 years was 13.8% (interquartile range [IQR] 4.9%-26.8%, n=286). Only 7 regions had ITN coverage of >=60%; all in countries that adopted ITNs for pregnant women >5 years ago (P=0.04) and in areas with a mean *Plasmodium falciparum* (Pf) prevalence among children 2-9 yrs of age between 10-49% (2007). Thirty nine countries have adopted IPTp (median year of adoption 2004, range 1993-2007). The median IPTp coverage (any source, any number of doses) was 17.0% (IQR 4.6-74.3%, n= 282 regions) in 36 countries that had an IPTp policy in place for ≥1 year at the time of survey; 49 regions from 9 countries had a coverage of ≥60%, 42 of these (85.7%) were in areas with a mean Pf prevalence among children 2-9 yrs (2007) of 10% or more, and 25 of them were in countries which had adopted IPTp >5 years ago (P<0.001). The median use of any drug for malaria prevention was 55.6 % (IQR 37.0-73.9%, 281 admin1 from 31 countries), and the median coverage of ANC (≥1 visit) was 88.1% (IQR 66.5-95.3%, n=342 admin1, 39 countries). In conclusion, ITN coverage is still below the Abuja target for many countries in sub-Saharan Africa. Considerable progress has been made for IPTp. The high utilisation of ANC and of use of drugs for malaria prevention in pregnancy indicates there is significant potential to improve malaria prevention among pregnant women.

750

IMPACT OF INTERMITTENT PREVENTIVE TREATMENT IN INFANTS WITH SULFADOXINE-PYRIMETHAMINE ON MORTALITY IN THE DISTRICT OF KOLOKANI, MALI

Alassane Dicko[1], Moussa Konare[1], Djibril Traore[1], Souleymane Diarra[1], Abdoulbaki I. Diallo[1], Ogobara K. Doumbo[1], Christophe Rogier[2]

[1]MRTC/FMPOS/University of Bamako, Bamako, Mali, [2]Institut de recherche biomédicale des armées-IRBA, Marseille, France

Intermittent Preventive Treatment in infants (IPTi) with Sulfadoxine-Pyrimethamine (SP) given during routine vaccinations is efficacious in preventing malaria disease and anemia. However the impact on mortality remained to be established. To evaluate the impact of IPTi- SP on the mortality, the 22 health sub-districts in the district of Kolokani, Mali, were randomized in a 1:1 ratio and starting in December 2006, IPTi - SP was implemented for 12 months in 11 health sub-districts (intervention zone) while the other 11 health sub-districts served as the control (non-intervention zone). A cross-sectional survey was conducted in 98 randomly selected locations in March-April 2009 to determine mortality in children in the target age group during the implementation period (Dec 2006-2007) in both intervention and non-intervention zones. Causes of death were assessed using a post-mortem questionnaire. A total of 3,122 children (1,556 in each zone) were surveyed. Preliminary results indicated that during the intervention period, there were 79 deaths in the intervention zone (mortality = 5.04 %, 95% CI of 3.89% - 6.20%) and 109 in the non intervention zone (mortality = 7.01%, 95% CI 5.62% -8.40%), giving a protective efficacy against all cause mortality of 27.5% (95% CI: 2.3%-46.4% (p= 0.035). The differences in disease specific mortalities between the two zones were not statistically significant. In conclusion, this study shows significant reduction in overall mortality in IPTi intervention zone compared to the control zone during the IPTi implementation period and supports the introduction of IPTi-SP alongside other malaria control interventions.

751

SOCIAL AND ENVIRONMENTAL DETERMINANTS OF CHILDHOOD MALARIA AND THE USE OF ITN: IMPLICATIONS FOR MALARIA CONTROL STRATEGIES IN THE DEMOCRATIC REPUBLIC OF CONGO

Olufunke A. Alaba[1], Gauthier Tshiswaka-Kashalala[2]

[1]University of Cape Town, Cape Town, South Africa, [2]University of Pretoria, Pretoria, South Africa

Various malaria control strategies have been applied overtime in countries, however, without taking into cognizance the environmental and social context of a country, these strategies may be difficult to achieve one of its intended goal; reduction of childhood malaria incidence. We therefore, want to examine social and environmental determinants associated with childhood malaria and the use of ITN in the Democratic Republic of Congo. Social and Environmental factors can negatively or positively influence individual's decisions and functions taken on behalf of children which may eventually affect the effectiveness of the various strategies to combat this scourging disease by the government. The Democratic Republic of Congo is just recovering from years of war and to combat malaria which is one of the biggest health treat in the country, the government has started with various control strategies, including appropriate case management in both community and health infrastructures, and scaling up the use of insecticide treated nets (ITNs). However, almost 47.3% of the children die annually from malaria. The analyses for this study will be based on the 2007 Demographic Health Survey of the Democratic Republic of Congo. Finding relevant literatures that look into the association of disaster/war with malaria control strategies are very sparse. Therefore, the findings of this study are expected to significantly augment knowledge on what influences malaria control strategies in a war-torn environment.

752

APPRAISING EFFECTS OF THE SUPPLY CHAIN OPERATIONS OF THE LONG-LASTING INSECTICIDAL NETS (LLINS) MASS CAMPAIGNS IN NIGERIA ON OWNERSHIP AND USAGE

Chukwuyem Okoh[1], Anne Ikwang[1], Ndukwe K. Ukoha[1], Osuolale Adekunle[2]

[1]United States Agency for International Development | DELIVER PROJECT, Abuja, Nigeria, [2]National Malaria Control Program, Abuja, Nigeria

Widespread use of long-lasting insecticidal nets (LLINs) within a community is a proven intervention for malaria control. Therefore, a widespread effort is being made to distribute 63 million LLINs in Nigeria, which will achieve universal coverage, before the end of 2010_a country that shoulders 25 percent of the Africa malaria burden. Between May 2009 and April 2010, the National Malaria Control Program, with support from various partners, procured and distributed 18.5 million LLINs in 11 states (representing over 30% of the country's total population). Despite rapidly increasing availability through mass campaigns, studies indicate that fewer than 70% of households receive the nets, while only 50.3% fulfil measures of *universal coverage*; the use rate is approximately 61.5%. Setbacks with other initiatives similar to these_which have been linked to a number of factors, including supply chain constraints at various levels_are also evident in other malaria interventions. To understand how the beneficiaries perceive the process and to understand the logistics environment in which the activities take place, a qualitative appraisal of implementation strategies is underway for the supply chain operations of the LLINs universal coverage campaigns in Nigeria. Thee are preliminary findings. Although most of the households are satisfied with the distance they have to travel to the distribution points, they have major concerns

about crowd control, waiting time to collect the bed nets, and the attitude of the personnel. The recipients also question the effectiveness of the nets. Currently, there is no specific strategy to conclusively address beneficiaries' complaints about acquisition and use of the LLINs. Even though the policies and guidelines are strong enough, program managers find it difficult to determine what needs to be done and to respond quickly to unforeseen challenges, which are frequently part of campaign activities. To address these constraints, program managers and the Roll Back Malaria partners are developing the necessary framework to deal with these issues and to improve LLIN ownership and use at the household level.

753

THE MALAWI NATIONAL MALARIA CONTROL PROGRAM'S "YEAR OF ACTION-2010": GAUGING PROGRESS TOWARD MALARIA CONTROL

Doreen Ali[1], Don Mathanga[2], Wilfred Dodoli[3]

[1]National Malaria Control Program, Lilongwe, Malawi, [2]Malaria Alert Centre, Blantyre, Malawi, [3]World Health Organization, Lilongwe, Malawi

Malawi is receiving support from the President's Malaria Initiative and the Global Fund to implement its National Strategic Plan (2005-2010). The primary strategic areas identified for the scale-up of malaria control activities include case management, intermittent preventive treatment of pregnant women with SP (IPTp) and malaria prevention with emphasis on the use of insecticide treated nets.

UNICEF's Multiple Indicator Cluster Survey in 2006 is the most recent nationally representative evaluation that has served as a baseline of select malaria control activities before increased resources were made available. In this "year of action," NMCP is undertaking an RBM supported Malaria Program Review, a study evaluating artemether-lumefantrine efficacy and the nation's first ever Malaria Indicator Survey (MIS) to help inform the new strategic plan. A recent household survey completed in 8 of Malawi's 28 districts in 2009 has revealed interval improvement since the MICS results. Select indicators document that net usage in children under 5 increased to 61% from 23% in 2006 and coverage with 2 doses of IPTp reached 72% compared to 46% of women in 2006. Malawi's 2010 MIS is underway. Fieldwork and data collection was completed in April 2010. Children under 5 from 3500 households were interviewed. Preliminary results will be available in June 2010.

754

REDUCTION IN ANEMIA IN CHILDREN UNDER TEN YEARS OF AGE AFTER DISTRIBUTION OF LONG-LASTING INSECTICIDAL NETS (LLIN) FOR CONTROL OF MALARIA AND LYMPHATIC FILARIASIS IN FOUR LOCAL GOVERNMENT AREAS (LGAS) IN SOUTHEAST NIGERIA

Patricia M. Graves[1], Emmanuel Emukah[2], Aryc W. Mosher[1], Jeremiah Ngondi[1], Emmanuel Miri[3], Obiezu Josephine[2], Okpala T. Njideka[2], Njoku Chidi[2], Nwordu Kenrick[2], Obasi Andrew[2], Frank O. Richards[1]

[1]The Carter Center, Atlanta, GA, United States, [2]The Carter Center, Owerri, Nigeria, [3]The Carter Center, Jos, Nigeria

In an integrated filariasis and malaria project, LLIN were distributed to all households in 4 LGAs in two states in South East Nigeria starting in April 2008. Two LGAs received LLIN for vulnerable persons (pregnant women and under fives) only while the others were targeted for full coverage.

At annual representative household surveys, household net ownership increased from a mean of 0.1 nets per household in 2007 (N=968) to 1.2 in 2008 (N=1078). However by 2009 net ownership had dropped to 0.8 per household (N=1294). Net use (all ages) showed a similar pattern, increasing from 2.0% of persons (N=5197) sleeping under nets in 2007 to 35.5% in 2008 (N=5200) but dropping to 21.4% in 2009 (N=5200). A higher proportion of children under five slept under nets in the full coverage arm (61.0%, 95% CI 50.7_70.4%) than the vulnerable arm (24.8%, 95% CI 18.2_ 32.8%) in 2008. Prevalence of malaria (any species) by blood slide declined significantly between 2007 and 2008: from 22.2% (N=1093; 95% CI 19.3-25.5%) in 2007 to 12.6% (N=1446; 9.7-16.2) in 2008. Within study arms, the decrease in malaria prevalence was significant only in the full coverage LLIN arm (22.3% (N=552) to 10.1% (N=681), $p<0.001$) but not in the vulnerable group arm (22.1% (N=541) to 14.9% (N=765), $p=0.79$). The mean hemoglobin (Hb) in children under 10 increased significantly in both study arms, from 9.5 g/dl (N=453; 95% CI 9.2 - 9.8) overall in 2007 to 10.6 g/dl(N=629; 10.4 - 10.8) in 2008; it remained higher at 10.3 g/dl (N=704; 10.1-10.5) in 2009. The proportion of children with moderate to severe anemia (defined as Hb<8 g/dl) declined significantly from 15.1% (95% CI 10.7-21.0) in 2007 to 2.3% (1.0% to 5.0%) in 2008 and 7.9% (5.8-10.6) in 2009. The difficulty of sustaining LLIN programs after initial increase is demonstrated by a drop in net ownership and use in the third year of the study. Nevertheless the results show that community-wide distribution of LLIN (as compared to only targeting vulnerable groups) is the best way to protect children in the study area from malaria and anemia.

755

ZEROVECTOR® DURABLE LINING (DL) - A PROGRAM EXPENDITURE MODEL

John H. Thomas[1], Johan den Arend[2], Thomas D. Soerensen[2]

[1]DART S.A., Bridgewater, NJ, United States, [2]Vestergaard Frandsen S.A., Lausanne, Switzerland

Community level malaria protection requires coverage and usage of an effective tool by >80% households. Durable Lining (DL) is an innovative technology for disease prevention that reduces vector densities and breaks transmission cycles. The product has residual efficacy that meets international standards for effectiveness, shown after two years of field use in Nigeria. Durable Lining (DL) may eliminate future need for IRS in rural communities. Measurements of room sizes, labor and installation activities, material and logistical requirements were collected from trials conducted in Mali, Ghana, and South Africa in 2008/09. The data were used to analyze prospective costs associated with an installation project for DL. The cost analysis was converted to a program where estimates for the various program components could be entered and an expenditure budget modeled. The extent of accuracy that the model is capable of providing is determined by the level of detail used for input. General information about project scale and timing can result in a cost estimate sufficient for project planning. When details about exact number of rooms where DL is to be installed, distances between houses and villages where installation is to occur, specific dates for the project start and completion, wage rates, and fuel costs are entered the model can produce a budget that can be used for program pricing considerations. An additional feature is that similar data for indoor residual spray programs (IRS) can be entered and a comparison between the options produced. The complexities of planning, preparing, implementing, and maintaining Durable Lining are incorporated into a model that estimates program expenditures and total budget requirements for an installation project. Expenditure components in the model include materials, delivery, training, labor, transportation, administration, monitoring, and community awareness activities (IEC). The model can be used to make direct comparisons with cost of alternative vector control options such as IRS.

756

PROXIMITY TO HEALTH SERVICES AND GEOGRAPHIC FEATURES DETERMINE INSECTICIDE TREATED NET USE AMONG 5-30-MONTH OLD CHILDREN IN MALAWI

Peter S. Larson[1], Mark L. Wilson[1], Don P. Mathanga[2], Carl H. Campbell[3]

[1]University of Michigan, Ann Arbor, MI, United States, [2]College of Medicine University of Malawi, Blantyre, Malawi, [3]Department of Community Health College of Medicine University of Malawi, Blantyre, Malawi

Malaria prevention with insecticide-treated nets (ITNs) depends on both their availability and proper use. Particularly in rural areas, distance to ITN providers may impede prevention efforts. We undertook a large-scale, household-level, population-based survey to determine ITN use and malaria intensity among 5-30 month old children throughout Malawi during May 2007. In addition to questions involving demographic and environmental risk, child ITN use during the preceding 24 hours was determined. Traditional multivariable statistical methods and propensity analysis were used to evaluate how proximity to health services was associated with ITN access and use. Rates of reported ITN use were negatively associated with distances to nearest health facility, both for the shortest linear, "crow-fly" distance and for travel by road. "Hockey-stick" regression indicated that probability of ITN use declined as distance to facility increased to a breakpoint of 2.1 km ("crow-fly") and 4.7 km (road distance). Among the most proximal 20% of households, ~65% reported using ITNs in the past 24 hrs. Beyond the breakpoint, only ~40% of households reported ITN use. Access to material resources appeared to have a confounding relationship with facility distance, but ITN patterns remained similar even after controlling for material wealth. Propensity analysis was used to estimate direct effects of health facility access using 2.1 and 4.7 km as proxy measures for accessibility. After controlling for all factors that might predispose households to be located beyond these breakpoints, reported ITN use was significantly lower (OR = 0.81) compared to those living closer to health facilities. Our findings demonstrate that proximity to health services was strongly correlated with reported ITN use. This could indicate that access not only results in more available health resources, but also may reinforce health behaviors. We suggest that equitable and regular access to health services would reduce the burden of malaria, and hence should be a priority for health policy makers.

757

CAUDAL CONTROLS VECTOR COMPETENCE FOR *PLASMODIUM FALCIPARUM* AS A REGULATOR OF THE TRIPARTITE INTERACTIONS BETWEEN THE INNATE IMMUNE SYSTEM, THE MICROBIOTA AND THE MALARIA PARASITE

April M. Clayton[1], Suchismita Das[1], Musapa Mulenga[2], Stefanie Trop[1], Chris Cirimotich[1], George Dimopoulos[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Malaria Institute at Macha, Macha, Zambia

Anopheles gambiae, the major vector for the human malaria parasite *Plasmodium falciparum* in sub-Saharan Africa, uses its innate immune system to defend against *Plasmodium,* mainly via the Toll and Imd (Immune Deficiency) signaling pathways. Interestingly, these immune pathways are also activated by the microbiota present in the mosquito midgut, which is the primary site for *Plasmodium* invasion and development (as reported previously). *Caudal* was first identified in *Drosophila* as a developmental transcription factor as well as a negative regulator of the Imd pathway -mediated activation of the Relish transcription factor (as reported previously). We have shown through RNAi-based silencing assays that depletion of the *An. gambiae Caudal* results in a significant reduction of the midgut microbiota as well as a change of its species composition. Interestingly, *Caudal* is also a highly potent regulator of vector competence for *P. falciparum* while its implication in the defense against the rodent parasite *P. berghei* was weak. Our previous studies have also shown that the Imd pathway more efficiently defends against *P. falciparum* than *P. berghei* (as reported previously). These findings suggest that the *An. gambiae Caudal* controls the finely tuned tripartite interactions between the innate immune system, the midgut microbiota, and the *Plasmodium* parasite as a factor of the Imd pathway. We are currently conducting comprehensive whole-genome microarray studies to better understand *Caudal's* relationship to the Imd and Toll pathways and to identify potent anti-*Plasmodium* effectors that are transcriptionally controlled by this immune regulator. We also present studies on *Caudal*'s role in regulating the midgut microbial load and composition in field-derived *Anopheles arabiensis* mosquitoes, a key vector of malaria in southern Zambia.

758

REACTIVE OXYGEN SPECIES-DEPENDENT CELL SIGNALING REGULATES THE MOSQUITO IMMUNE RESPONSE TO *PLASMODIUM FALCIPARUM*

Win Surachetpong, Nazzy Pakpour, Kong Chueng, Shirley Luckhart

University of California Davis, Davis, CA, United States

Plasmodium parasites undergo a series of complicated transformations inside the mosquito vector during which they experience significant losses. This is due in part to the mosquito innate immune response, yet the details of the cell signaling pathways that regulate this response are poorly understood. We have previously shown that provision of human insulin in a blood meal leads to increased reactive oxygen species (ROS) and decreased antioxidant production in the mosquito midgut. ROS have been implicated in direct killing of pathogens, increased tissue damage, and regulation of immune signaling pathways; all of which could affect the establishment of *Plasmodium* infection in mosquitoes. Here, we demonstrate that provision of human insulin in *P. falciparum* infected blood meals fed to *Anopheles stephensi* resulted in a trend towards increased parasite development. The addition of antioxidant significantly decreased parasite numbers in insulin-fed mosquitoes, suggesting that insulin-induced ROS are involved in the establishment of parasite infection. Our data suggest that the effect of human insulin on parasite development is not a result of ROS-induced parasite killing or tissue damage. Rather, our studies demonstrate a role for ROS in mosquito cell signaling. ROS scavenging by antioxidants resulted in decreased phosphorylation of downstream effectors of the MAPK and PI3K/Akt signaling pathways in mosquito cells. Although ROS are required for signaling downstream of insulin stimulation, scavenging of ROS had no effect on TGF-beta1-dependent MAPK activation. Furthermore, we found that ROS alone can directly activate both the MAPK and PI3K/Akt signaling pathways in mosquito cells. Taken together, these data highlight a novel and specific role for ROS as mediators of *A. stephensi* cell signaling processes that are involved in the innate immune response to *Plasmodium* parasites.

759

SEMINAL FLUID PROTEIN IDENTIFICATION AND POTENTIAL FUNCTIONS IN THE DENGUE VECTOR, *AEDES AEGYPTI*

Laura C. Harrington, Laura K. Sirot, Melissa C. Hardstone, Michelle E. Helinski, Mari Kimura, Prasit Deewathanawong, Mariana F. Wolfner

Cornell University, Ithaca, NY, United States

New tools that target mosquito control may reduce vector populations and break the cycle of dengue transmission. Male mosquito seminal fluid proteins (Sfps) are one such target since these proteins, in aggregate, modulate the reproduction and feeding patterns of the dengue vector, Ae. aegypti. We identified 95 proteins of *Aedes aegypti* ejaculate that are transferred to females during mating. Using a stable isotope labeling

method, we identified sperm proteins and Sfps transferred from males to females. Sperm proteins were distinguished from Sfps by comparing the transferred proteins to sperm-enriched samples we analyzed from testes or seminal vesicles. We identified and confirmed transfer to females during mating for 56 Sfps and 39 predicted sperm proteins. The Sfp classes detected suggest roles in sperm fertility and protection from oxidative stress, semen coagulation, ecdysteroidogenesis, and protein activation/inactivation. Many of the *Ae. aegypti* predicted sperm proteins were homologous to Drosophila melanogaster sperm proteins, suggesting conservation of their sperm-related function across Diptera. This is the first study to directly demonstrate transfer of seminal fluid proteins from male *Ae. aegypti* to females.

760

CAN HUMAN INSULIN AND IGF-1 SURVIVE THE MOSQUITO MIDGUT?

Andrew Nuss, Mark R. Brown

The University of Georgia, Athens, GA, United States

Insulin and insulin-like growth factors (IGF) present in a mosquito blood meal have insect homologues, which opens the possibility of cross-talk between vertebrate hormones and insect receptors. Bovine insulin, for instance, has been demonstrated to stimulate ovaries of *Aedes aegypti* to produce ecdysteroids, as reported previously. Insulin signaling is relevant to disease transmission as activation of the insulin/insulin-like growth factor signaling pathway affects mosquito longevity and therefore the amount of time an infected mosquito can vector pathogens, as reported previously. In this study, the fate of human insulin and IGF-1 was investigated to determine their viability as signaling molecules after ingestion by *Anopheles stephensi*. Female *An. stephensi* were fed washed red blood cells spiked with physiological concentrations of radiolabeled insulin or IGF-1 through artificial feeders. Mosquitoes were dissected every 6 h for 48 h and autoradiography was used to estimate the amount of intact insulin or IGF-1 present in the midgut and hemolymph over time. Insulin and IGF-1 persisted intact in the midgut for up to 18 h. Intact insulin and IGF-1 were also detected in hemolymph samples showing that these molecules can cross the midgut and exist as viable signaling molecules within the mosquito.

761

TISSUE DISTRIBUTION, BIOACTIVITY AND SIGNALING OF OVARY ECDYSTEROIDOGENIC HORMONE IN THE YELLOW FEVER MOSQUITO, *AEDES AEGYPTI*

Animesh Dhara, Jai Hoon Eum, Michael R. Strand, Mark R. Brown

University of Georgia, Athens, GA, United States

Egg maturation in female *Aedes aegypti* is dependent on the release of neuropeptides from the medial neurosecretory cells in the female brain after blood ingestion. Ovary ecdysteroidogenic hormone (OEH) is one such peptide which shows gonadotropic activity when injected in blood-fed decapitated females and ecdysteroidogenic activity when incubated with dissected ovaries *in vitro*. A previous molecular characterization of *Ae. aegypti* OEH focused on a truncated version of the full length protein. In this study, we compare the bioactivity of short and long forms of OEH and determine the tissue distribution of OEH transcript and peptide in all life stages and during a gonadotropic cycle. In addition, we seek to identify a receptor or binding protein for OEH and determine whether there is any interaction between OEH and insulin-like peptides, also known to stimulate egg maturation. Insight into this endocrine cascade may lead to novel controls for mosquito reproduction.

762

ROLE OF INSULIN SIGNALING IN BLOOD MEAL NUTRIENTS STORAGE AND VITELLOGENESIS IN *AEDES AEGYPTI*

Monika Gulia, Micheal R. Strand, Mark R. Brown

University of Georgia, Athens, GA, United States

Reproduction in mosquitoes encompasses a highly regulated sequence of behavioral, metabolic, and synthetic processes that result in the production of eggs. As in all other animals, peptide hormones bind to cell receptors that activate signal transduction pathways to provide precise regulation of these physiological processes. In vertebrates, insulins are important growth factors and multifunctional regulators of metabolism. However, insulin like peptides (ILPs) are not restricted to vertebrates, but have also been identified in invertebrates including mosquitoes. Earlier studies from mosquitoes indicated that blood feeding stimulates egg development by triggering the release of peptide hormones from neurosecretory cells in mosquito brain. It has also been demonstrated that at least one ILP in *Aedes aegypti* regulates carbohydrate and lipid stores in the same way as insulin in vertebrates. An insulin receptor (IR) has also been identified from several mosquito species including yellow fever mosquito, *Ae. aegypti.* Knock down of IR expression by RNAi decreased metabolic stores and yolk synthesis in blood fed mosquitoes. Our results indicate that insulin signaling regulates mosquito reproduction and metabolism.

763

ANOPHELES GAMBIAE: MULTIPLE IMMUNE SURVEILLANCE MECHANISMS FOR GUT COMMENSAL MICROBES

Phanidhar Kukutla, Matthew Steritz, David Louis, Monica Baldauf, Celeste Alvarez, Ying Wang, Jiannong Xu

New Mexico State University, Las Cruces, NM, United States

All life stages of mosquitoes are in close association with commensal bacteria. This relationship plays a prominent role in the regulation of the mosquito's basal immunity. In an attempt to understand how mosquitoes cope with the gut commensal microbes, we monitored the gut expression of antimicrobial peptide (AMP) genes *Defensin 1, Defensin 4,* and *dual oxidase (Duox)* that mediates the reactive oxygen species (ROS) immunity as well as immune regulators *Caudal* and *PGRP-LB*. *Def 1* transcription is mediated by NF-κB (IMD and/or Toll pathways), and Caudal can reduce the NF-κB activity by occupying the NF-κB site at the promoter of an AMP gene. In the gut *Def1* and *Caudal* showed opposite transcription pattern, suggesting a balanced NF-κB mediated AMP production. *Def 4* transcription is regulated by an unknown mechanism. Gut *Def 4* and *Duox* showed a similar expression pattern throughout the life-stages of the mosquito, which suggests that they both are required to maintain the gut homeostasis. *PGRP-LB* is constitutively expressed throughout all stages. As an amidase PGRP-LB reduces the peptidoglycan load to ensure an appropriate level of IMD signaling to the commensal habitants in the gut. Our data suggest the gut homeostasis is maintained by multiple immune surveillance mechanisms.

764

GENERAL PRINCIPLES AND NOVEL POSSIBILITIES FOR SINGLE-CONSTRUCT GENE DRIVE

John M. Marshall, Bruce A. Hay

California Institute of Technology, Pasadena, CA, United States

Mosquito-borne diseases such as malaria and dengue fever continue to pose a major health problem through much of the world. Several new approaches to disease control utilize gene drive systems to spread refractory genes into mosquito populations. Recently proposed gene drive systems include Medea, homing endonuclease genes and underdominance constructs. Through mathematical analysis of the population genetics of single-construct systems, we show that the gene

drive systems currently under consideration only represent a fraction of the full range of possibilities. We summarize the conditions that must be satisfied for a single-construct system to spread to fixation in a population or to induce a population crash. We also describe basic properties of the release threshold, above which a gene drive system is expected to spread into a population. Both autosomal and X-linked constructs are considered. Many of these hypothetical gene drive systems are prohibitively difficult to engineer; however several can be engineered with simple combinations of toxins and antidotes. We highlight a number of novel possibilities including inverse Medea constructs, which consist of a zygotic toxin linked to a maternal antidote; and Semele constructs, which encode semen-based lethality for which transgenic females possess an antidote. Implications for disease control are discussed.

765

THE REGULATORY ROLE OF VITELLOGENIN PROMOTER DRIVEN *ANOPHELES GAMBIAE* NF-KB REL2 TRANSGENE IN THE DEFENSE AGAINST *PLASMODIUM* PARASITES AND OTHER MICROBES

Suchismita Das, Yuemei Dong, Jayme Souza-Neto, Chris Cirimotich, George Dimopoulos

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

The innate immunity of mosquitoes is the primary line of defense against the malaria parasite *Plasmodium* and other microbes. It mainly comprises of the TOLL and IMD pathways where the two NF-kappa B-like transcription factors, Rel1 and Rel2 translocates to the nucleus and activates the transcription of several antimicrobial peptides and many other effector genes. IMD pathway is the major player in regulating resistance of several *Anopheles* species to numerous malaria parasites and is more likely appealing for the generation of genetically modified mosquitoes over-expressing Rel2 that are resistant to *Plasmodium* species. The Rel2 gene (orthologous to Drosophila Relish) of the malaria vector *Anpoheles gambiae*, has been shown to control the expression of several immune genes (LRIM1, CLIPB14, KIN1, FBN etc) and antimicrobial peptides and also regulate the bacterial and *Plasmodium* infections. The *A. gambiae* Rel2-S (Rel2 short form lacking the inhibitory ankyrin repeats and death domain) transcript has been cloned under the *A. gambiae* vitellogenin promoter to generate blood-fed inducible Rel2 transgenic mosquitoes (fat-body specific) in *Anopheles* stephensi. We have observed a decreased *Plasmodium falciparum* infection phenotype (~50% lower oocyst intensity); upon activation of Rel2 transgene (and the IMD pathway) after feeding on infectious blood-meal. Upon injection of gram positive and gram negative bacteria in blood-fed Rel2 transgene induced mosquitoes; their survival was better when compared to wild-type mosquitoes. The vitellogenin driven Rel2 transgenic mosquitoes were found to lay less number of eggs compared to the wild-type, however their longevity were very much comparable. We have furthermore explored the regulatory role of Rel2 in mosquito innate immunity in activation of other immune genes and effector molecules; Tep1, Defensin, LRIM1 were among the few which were found to be up-regulated in Rel2 transgene induced mosquitoes. Studies are ongoing to look at the *Plasmodium* infection phenotype after silencing of various immune genes in the Rel2 transgene induced mosquitoes.

766

IDENTIFICATION OF AN IMMUNE-RELATED *ANOPHELES GAMBIAE* THREONINE- AND TRYPTOPHAN-RICH REPEAT (*AGTWRR*) MIDGUT PROTEIN

Sung-Jae Cha, Marilyn Donnelly-Doman, Marcelo Jacobs-Lorena

Johns Hopkins University, Baltimore, MD, United States

Soon after ingestion by the female mosquito, the malaria parasite undergoes gametogenesis followed by fertilization and differentiation of the resulting zygote into motile ookinetes. The ookinete traverses the mosquito midgut to form an oocyst that when mature, releases thousands of sporozoites into the haemocoel. In turn, the sporozoites invade the salivary gland from where they move to the next human host when the mosquito bites again. Ookinete invasion of the mosquito midgut contributes to one of the biggest population bottlenecks of the *Plasmodium* spp. infection cycle. Here we report on a previously uncharacterized gene (AgTWRR), which contributes to this bottleneck. It encodes an unusual 515 amino acids-long protein that is rich in threonine (21.6%) and tryptophan (8.9%). Feeding mosquitoes *P. berghei*- or *P. falciparum*-infected blood or Gram-negative bacteria (*Escherichia coli*) greatly upregulated AgTWRR expression in the midgut. RNAi silencing of AgTWRR significantly increased the number of *P. berghei* parasites that developed into oocysts, indicating that this gene is part of the mosquito defense network against *Plasmodium*.

767

MOSQUITO GUT-MICROBIOME-DENGUE TRIPARTITE INTERACTIONS INFLUENCES VIRUS INFECTION

Jose L. Ramirez, Jayme Souza-Neto, George Dimopoulos

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

Dengue virus is one of the most important arboviral pathogens and the causative agent for dengue fever, dengue hemorrhagic fever and dengue shock syndrome. Dengue virus is transmitted among humans by the mosquitoes *Aedes aegypti* and *Aedes albopictus* and it is estimated that at least 2.5 billion people are at daily risk of infection. During their lifecycle, mosquitoes are exposed to a range of microbes, some of which are needed for the successful growth into adulthood. Nevertheless, new evidence suggests that the microbial flora also plays an important role in influencing the mosquito's susceptibility to infections by other pathogens. Here we present an analysis of the interactions between the dengue virus, the mosquito and seven bacterial types isolated from midguts of field-collected mosquitoes. We observed a marked decrease in viral loads in mosquitoes infected with certain bacterial isolates as well as a dynamic modulation of the mosquito's immune system. Transcript abundance analysis of selected antimicrobial peptides suggests that the mosquito's microbial flora plays a critical role in the elicitation of immune activity that is in part responsible for the lower viral load. In short, this study assessed the effects of the endogenous microbial flora on mosquito dengue virus infection as well as the modulation of the mosquito's innate immune system, a tripartite interaction that likely defines viral transmission dynamics.

768

CHARACTERIZATION OF ENDOGENOUS *HSP70* BASED INDUCIBLE PROMOTERS IN *AEDES AEGYPTI* EMBRYOS

Tiffany L. Gross, Kevin M. Myles, Zach N. Adelman

Virginia Polytechnic Institute and State University, Blacksburg, VA, United States

While inducible promoters capable of controlling transgene expression in specific tissues have been characterized for *Aedes aegypti*, no whole body, whole life stage inducible promoter has been described for this mosquito. Previously we characterized the gene structure and expression of heat shock 70 genes in *Ae. aegypti*. Preliminary experiments using *hsp70*-derived genomic fragments to drive transgene expression demonstrated high levels of transcription under stress conditions, as well as repression under control conditions for constructs containing the entire intergenic region between the *AaHsp70Aa/AaHsp70Ab*, and *AaHsp70Ba/AaHsp70Bb* genes. In order to further define an optimal *Aedes aegypti* Hsp70 promoter, deletion constructs of regions between *AaHsp70Aa/AaHsp70Ab* and *AaHsp70B/AaHsp70Bb* were produced and cloned into luciferase reporter constructs. Constructs were injected into *Aedes aegypti* embryos along with a Renilla luciferase control plasmid. Embryos were subsequently heat shocked at 39 C and harvested after 24 hours. Dual luciferase assays

were performed to compare the activity of each promoter construct. Significant differences in induction and repression were observed based on construct size. Isolating inducible *AaHsp70* promoter elements would be valuable for transgenesis and gene function studies, particularly when it is important to minimize the presence of exogenous sequences.

769

ANOPHELES GAMBIAE SAGLIN AND SPOROZOITE INVASION: A TRANSGENIC ANALYSIS

Robert Alford[1], Robert A. Harrell, II[1], Channa Aluvihare[1], David A. O'Brochta[1], Abraham Eappen[2], Adam Richman[2], Tao Li[2], Sumana Chakravarty[2], B. Kim Lee Sim[2], Stephen L. Hoffman[2], Peter F. Billingsley[2]

[1]University of Maryland, College Park, College Park, MD, United States, [2]Sanaria, Inc., Rockville, MD, United States

Sporozoite invasion of mosquito salivary glands is thought to involve specific receptor-ligand interactions. Saglin, a 100 kDa protein of *Anopheles gambiae* has been implicated as a target for *Plasmodium falciparum* sporozoite binding during salivary gland invasion. The hypothesis that saglin is directly involved in *P. falciparum* invasion of the salivary glands was tested by: 1) indirect immunofluorescence assays to determine the distribution of saglin on the surface of the salivary glands of female *An. gambiae* mosquitoes; and 2) by creating transgenic *An. stephensi* expressing *An. gambiae* saglin in the distal lateral lobes of the salivary glands and measuring *P. falciparum* abundance in transgenic salivary glands. Indirect immunofluorescence using an *An. gambiae* saglin-specific monoclonal antibody (mAb2A3) revealed the presence of saglin in the medial and proximal lateral lobes of the salivary glands of *An. gambiae*. This pattern of saglin localization was independent of the age of adult females or their gravid state. mAb2A3 did not recognize saglin in wild type *An. stephensi*. Transgenic *An. stephensi* expressing *An. gambiae* saglin under the control of an antiplatelet protein gene promoter exhibit strong constitutive transgene expression in the distal lateral lobes of the salivary glands. These transgenic mosquitoes showed no difference in their susceptibility to *P. falciparum* sporozoite invasion compared to salivary glands from non-transgenic *An. stephensi*. These observations do not support the hypothesis that saglin is directly involved in sporozoite invasion of the salivary glands.

770

THE *AEDES AEGYPTI* TRANSCRIPTOME BY RNASEQ: A TOOL FOR VECTOR DISEASE CONTROL?

Mariangela Bonizzoni[1], William A. Dunn[2], Osvaldo Marinotti[2], Corey L. Campbell[3], Anthony A. James[4]

[1]Program in Public Health and Department of Molecular Biology and Biochemistry, University of California Irvine, Irvine, CA, United States, [2]Department of Molecular Biology and Biochemistry, University of California Irvine, Irvine, CA, United States, [3]Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, United States, [4]Department of Molecular Biology and Biochemistry and Department of Microbiology and Molecular Genetics, University of California Irvine, Irvine, CA, United States

Dengue is the most important arboviral disease with 50-100 million people infected annually and cases continuing to rise. The principal vector for Dengue viruses (DENV) is the mosquito, *Aedes aegypti*. The current lack of a vaccine and ineffective vector control create an urgent need for new control strategies. Recent advancements in mosquito molecular biology support the development of genetic control strategies whereby DENV-competent vector populations are replaced by mosquitoes unable to transmit viruses. Key to these strategies are the identification of promoters for expressing antiviral effector molecules, the synthesis of genes with anti-pathogen properties, the means to genetically modify mosquitoes in a stable manner with a minimum fitness load and the development of ways to introgress the antiviral-effector genes into field vectors. Illumina RNAseq technology was used to compare variation in gene expression profiles between blood- and sugar-fed mosquitoes. The results allow us to 1) refine the annotation of the *Ae. aegypti* genome, 2) analyze biochemical pathways and biological processes elicited by a blood meal, with particular attention to genes previously described as important for pathogen interactions, and 3) identify promoters and/or regulatory regions of genes highly-activated after a blood meal as candidates for driving the expression of anti-pathogen effector molecules. More than 40% of the transcripts detected were expressed differentially between blood- and sugar-fed mosquitoes. This variation in transcription corresponds with an enhancement of digestive activity and a down-regulation of genes involved in stimuli perception. Several genes previously linked to pathogen interaction also were expressed differentially. Putative cis-regulatory elements (CREs) were identified at the 5'-end flanking sequences of selected blood-meal activated genes. These CREs may be essential for accurate temporal and spatial promoter activity.

771

UTILIZING TISSUE-ENRICHED EXPRESSION PROFILES TO ELUCIDATE HEMOCYTE TRANSCRIPTOME RESPONSES TO INFECTION IN *AEDES AEGYPTI*

Young-Jun Choi, Jeremy F. Fuchs, Bruce M. Christensen

University of Wisconsin-Madison, Madison, WI, United States

Hemocytes are fundamental elements of the mosquito host defense. They mediate immune mechanisms such as phagocytosis, melanization, and production of antimicrobial peptides. Previous studies investigating tissue-enriched expression of hemocyte transcriptome have not adequately addressed changes associated with infection status. Consequently, it has been difficult to identify specific transcriptional responses unique to hemocytes. Using genome-wide microarrays, we identified transcripts with enriched expression in circulating hemocytes with respect to remaining carcass (body minus hemocytes) in both naïve and immune-challenged adult female mosquitoes, and showed that infection response significantly alters the tissue enrichment ratios. Taking this effect into account, we took a combinatorial approach through integration of infection responsive expression profiles with tissue enrichment ratios, and resolved patterns of transcriptional response unique to hemocytes from those that are likely shared by other immune responsive tissues. This analysis contributes to the molecular characterization of hemocytes, reveals new insights into the distinctive features of the hemocyte transcriptome response to infection, and provides valuable resources for designing RNAi experiments specifically targeting hemocyte function.

772

METABOLOMIC AND TRANSCRIPTIONAL DIFFERENCES IN HIGH AND LOW NUTRIENT *AEDES AEGYPTI*

David Price, Wayne Van Voorhies, Immoh Hansen

New Mexico State University, Las Cruces, NM, United States

When raised under high population density and nutritional constraints, mosquitoes emerge smaller and with less nutrient reserves than those raised under low population density. Under field conditions large, high-nutrient mosquitoes occur less frequently than small, low-nutrient mosquitoes. Such mosquitoes do not have enough nutrient stores to successfully complete egg production from a single blood meal and therefore need a second blood meal in order to produce eggs. This can raise their vectorial capacity. This study focuses on the molecular differences between high- and low-nutrient mosquitoes and on the regulation of nutrient accumulation in the fat body. We performed transcriptome sequencing and metabolomics analysis of mosquito fat body tissue and compared the metabolic rate of individual low- and high nutrient mosquitoes. The results of this study will further our understanding of the molecular basis of mosquito nutrient metabolism and pave the way for creation of transgenic lines with altered nutrient use and increased reproductive fitness.

773

TARGETED KNOCKDOWN OF SRPN6 IN TRANSGENIC *ANOPHELES STEPHENSI*

David A. O'Brochta[1], Robert Alford[1], Robert A. Harrell, II[1], Channa Aluvihare[1], Kristin Michel[2], Abraham Eappen[3], Adam Richman[3], Tao Li[3], Sumana Chakravarty[3], B. Kim Lee Sim[3], Stephen L. Hoffman[3], Peter F. Billingsley[3]

[1]University of Maryland, College Park, College Park, MD, United States, [2]Kansas State University, Manhattan, KS, United States, [3]Sanaria, Inc., Rockville, MD, United States

SRPN6 is a gene encoding a serine protease inhibitor (serpin) whose expression is induced in the midgut during invasion of *Plasmodium berghei* ookinetes through the midgut wall. In *Anopheles gambiae, SRPN6* is also expressed in the salivary glands at the onset of sporozoite invasion. Functional roles in the invasions of both epithelia were proposed after injection of *SRPN6* double-stranded RNA (dsRNA) into the hemolymph of infected adult female mosquitoes resulted in increased numbers of oocysts and sporozoites. We tested the hypothesis that *SRPN6* expression in midgut and salivary gland epithelia of adult female mosquitoes has an antagonistic affect on parasite invasion. Transgenic *An. stephensi* were created that produce ds-*SRPN6*-RNA in a tissue specific manner. Expression in the midgut was under the control of the promoter from the midgut carboxypeptidase gene, while expression in the distal lateral lobes of the salivary gland was under the control of the promoter from the salivary antiplatelet protein gene. Using these novel constructs, knockdown of endogenous SRPN6 expression was confirmed in both midguts and salivary glands of *An. stephensi*. The effects of the knockdowns on transmission - i.e. oocysts in the midguts and sporozoites in the salivary glands, of the human malaria parasite *P. falciparum* and the rodent malaria parasites, *P. berghei* and *P. yoelii* will be reported.

774

EVOLUTIONARY RATES OF SIRNA AND MIRNA GENES IN GEOGRAPHICALLY DIVERSE *AEDES AEGYPTI* MOSQUITO POPULATIONS

Scott A. Bernhardt[1], Carol D. Blair[2], Kenneth E. Olson[2], Mark P. Simmons[2], Brian J. Geiss[2], William C. Black, IV[2]

[1]Utah State University, Logan, UT, United States, [2]Colorado State University, Fort Collins, CO, United States

The *Aedes aegypti* mosquito is one of the most significant transmission vectors of dengue viruses. RNA silencing has been shown to act as an effective antiviral mechanism and as an innate immune response to protect adult mosquitoes from alphavirus and flavivirus infection and unregulated dissemination. It is generally believed that a molecular co-evolutionary arms race exists between viruses and their arthropod hosts. Research has demonstrated that the rate of evolution of amino acid sequences is substantially elevated in antiviral RNA interference (siRNA) genes (Dicer2, Ago2, R2D2) in *Drosophila* compared to the genes in the microRNA (miRNA) pathway (Dicer1, Ago1, R3D1) and to the average rate in the entire genome. To quantify *Ae. aegypti* evolution in the siRNA genes, we generated sequence data for most of the exon regions of *ago2*, *dcr2*, *r2d2*, *ago1*, *dcr1* and *r3d1* in 104 *Ae. aegypti* mosquitoes from three populations from Mexico, two populations from Senegal, Africa, and one population from Thailand. Collection sites were chosen based on their diverse geographic distribution, vector competence for DENV-2 and *Ae. aegypti* subspecies. The intraspecific rates of siRNA and miRNA gene evolution were then compared. The rates of amino acid evolution and the ratio of non-synonymous to synonymous nucleotide differences were determined to be significantly elevated in siRNA genes (*ago2*: 0.306, *dcr2*: 0.230, *r2d2*: 0.221) compared to miRNA genes (*ago1*: 0.039, *dcr1*: 0.193, *r2d1*: 0.131). Phylogenetic analysis using maximum likelihood demonstrated substantial variation in all genes and distinct clade formation with one *Ae. aegypti* collection from Senegal, Africa. Likelihood ratio test for positive selection also identified a substantially higher number of positively selected sites in the siRNA genes. Even though the collection sites were selected based on *Ae. aegypti's* role in arbovirus transmission, our findings do not conclusively demonstrate a role for arbovirus infection in driving RNAi gene evolution rates.

775

GENETIC STRUCTURE OF *CULEX RESTUANS* IN THE EASTERN UNITED STATES

Andrea Egizi, Dina M. Fonseca

Rutgers University, New Brunswick, NJ, United States

Studies of the population genetics of disease vectors can help explain the spread of vector-borne disease. *Culex* mosquitoes are known to be important vectors of West Nile Virus (WNV). *Cx. restuans* is a native species of mosquito and its role relative to other species in WNV transmission is not well understood. We examined the genetics of *Cx. restuans* from several populations in the eastern US using a panel of 17 microsatellite loci. Populations from distinct geographical areas were found to be highly similar, indicating a high degree of gene flow and thus the potential for arboviruses such as WNV to expand their range via movement of these mosquitoes. A similar trend has been documented in populations of *Cx. tarsalis* in the western US. Additionally, blood meal analysis (BMA) was performed on blooded specimens of *Cx. restuans* to determine feeding preferences and better understand their role in disease transmission cycles.

776

THE POPULATION STRUCTURE OF THE MALARIA VECTOR *ANOPHELES MELAS* IN WEST AFRICA

Kevin C. Deitz[1], Michael P. Reddy[2], Hans J. Overgaard[3], Neha Satyanarayana[1], Abrahan Matias[4], Musa Jawara[5], Alessandra della Torre[6], Simon Abaga[7], Joao Pinto[8], Adalgisa Caccone[9], Michel A. Slotman[1]

[1]Texas A&M University, College Station, TX, United States, [2]Yale University, New Haven, CT, United States, [3]Norwegian University of Life Sciences, Ås, Norway, [4]Medical Care Development International, Silver Spring, MD, United States, [5]Medical Research Council Laboratories, Fajara, Gambia, [6]Università "La Sapienza", Rome, Italy, [7]Ministry of Health and Social Welfare, Malabo, Equatorial Guinea, [8]Universidade Nova de Lisboa, Lisbon, Portugal, [9]Yale University, College Station, CT, United States

Anopheles melas, a brackish-water breeding mosquito belonging to the *An. gambiae* complex, inhabits West-African coastal marshlands from Senegal to Angola. Although it is not generally considered a major malaria vector due its limited distribution, it is frequently an important local vector, as is the case in several locations on Bioko Island, Equatorial Guinea. Given its role in malaria transmission, knowledge of *An. melas* migration patterns is important in informing vector control efforts. To this end, we are using microsatellite markers, as well as a portion of the mitochondrial ND 4 and 5 genes, to investigate the population structure of *An. melas* across its range. In addition to studying the effect of *An. melas'* patchy distribution on its population structure, we are placing a particular emphasis on estimating migration levels of *An. melas* between the African mainland and Bioko Island, where *An. melas* is the target of extensive vector control efforts under the Bioko Island Malaria Control Project II in support of the National Malaria Control Program of Equatorial Guinea. Our results thus far suggest that the *An. melas* populations on Bioko Island belong to a single larger population. In contrast, highly significant genetic differentiation was found between the mainland and Bioko Island populations, suggesting that migration between them is highly restricted. Thus far, two geographically close mainland populations did not show any significant differentiation, however analyses of samples from a wide range is underway and will provide real insight in patterns of migration across the range of this disease vector.

777

THE MOLECULAR EVOLUTION OF OLFACTION GENES IN THE *ANOPHELES GAMBIAE* COMPLEX

Giridhar Athrey, Alexander Badamchi-Zadeh, Michael Lindsey, Larry Balle, II, Jonathan Castro, Vamsi P. Reddy, **Michel A. Slotman**

Texas A&M University, College Station, TX, United States

The main malaria vector of sub-Saharan Africa, *Anopheles gambiae s.s.,* has a strong preference for blood feeding on humans. This distinct host preference is a major reason this species is such a devastating malaria vector. This marked anthropophily is the result of the mosquito's attraction to components of human sweat and involves two classes of genes in its olfaction system; the olfaction receptors and the odorant binding proteins. It is thought that the adaptation of *An. gambiae* to human hosts has occurred after the relatively recent increase in human population size in Africa following the introduction of agriculture. We expect that this recent adaptation has left a signature of selection in the genes directly involved in this process. Therefore, we are examining patterns of genetic variation of olfaction receptors and odorant binding proteins in the species of the *An. gambiae* complex. Our goal is to identify candidate anthropophily genes by determining which genes show signatures of selection in the *An. gambiae* s.s., but not in other species of the complex.

778

THE SYSTEMATICS OF THE *CULEX PIPIENS* COMPLEX, 40 YEARS LATER

Dina M. Fonseca, Emilie C. Cameron, Motoyoshi Mogi, Craig Williams, Mark Bullians, Richard C. Wilkerson, Laura D. Kramer

Center for Vector Biology and Entomology Department, Rutgers University, New Brunswick, NJ, United States

Mosquitoes in the *Culex pipiens* Complex are important vectors of human encephalitis, parasitic filaria, and avian malaria. Their geographical distribution overlaps that of humans and they are currently the most ubiquitous mosquitoes on Earth. The complex includes *Cx. pipiens pipiens* (two forms differentially domesticated)*, Cx. pipiens pallens, Cx. quinquefasciatus*, *Cx. australicus*, and *Cx. globocoxitus.* The difficulties in distinguishing the different species morphologically and the existence of hybrid zones between several of them have made this species complex "…one of the major outstanding problems in mosquito taxonomy". Hybridization between some of the members of the complex appears to have been influenced or even driven by infection with multiple *Wolbachia* strains and some hybrid zones exhibit unusual patters of generic exchange. To examine the phylogenetic relationships between the species, subspecies and forms in the Complex we used methods originally developed for population level analysis: we analyzed the microsatellite diversity at eight loci, sequenced part of the mitochondrial NADH4 gene and cloned and sequenced nuclear introns in acetylcholinesterase 2, triosephosphate isomerase and wingless. In all analyses we included specimens from several populations of each species and subspecies. As outgroups we used species morphologically similar such as *Cx. torrentium* and *Cx. pervigilans* as well as *Cx. vagans*, *Cx. restuans* and *Cx. salinarius.* We include in some of the analyses specimens of *Cx. pipiens* not infected with *Wolbachia* reported from South Africa in 2003. We present statistical and phylogenetic analyses using MicroSat 1.5b, Phylip 3.573c, and PAUP 4.0b2, of the appropriate data sets. We hypothesize on the biogeographical expansion and evolution of the Complex, as well as on the status of the above taxonomic names.

779

FACTORS ASSOCIATED WITH *PLASMODIUM FALCIPARUM* INFECTIOUSNESS DETERMINED BY MEMBRANE FEEDING AND QT-NASBA

Andre Lin Ouedraogo[1], Teun Bousema[2], Will Roeffen[3], Bienvenu Sodiomon Sirima[1], Marga Van de Gegte-Bolmer[3], Karina Teelen Teelen[3], Sake J. De Vlas[4], Issa Nebie[1], Jan Peter Verhave[3], Robert Sauerwein[3], Adrian J.F. Luty[3]

[1]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [2]Department of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Department of Medical Microbiology, Radboud University Medical Centre, Nijmegen, The Netherlands, [4]Department of Public Health, Erasmus MC, University Medical Centre, Rotterdam, The Netherlands

Plasmodium falciparum gametocyte carriage is essential for mosquito infection and spread of the parasite. Human immune factors can reduce infectiousness and form the basis of transmission blocking vaccines. The occurrence and importance of this sexual stage immunity for low density infections has never been studied in natural settings. One hundred volunteers from an area of seasonal malaria transmission provided a total of 307 blood samples at the start, peak and end of the transmission season. Infectiousness was determined by membrane feeding assays and the same samples were used for gametocyte detection by Pfs25-QT-NASBA and assessment of Pfs45/45 and Pfs230 antibody responses. Natural human antibodies were purified from the same plasma samples and tested in the Standard Membrane Feeding Assay for transmission reducing activity. At least one mosquito was infected in 32.6% (100/307) of the experiments. In total, 7.5% (916/12,079) of the mosquitoes were infected with 1-97 oocysts per midgut. Human individual infectiousness and the proportion of infected mosquitoes were negatively associated with age ($p<0.001$ and $p=0.001$ respectively) after adjustment for confounding factors. Individual infectiousness also declined over time with a significant change at the peak (OR=0.56; $p<0.001$) and end of the transmission season (OR=0.20; $p<0.001$) compared to the start. Submicroscopic gametocyte carriers, as detected by Pfs25 QT-NASBA, were infectious to mosquitoes in 32.8% of the feeds. Purified antibodies appeared to completely block or reduce mosquito infection when tested in the Standard Membrane Feeding Assay. This study thus revealed age- and season-dependent patterns of gametocyte infectiousness in residents of an endemic area. Naturally acquired antibodies were found able to reduce the parasite infectiousness. These findings are relevant for the characterization of the infectious reservoir and may provide new opportunities for malaria control.

780

USE OF 'ROLLING' MALARIA INDICATOR SURVEYS (RMIS) AS A MONITORING AND EVALUATION (M&E) TOOL IN MALARIA ENDEMIC SETTINGS WITH MARKED SEASONALITY

Arantxa Roca-Feltrer[1], Juan Barranco[2], Paul Chipeta[3], Kamija Phiri[3], David Lalloo[2], Dianne J. Terlouw[2]

[1]Liverpool School of Tropical Medicine and Malawi Liverpool Wellcome Trust Programme, Blantyre, Malawi, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]Malawi Liverpool Wellcome Trust Programme, Blantyre, Malawi

In recent years, malaria control interventions have scaled up considerably. To guide control efforts, new M&E tools are needed to assess rapid changes in intervention coverage and malaria burden. Currently, M&E tools at the community level rely largely on national cross-sectional household surveys such as the Malaria Indicator Survey (MIS). However, these are logistically demanding, costly, and burden estimates can be susceptible to annual and seasonal variability. Growing evidence suggests that malaria seasonality also changes over time as transmission levels decrease. Monitoring these changes will be key to maximize the impact

of available control options, particularly to assess the optimal timing of seasonally targeted interventions such as Indoor Residual Spraying (IRS) or seasonal Intermittent Preventive Treatment (sIPT). To address this we developed a novel ongoing (rolling) cross-sectional survey tool: the 'rolling' MIS (rMIS). As part of a randomized trial assessing the safety and effectiveness of two different ACTs, a monthly rMIS is conducted from May 2010 onwards in 51 villages in Chikwawa district, southern Malawi (an area of perennial transmission with marked seasonality). Each month, a random sample of households will be visited covering each village at least twice a year. This rMIS will evaluate coverage of malaria control interventions and parasite and anaemia prevalence in under-fives. Most importantly, we will assess the potential role of rMIS to monitor short-term changes in the burden of malaria accounting for the potential role of malaria seasonality. Preliminary results from the rMIS will be presented based on a comparison with the standard national MIS, conducted in April-May 2010. Findings will focus on the logistical, user-friendliness and costs aspects as well as on the applicability of this tool to monitor rapid changes in the burden of malaria accounting for the effect of seasonality

781

A COMPARISON OF SCHOOL- AND COMMUNITY-BASED CROSS-SECTIONAL SURVEYS FOR COLLECTION OF SEROLOGICAL MEASURES OF MALARIA TRANSMISSION

Chris Drakeley[1], **Jennifer Stevenson**[1], Carol Gitonga[2], Lynn Spencer[1], Albert Oduor[3], Robin Oriango[3], Simon Kariuki[3], Mary Hamel[4], Kayla Laserson[4], John Vulule[5], Simon Brooker[1], Jonathan Cox[1]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]Kenya Medical Research Institute/Wellcome Trust Collaborative Programme, Nairobi, Kenya, [3]Kenya Medical Research Institute/Centers for Disease Control and Prevention, Kisumu, Kenya, [4]Centers of Disease Control and Prevention, Kisumu, Kenya, [5]Kenya Medical Research Institute, Kisumu, Kenya

Antibody responses to malaria antigens represent markers of exposure to infection and can be used to monitor transmission and the effect of interventions. As antibodies can persist for many years they are particularly useful at low transmission intensities. Samples are usually collected during community based cross sectional surveys; however, these surveys can be logistically demanding and expensive if conducted frequently. An alternative approach is to use school surveys, which are long established in helminth research and are being used increasingly within malaria studies. Here we compare antibody responses to the *Plasmodium falciparum* merozoite antigen MSP-119 in samples collected in community surveys with those collected during contemporaneous school surveys conducted in Rachuonyo and Kisii districts in Western Kenya. Age specific sero-prevalences and estimates of the sero-conversion rates were similar between the two survey approaches, with both discriminating areas of high and low transmission. The broader application of school-based sampling, and its potential advantages for monitoring and evaluating variations in malaria transmission, are discussed.

782

EVALUATION OF DIFFERENT INDICES TO MONITOR MALARIA IN THE GAMBIA, WEST AFRICA

Abraham R. Oduro[1], Kalifa Bojang[2], David Conway[1], David Schellenberg[3]

[1]Medical Research Council, The Gambia/London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]Medical Research Council, The Gambia, Banjul, Gambia, [3]London School of Hygiene and Tropical Medicine, London, United Kingdom

For over half a century malaria research and control measures have been taking place in The Gambia. Recent analysis of malaria indices from several facilities suggests a significant decrease in disease burden in the country. There is a need to confirm this finding as it has implications on the current control measures and design of new interventions. Although important, the available indices are constrained in determining the characteristics of the emerging trends, the impact of the expanding control measures and assessing novel tools that may appear in the future. Robust indices of the current malaria epidemiology will help prioritize malaria control measures and research activities. This was a countrywide study that evaluated clinical, parasitological and serological indices in varied transmission settings for monitoring malaria over time and space. It also examined the usefulness of health facility surveys as a method for collecting data to describe area specific malaria epidemiology. The presentation will focus on the utility of data from different sources and indices for monitoring malaria. Data analysis is ongoing however provisional results shows significant shift in demographic and gender patterns of malaria infections. Young teens compared to young children [19.9% vs.10.0%, 95% 5.8, 13.9; p<0.001] currently have the highest burden of infection. Again there is preponderance of more infections in adolescent males (23.9% 95%CI 19.3, 28.8) compared to females[15.2% 95% CI 11.8, 18.9] due to behavioural and differential ITN use. Additional information will be presented on how malaria indices from health facility surveys correlate and explain current infection burden in their catchment communities and further issues on the utility of filter paper for collecting serum to monitor transmission intensity will also be considered. Malaria is changing hence the continuous use of existing malaria related indices in children as a proxy for defining malaria burden in endemic regions may need review. Moreover, integrated approach to data collection including health facility surveys and filter paper based specimen antibody assays present potential tools for monitoring area specific changes in malaria.

783

MALARIA SURVEILLANCE IN HAITI, POST-EARTHQUAKE, 2010

David A. Townes[1], Ribka Amsalu[2], Roc Magloire[3], Michelle Chang[1], Meredith McMorrow[1], S. Patrick Kachur[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Save the Children, Washington, DC, United States, [3]Ministry of Public Health and Population, Port-au-Prince, Haiti

On January 12, 2010, a 7.0 magnitude earthquake struck Haiti. The earthquake's epicenter was 10 miles west of the Haiti capital city of Port-au-Prince. According to the Haitian government, approximately 200,000 persons were killed, and over 2 million were displaced. *Plasmodium falciparum* malaria is endemic in Haiti where the principal vector is the *Anopheles albimanus* mosquito, which frequently bites outdoors. Thus, displaced persons living outdoors or in temporary shelters in Haiti are at substantial risk for malaria. We conducted a survey of 1,629 consecutive suspected malaria patients presenting to medical clinics managed by Save the Children in the earthquake affected areas of Leogane and Jacmel from March 4 to April 9, 2010. Suspected malaria accounted for 3.0% of all consultations. Females accounted for 59% of suspected malaria consultations. A malaria rapid diagnostic test (RDT) was performed on 96% (1,564/1,629) of these patients with an overall positivity rate of 20.3% (317/1,564). Among 341 children less than five years of age, 7.6% were RDT positive, 87.7% were RDT negative, and 4.7% had no RDT result recorded. Among 1288 individuals five year of age and older, 22.6% were RDT positive, 73.6% were RDT negative, and 3.8% had no RDT result recorded. Among 463 women aged 15-49 years, 21.0% were RDT positive, 75.6% were RDT negative, and 3.5% had no RDT result recorded. This included 40 pregnant women among whom 27.5% were RDT positive, 65.0% were RDT negative, and 7.5% had no RDT result recorded. Of the 317 patients with a positive RDT, 87.7% received chloroquine, 2.5% received quinine, and 9.8% had no anti-malarial documented. Malaria is an important public health problem in Haiti post-earthquake with the potential for an increase in cases given the large number of displaced individuals and the onset of the rainy season. Continued malaria surveillance is essential to monitor prevalence, identify areas of potential increased transmission, detect epidemics should they occur, and help direct and monitor interventions and response.

784

RECURRENT AND SUB-PATENT INFECTIONS ARE A COMMON OCCURRENCE IN *PLASMODIUM VIVAX* PATIENTS TREATED WITH CHLOROQUINE AND PRIMAQUINE: A ONE-YEAR COHORT STUDY IN PERU

Peter Van den Eede[1], Christopher Delgado[2], Veronica E. Soto-Calle[2], Dionicia Gamboa[2], Jozef Anné[3], Annette Erhart[1], Umberto D'Alessandro[1]

[1]Institute of Tropical Medicine Antwerp, Antwerp, Belgium, [2]Instituto de Medicina Tropical Alexander Von Humboldt, Lima, Peru, [3]Catholic University of Leuven, Leuven, Belgium

Worldwide, *Plasmodium vivax* has re-emerged and developed in a major problem in areas where it had been eradicated, and has become increasingly prevalent in areas where it is sympatric with *P. falciparum*. It has the ability of producing relapses originating from dormant liver forms. Fifty one *P. vivax* patients living in communities around Iquitos, in the Peruvian Amazon region, where treated with chloroquine and primaquine and then followed up monthly for 1 year. Passive detection of malaria cases was also carried out throughout the study period. At each visit a blood sample was systematically collected and screened with species-specific PCR. Positive samples were then genotyped using 16 polymorphic microsatellites. Eighty four recurrent infections were identified, 61% within 6 months after treatment (median time 203 days), 22 of them positive also by microscopy. The majority (71%) of recurrences was asymptomatic and in 13 patients the infection persisted for several months at sub-patent level. The genotype of most (75%) recurrent infections was different from that at day 0; 41% of recurrent infections carried different alleles as compared to any previous episode. Only 8 infections were polyclonal. The average expected heterozygosity was 0.55. There was strong linkage disequilibrium (*ISA* = 0.29, $p < 1.10\text{-}4$) which remained also when analyzing only the unique haplotypes, suggesting common inbreeding. In Peru, similarly to Brazil and Vietnam, *P. vivax* recurrent infections, despite the low transmission intensity, were common and displayed a high turnover of parasite genotypes. Most infections were asymptomatic, persisting for several months and detectable only by PCR. *Plasmodium vivax* patients, even when appropriately treated, may still represent an important parasite reservoir from which transmission can be maintained. Therefore, any elimination effort should consider approaches able to identify and treat this hidden reservoir.

785

THE FEASIBILITY OF MALARIA ELIMINATION: A FRAMEWORK FOR ANALYSIS

Bruno Moonen[1], **Justin M. Cohen**[2], David L. Smith[3], Andrew J. Tatem[3], Simon I. Hay[4], Oliver J. Sabot[2]

[1]Clinton Health Access Initiative, Nairobi, Kenya, [2]Clinton Health Access Initiative, Boston, MA, United States, [3]University of Florida, Gainesville, FL, United States, [4]University of Oxford, Oxford, United Kingdom

The realization that the Global Malaria Eradication Program was unable to interrupt transmission in every region due to a variety of technical, operational, administrative, or socioeconomic factors led to understanding of the importance of carefully assessing the feasibility of such a campaign - with all its significant programmatic, financial, and technical implications - before embarking upon it. However, specific criteria for such an evaluation were never clearly proposed. Today, as many countries again contemplate the potential for malaria elimination within their borders, advice on how to conduct such a complex assessment remains scant. To establish a rigorous, quantitative framework for evaluating the feasibility of malaria elimination, a comprehensive evaluation was undertaken to provide explicit guidance for those grappling with this decision. Following a thorough historical review, the feasibility of malaria elimination was defined along three dimensions: technical, operational, and financial. Technical feasibility was defined in relation to two key concepts: the rate of importation of infections from neighboring regions and the probability of these infections leading to onward transmission. These parameters are quantified for a specific context using observational evidence and mathematical models that can estimate reductions in transmission achievable with available tools as well as the potential for maintaining elimination. The operational feasibility component can then evaluate whether the interventions needed to achieve and sustain elimination according to the technical models can be implemented given the capacity of the national malaria program and the health system. Finally, the financial feasibility component should evaluate whether the costs of these required elimination interventions can be sustained over time and compare these costs to those required to control endemic transmission without eliminating it altogether. Together, these analyses will allow malaria programs to make informed decisions and set appropriately evidence-based strategies.

786

CD23-BOUND IGE AUGMENTS AND DOMINATES RECALL RESPONSES THROUGH HUMAN NAÏVE B CELLS

Qyana K. Griffith[1], YanMei Liang[1], Daniel Onguru[2], Taslima Shah[1], Helmut Haas[3], Pauline Mwinzi[2], Lisa Ganley-Leal[1]

[1]Boston University School of Medicine, Boston, MA, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]Research Center Borstel, Borstel, Germany

Peripheral blood human naïve B cells express high levels of CD23 and circulate pre-loaded with IgE. The antigen specificity of CD23-bound IgE presumably differs from the B cell receptor (BCR) and may reflect the antigen specific mix of free serum IgE. CD23-bound IgE is thought to enhance B cell antigen presentation raising the question of how a B cell might respond when presented with a variety of antigens and CD23-bound IgE specificities. We recently reported that an increase in CD23+ B cells is associated with resistance to schistosomiasis highlighting the potential importance of CD23-bound IgE in immunity. We sought to determine the relationship between BCR and CD23-bound IgE mediated B cell activation in schistosomiasis. Crude schistosome antigens downregulated basal B cell activation levels in individuals hyper-exposed to infectious worms. However, schistosome-specific IgE from resistant, occupationally exposed Kenyans recovered responses of naïve B cells to schistosome antigen. Furthermore, cross-linking of CD23 overrode intracellular signals mediated via the BCR illustrating its dominating role in B cell activation. Notably, the nature of the cognate antigen appeared to dictate the threshold of antigen-specific CD23-bound IgE required for B cell activation suggesting that CD23-bound IgE functions as a rheostat for B cell responses to antigenic stimuli. These results suggest that CD23-bound IgE augments and dominates host recall responses through naïve B cells.

787

A SOMATICALLY DIVERSIFIED LECTIN (FREP3) IS INVOLVED WITH RESISTANCE OF SNAILS TO DIGENETIC TREMATODE INFECTION

Patrick C. Hanington, Michelle A. Forys, Coen M. Adema, Eric S. Loker

Center for Evolutionary and Theoretical Immunology, University of New Mexico, Albuquerque, NM, United States

Transcriptional analysis of *Biomphalaria glabrata* snails following challenge with the digenetic trematodes *Schistosoma mansoni* and Echinostoma paraensei revealed a number of transcripts associated with snail resistance to infection. Comparison of the transcriptional profiles expressed by snails resistant to infection because of size, strain (BS-90 resistant/M-line susceptible) or prior exposure to homologous parasites (acquired resistance) yielded a small group of resistance-associated transcripts that were commonly up regulated in all three resistance models. Fibrinogen related protein 3 (FREP3) was identified as one such transcript, demonstrating an increased expression beginning as early as 12 hours

post exposure (dpe), and continuing until 8 dpe. The common recurrence of FREP3 in all of our transcriptional studies of snail resistance and the sequence heterogeneity that arises in FREP3 molecules due to a high rate of point mutation and gene conversion events made FREP3 a high priority for further functional analysis. In situ hybridization studies co-labeling for newly produced hemocytes (BrdU) and FREP3 suggested that newly developed hemocytes are major producers of FREP3. Using an anti-FREP3 antibody we purified native FREP3 from *B. glabrata* plasma and used both FREP3 and the antibody to it, to analyze FREP3 function. We have identified that FREP3 is involved in binding and recognition of galactose sugars, and that it can act as an opsonin to enhance phagocytosis of bound targets. Injection of FREP3-specific small interfering RNA into size resistant snails and subsequent challenge of these normally resistant snails with *E. paraensei* results in a partial loss of the resistance phenotype, suggesting FREP3 is important for immune-associated resistance to trematode infection.

788

SUBVERSION OF INNATE IMMUNE SIGNALS BY *SCHISTOSOMA MANSONI* PERMITS WORM DEVELOPMENT

Diana K. Riner, Sean K. Maynard, Stephen J. Davies

Uniformed Services University of the Health Sciences, Bethesda, MD, United States

Blood flukes of the genus *Schistosoma* infect 200 million people. As a result of host parasite co-evolution, *S. mansoni* has evolved to exploit host immune factors as signals to coordinate its own development within the host. Worms fail to develop normally in RAG-/- mice that lack all T and B cells, while development is restored when CD4+ T cells are transferred into RAG-/- mice, suggesting that CD4+ T cells play a central role in regulating parasite development. Recent findings suggest the role of CD4+ T cells in this process is indirect, limited to provision of non-cognate T cell help for innate responses which, in turn, facilitate parasite development. In support of this hypothesis, we have found that administration of LPS to RAG-/- mice, in the absence of CD4+ T cells, also restores worm development, indicating that innate immune signals are sufficient for parasite development to proceed normally. LPS, a pathogen-associated molecular pattern (PAMP), activates toll-like receptor 4 (TLR-4), resulting in signaling through both MyD88-dependent and TRIF-dependent pathways. Interestingly, specific stimulation of TRIF-dependent signaling failed to restore worm development in RAG-/- mice suggesting that worm development is not dependent on TRIF-mediated induction of type I interferon expression. However, high levels of PAMPS are not present during the normal course of a *S. mansoni* infection. We therefore hypothesize that, during schistosome infection, endogenous danger-associated molecular patterns (DAMPs) induce the innate responses required for parasite development, following their release by damaged host cells such as hepatocytes. In support of this, we show that stimulation of the NALP3 inflammasome, a MyD88-dependent sensor of endogenous DAMPs, restores worm development in RAG-/- mice. Current research efforts are focused on dissecting the MyD88-linked signaling events that influence schistosome development. Elucidation of the innate immune signals that control schistosome development may help in the development of new drug targets and vaccine strategies.

789

GLYCOPROTEIN CHARACTERIZATION OF THE *TAENIA SOLIUM* ONCOSPHERE AND ITS ROLE IN ADHERENCE MECHANISMS

Manuela R. Verastegui[1], Yanina Arana[1], Nancy Chile[1], Sandra Palma[1], Cesar M. Gavidia[2], Anamelva Aquise[1], Elizabeth Quispe[1], Charles R. Sterling[3], Hector H. Garcia[1], Armando E. Gonzalez[2], Robert H. Gilman[4]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]University of San Marcos, Veterinary School, Lima, Peru, [3]Department of Veterinary Science and Microbiology, University of Arizona, Tucson, AZ, United States, [4]Bloomberg School of Public Health, The Johns Hopkins University, Baltimore, MD, United States

The molecules by which oncospheres of *Taenia solium* attach to the intestine prior to invasion have not been studied. Knowledge of these molecules could greatly aid in the design of novel preventive and interventional strategies against this parasite. We characterized the carbohydrate/lectin surface membrane of the *T. solium* oncosphere and used an *in vitro* adhesion CHO cell system and polysaccharide or glycoconjugates, laminin and fibronectin substrate assay to investigate the molecules responsible for *T. solium* oncosphere adherence to host cells. This study demonstrated that the activated oncosphere had a lectin with a strong affinity for sialic acid, and that the predominant carbohydrates on the *T. solium* oncosphere are α-D-galactose, α-galNAc and α-D-Mannose. We found that the oncospheres bound to CHO cells and that this binding was augmented by laminin and to a lesser extent by fibronectin and fetal bovine serum. Laminin even at low doses increased oncosphere adherence but exhibited decreased adherence at higher concentrations. Adherence by laminin and fibronectin was specific since antibodies to both inhibited oncosphere adherence. Also adherence of oncospheres to CHO cells even in the presence of laminin was significantly inhibited by heparin. Furthermore, as demonstrated by immunofluorescense heparin bound strongly to activate oncospheres. This study provides us with significant insight into the mechanism of activated *T. solium* oncosphere binding, and will be useful for the development of vaccines that will prevent attachment of oncospheres to the intestinal wall.

790

A RECOMBINANT ATTENUATED *SALMONELLA* VACCINE SYSTEM FOR *TAENIA SOLIUM* CYSTICERCOSIS INFECTION IN PIGS

Maria E. Silva[1], Kathy Hancock[2], Roy Curtiss III[3], Bonnie Gunn[3], Armando E. Gonzalez[4], Hector H. Garcia[5], Robert Gilman[6], Victor C. Tsang[2]

[1]Georgia State University, Atlanta, GA, United States, [2]Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Biodesign Institute, Center for Infectious Disease and Vaccinology, School of Life Science, Arizona State University, Tempe, AZ, United States, [4]School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Lima, Peru, [5]Department of Microbiology, Universidad Peruana Cayetano Heredia, Lima, Peru, [6]Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Cysticercosis is a parasitic infection produced by the larval stage of *Taenia solium*. The clinical importance of the disease appears when the larvae develop in the central nervous system of humans producing neurocysticercosis. Although a number of intervention trials have demonstrated that transmission of *T. solium* can be inhibited temporarily; recrudescence occurs as a consequence of tapeworm reintroduction through infection of a susceptible intermediate host. Vaccination of the intermediate host will remove the source of human infection and interrupt the *T. solium* life cycle. As reported previously, the oncosphere antigen Tsol18 can confer protection when it is administrated as a recombinant GST fusion protein. However, production of a recombinant protein is

expensive and therefore impractical for large-scale use. Consequently, the potential of a live vector vaccine system to deliver *Taenia solium* Tsol18 was investigated. An attenuated strain of *Salmonella enterica* serovar Typhimurium χ9402 was used to develop an oral delivery system for Tsol18 antigen. Tsol18 gene was cloned downstream from the β-lactamase signal sequence in a multicopy Asd+ plasmid vector pYA3620 to yield plasmid pYA3620/Tsol18 and then transformed into the vaccine strain. The recombinant attenuated *Salmonella* vaccine harboring Tsol18 was stable and expressed rTsol18. Immunization of mice with either one or two doses of 109 CFU of the recombinant vaccine strain carrying plasmid pYA3620/Tsol18 elicited specific IgG responses to *Salmonella* self antigens (LPS and SOMPs) and to rTsol18. Moreover, oral immunization of piglets with a single dose of 1012 CFU reduced the numbers of viable cysts after challenge. The use of a recombinant attenuated *Salmonella* vaccine will not only reduce the cost of vaccine production but also the number of vaccine doses needed per animal. The data we present provides the basis for an affordable and easy vaccine delivery system that can be used as an adjunct in cysticercosis/taeniasis control programs.

791

INVESTIGATION OF POST-TREATMENT INFLAMMATORY RESPONSES IN A RAT MODEL OF NEUROCYSTICERCOSIS

Siddhartha Mahanty, Andrew Schwendeman, Elise Madrid, Fernando Torres-Velez, Abby Berns, Erick Scott, Martin Lizak, Theodore Nash

National Institutes of Health, Bethesda, MD, United States

Neurocysticercosis (NCC) resulting from infection by the cestode *Taenia solium* is the leading cause of epilepsy in endemic regions. Anthelmintic treatment leads to inflammation around the dying cysts, often resulting in a worsening of symptoms. In an intracerebral (IC) infection model using metacestodes of *T. crassiceps* (Tc) in rats, we studied the effects of anthelmintics on parasite-host interactions. Tc metacestodes were surgically implanted in the brain parenchyma, and growth of IC cysts were tracked and quantified by magnetic resonance imaging (MRI). IC infection resulted in 60% mortality by day 80 post-infection (PI). Six to eight weeks PI the rats received 18 days of treatment with praziquantel, albendazole, or a combination thereof. Treatment with PZQ and PZQ plus ABZ resulted in growth arrest of the cysts by MRI, suggesting damage or death of the parasite. Histopathlological examination of pericystic brain revaled patchy areas of mononuclear inflammatory cell infiltrates. A majority of rats had detectable serum IgG responses to cyst antigens (Ags), and high titers of Ag-specific IgG1, IgG2a, and IgG2b consistent with a mixed Th1/Th2-type response to the parasite. Preliminary studies of whole brain samples for gene expression of pro-inflammatory and regulatory genes by RT-PCR did not reveal significant differences between the treated and untreated rats. Focused gene expression analysis of the pericystic region using laser dissection microscopy and characteriation of cellular immune responses to the IC cysts, including cell phenotyping by immunohistochemistry and flow cytometry are ongoing, aiming to determine the mechanisms underlying parasite-associated immune regulation and post-treatment neurological infalmmation. This model reflects cerebral infection in humans and may have utility in the investigation of novel anti-inflammatory therapies or the control of post-treatment inflammation associated with anthelmintics.

792

IN VITRO EFFECTS OF ANTHELMINTICS ON *TAENIA CRASSICEPS* REVEAL LIMITATIONS OF ITS USE AS A MODEL FOR *T. SOLIUM* CYSTICERCOSIS

Elise Madrid[1], Pierre Dorny[2], Theodore E. Nash[1], Siddhartha Mahanty[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Institute of Tropical Medicine, Antwerp, Belgium

Taenia crassiceps (Tc) cysts have been commonly used as a substitute for cysts of T. solium (Ts) *in vitro* and *in vivo* in a rodent model of cysticercosis. Although there are common features and characteristics of both organisms, some studies suggest that the two parasites differ in their biology, and that not all experimental conclusions from one can be applied to the other. One area of investigation for which Tc is particularly attractive is in the evaluation of anthelmintic drug efficacy. We investigated the utility of secreted/released parasite proteins for the evaluation of drug effects on Tc. Using changes in AP secretion as a measure of drug activity, as previously applied to the study of Echinococcus spp., we investigated the effects of the anthelmintics albendazole sulfoxide (ABZ) and praziquantel (PZQ) on Tc cysts. In contrast to the results using Ts cysts, we observed an initial increase of AP secretion followed by a significant decrease in secretion of AP with both ABZ and PZQ. Higher doses of both drugs caused earlier maxima of AP secretion; for example, peak secretion changed from day 4 to day 2 with PZQ concentrations of 10 ng/nml and 100 ng/ml, respectively. Overall, the release of AP on exposure of Tc to PZQ and ABZ resembled the patterns reported for *Echinococus* spp., but was observed at significantly lower concentration of both drugs. The secretion/release of a parasite specific antigen found in the serum and cerebrospinal fluid of Ts-infected patients. was evaluated using immunofluorescence microscopy with Tc cysts and monoclonal antibodies. These monoclonal antibodies were observed to cross-react with Tc, and studies aimed at characterizing the effects of drugs on the localization of the target antigen on Tc before and after exposure to anthelmintics are under way. These data suggest that secreted/released enzymes and immunoreactive antigens may provide sensitive measures of drug effects on Tc and also suggest that results of studies with Tc differ significantly from those reported with Ts.

793

43KDA EXCRETORY-SECRETORY ANTIGENIC PEPTIDE OF *TAENIA SOLIUM* METACESTODE AS A POTENTIAL DIAGNOSTIC MARKER IN HUMAN NEUROCYSTICERCOSIS

Priyadarshi S. Sahu, Subhash C. Parija, Sunil K. Narayan

Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, India

Taenia solium taeniasis/cysticercosis is being reported increasingly from India and that it is an important constraint for regional agricultural and health development. Diagnosis of neurocysticercosis (NCC) is complicated because of the variability in clinical presentations and course of the disease where viability of parasite is a major determinant. The living parasite continues releasing metabolic byproducts or excretory-secretory (ES) substances. Hence detection of ES substances in body fluids or an antibody response to the ES substances might help in diagnosing an early stage of infection before to the onset of parasite degeneration in typical symptomatic NCC in human. The objective of the present study was to characterize the ES substances collected from *in vitro* culture of *T. solium* metacestode larvae, and to identify specific ES peptides as diagnostic markers. Three ES peptides viz., 67kDa, 43kDa and 32kDa, were found to be diagnostic for NCC based on high sensitivity and specificity of their detection in either serum or cerebrospinal fluid (CSF) specimens. More remarkably, the 43kDa ES peptide was found reactive with CSF and serum specimens from confirmed NCC patients with absolute specificity and a high sensitivity (88.23% in serum and 89.28% in CSF). This peptide was also detected by sera and CSF from clinically suspected NCC patients but with a decreased sensitivity correlating with the decreasing order of the certainty of diagnosis. The 43kDa ES peptide is suggested to be an important peptide of diagnostic utility in NCC with an application in either clinical laboratory practice or mass screening studies in endemic areas.

794

FIELD-APPLICABLE COPROANTIGEN ASSAY TO DETECT *TAENIA SOLIUM* CARRIERS

Silvia Rodriguez[1], Catherine Guillen[2], Yesenia Castillo[2], Patricia Arias[2], Ricardo Gamboa[3], Guillermo Gozalvez[3], Victor Tsang[4], Robert Gilman[5], Armando E. Gonzalez[6], Hector H Garcia[2], Cysticercosis Working Group in Peru[7]

[1]Instituto Nacional de Ciencias Neurológicas, Lima, Peru, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Centro de Salud Global – Tumbes, Universidad Peruana Cayetano Heredia, Tumbes, Peru, [4]Georgia State University, Atlanta, GA, United States, [5]Johns Hopkins University, Baltimore, MD, United States, [6]Facultad de Medicina Veterinaria Universidad Mayor de San Marcos, Lima, Peru, [7]UPCH, Lima, Peru

Taeniasis by *Taenia solium* occurs only in humans and is the unique source of cysticercosis infection to humans and pigs. Cysticercosis causes economic losses to pig farmers and morbidity and mortality in humans, being the most important infectious factor increasing epilepsy burden in endemic areas. Diagnosis by microscopy or molecular techniques requires the presence of eggs or progglotids, which is infrequent. Serum antibody detection doesn't indicate active infection. Coproantigen detection is the best alternative, with 96.5% sensitivity and 98% specificity. A major limitation of coproantigen ELISA detection is its limited availability and technical requirements: ultralow temperature (-70ºC) storage, expensive blocking agents, ultra pure water and the use of a spectrophotometer to interpretation of the results. We initially measured the stability of polyclonal antibodies kept at -20ºC for 1 month, with no decrease in activity. Then we compared use of bottle commercial water and powder commercial milk instead of ultrapure water and fetal bovine serum respectively, again without major changes in the ratios (19.4 vs 18.9, OD pool positive / Mean OD + SD of 6 negative samples). Finally, applying both assay versions to 308 stool samples from patients with *T. solium* taeniasis and 78 from negative controls we found a Pearson's r of 0.97 comparing the OD results of a field-applicable version versus the standard coproantigen test. Additionally, using a visual color gradient chart we compared the classification of cases and controls as positive (taeniasis by *T. solium*), indeterminate, and negative cases with the results obtain from the spectrophotometer. Categorization was the same in all cases. Development of an ELISA test with minimal technical requirements and maintaining the sturdiness of the original assay will provide a simple, cheap, available tool to detect and follow up *T. solium* carriers. This assay will greatly facilitate surveys, epidemiological studies, control/eradication programs and individual management of patients in endemic areas for taeniasis/cysticercosis.

795

A RETROSPECTIVE REVIEW OF CALCIFIED NEUROCYSTICERCOSIS IN A NEW YORK CITY MUNICIPAL HOSPITAL

Aaron D. Storms[1], Laura R. Greisman[2], Jessica Furst[2], Phyllis Andrews[1], George Lantos[1], Christina M. Coyle[3]

[1]Jacobi Medical Center, Bronx, NY, United States, [2]Albert Einstein College of Medicine, Bronx, NY, United States, [3]Jacobi Medical Center; Albert Einstein College of Medicine, Division of Infectious Diseases, Bronx, NY, United States

Neurocysticercosis (NCC) is the most common cause of acquired adult onset seizures and epilepsy in endemic countries. Calcified lesions are the most common cerebral findings and previously had been classified as "inactive disease", but over the past decade there have been increasing reports demonstrating this is a frequent finding and associated with episodic seizures. The objective of this study was to document the frequency of perilesional edema associated with calcifications and to define clinical symptoms associated with the finding in an immigrant population seen in a nonendemic region. A retrospective chart review of patients with calcified lesions with NCC since 1995. MRIs and CT scans were reviewed by a neuroradiologist to identify the presence of calcified lesions and perilesional edema. Charts were reviewed for demographic data and the presence of edema related symptoms. 44 patients had calcified lesions consistent with NCC, of which 77% had positive W. blots. 22 (50%) patients were female and the mean age was 35 $\pm$13 years. All but one patient was an immigrant from an endemic region. The mean time from immigration was 6.6 $\pm$8 years. In descending order of frequency, patient's geographic locations of origin were Mexico, South America, Caribbean islands, and Central America. Additionally, 2 patients were from India, 1 was from Korea and there was one patient from the US who was a short term traveler to India. Of the 44 patients, 15 (34%) had perilesional edema, all of whom were symptomatic at the time of the scan. Symptoms in decreasing order of frequency were headache, seizure, hemiparesis and altered mental status. Recurrent perilesional edema was evident in 7 patients, with two patients having 2 or more recurrences. In conclusion, this study found that 34% of patients with parenchymal NCC and calcifications were found to have perilesional edema on retrospective review and is congruent with findings at other institutions. Calcified perilesional edema seems to be a frequent phenomenon in NCC, often associated with headaches and seizures.

796

GEOSTATISTICAL MAPPING OF MALARIA AND LYMPHATIC FILARIASIS CO-ENDEMICITY IN AFRICA

Hannah Slater, Edwin Michael

Imperial College, London, United Kingdom

Malaria and lymphatic filariasis (LF) cause the largest public health burden of all diseases worldwide. Some 350-500 million clinical episodes and 1 million deaths each year are caused by malaria, of which approximately 60% and 80% respectively occur in Africa. More than 50 million people are also thought to be infected with lymphatic filariasis in 39 endemic countries in sub-Saharan Africa, with approximately 14.6 million individuals living in these endemic countries estimated to suffer from the two major filarial debilitating conditions, lymphodema or hydrocoele. Both diseases are vector-borne and in many parts of sub-Saharan Africa are transmitted by the same vector, namely the *Anopheles* mosquito. Recently there have been calls for an integrated approach to disease control, however, to successfully achieve this, accurate maps of the geographic distributions of the infections, and maps highlighting co-endemic regions are crucial for 1) guiding the planning of control programmes and 2) assessing whether an integrated control strategy is cost-effective. We attempt to map the prevalence of LF and malaria infection across Africa using a Bayesian generalised spatial linear model in conjunction with community-level infection data obtained from the published literature. We use the package 'geoRglm' implemented in 'R' to create a disease prevalence distribution model with spatially correlated random effects. The model parameters are estimated using Markov chain Monte Carlo techniques. We use the model to assess how different environmental risk factors influence the distribution of LF and malaria, and how this could be affected by the expected changes in climate using future predictions from the global climate models. We apply simple overlay map functions to these maps to identify areas of co-infection, estimate the number of co-infected people and assess the implications for an integrated control strategy, specifically, whether this approach is more cost-effective and it is more suited to some regions than others based on their environmental conditions and current levels of endemicity.

797

COST SAVINGS OF SCALING UP TO INTEGRATED, CO-ADMINISTRATION OF IVERMECTIN, ALBENDAZOLE, AND PRAZIQUANTEL: TRIPLE DRUG ADMINISTRATION IN NIGERIA

Darin Evans[1], Frank Richards[1], William Adamani[2], Abel Eigege[2], Deborah McFarland[3], Michael Anibueze[4], Emmanual Miri[2]

[1]The Carter Center, Atlanta, GA, United States, [2]The Carter Center, Jos, Nigeria, [3]Emory University, Atlanta, GA, United States, [4]Federal Ministry of Health, Abuja, Nigeria

Nigeria is one of the first countries to implement co-administration of the drugs ivermectin (IVR), albendazole, (ALB) for lymphatic filariasis and praziquantel (PZQ) for schistosomiasis at scale. For safety reasons, one year of separate, stand-alone treatment was needed, after which all three drugs were given concomitantly in what has come to be known as Triple Drug Administration (TDA). In 2008, 9 Local Government Areas (LGAs) in north central Nigeria received a stand-alone treatment of IVR with ALB given to 1,367,927 adults and children, followed a week later by treatment with PZQ to 295,641 children. The next year IVR+ALB was administered to 1,355,455 persons, 303,346 of whom were children also receiving PZQ. Costing of the program was done through the use of work and travel logs, retrospective surveys and finance records for both MoH and partner organizations. Data collected included capital costs, salaries, transport, supplies, per diems, intervention materials, overheads, and time. Operational data were also collected for specific activities. These included advocacy, data management and reporting, drug delivery and distribution, field supervision, health education and community mobilization, M & E, morbidity control, planning and budgeting, procurement, and training. Total costs and costs per treatment were compared and cost curves examined for economies of scale and scope. Efficiency was measured using DEAP v2.1. From year 1 to year 2, total costs had reduced by nearly 60%. The greatest cost savings were seen in recurrent costs such as transportion and administration. Some diseconomies of scale were witnessed in districts with large populations due to distribution requirements in those areas; however costs were still lower than during the previous year and the cost curve had flattened from year 1 to year 2. Preliminary analysis of efficiency showed minor improvements in 2009 when compared to 2008, however consideration should be given to making intervention "packages" too complex. The up-front cost of a stand-alone distribution requires a higher, initial financial commitment before any cost savings can be realized.

798

THE CHALLENGES OF THE 'END-GAME' OF AFRICAN LF MDA PROGRAMS WITH RESPECT TO COENDEMICITY OF ONCHOCERCIASIS: MUST IVERMECTIN TREATMENT FOR ONCHOCERCIASIS CONTINUE WHEN LF TRANSMISSION HAS BEEN INTERRUPTED?

Frank Richards[1], A. Eigege[2], C. Gonzales-Peralta[3], A. Kal[2], Y. Sambo[2], S.E. Adelamo[2], J. Danboyi[2], B. Ibrahim[4], D. Pam[5], H. Mafuyai[5], G. Ogah[6], D. Goshit[4], J. Umaru[2], L. Rakers[1], J. Jiya[7], M. Anibueze[7], E. Miri[2]

[1]The Carter Center, Atlanta, GA, United States, [2]The Carter Center, Jos, Nigeria, [3]RB/CCI, Chula Vista, CA, United States, [4]Plateau State Ministry of Health, Lafia, Nigeria, [5]University of Jos, Jos, Nigeria, [6]Nasarawa State Ministry of Health, Lafia, Nigeria, [7]Federal Ministry of Health, Abuja, Nigeria

A strategy for the elimination of lymphatic filariasis (LF) in Africa is to provide combined mass drug administration (MDA) with ivermectin (IV) and albendazole (ALB). If MDA is administered with good treatment coverage, it is believed that LF transmission will be interrupted after 5-7 years and MDA can cease. LF occurs in all 30 local government areas (LGA) of Plateau and Nasarawa states of central Nigeria, while onchocerciasis occurs in just 12 of them. MDA with ivermectin (IV) has been administered in these 12 LGAs beginning in 1993, and together with ALB (for coendemic LF) since 2000. Assessments of LF in 2008 indicated that MDA with IV/ALB could be halted for LF in five of these coendemic LGAs. However, that led to the question of whether IV MDA as monotherapy needed to continue to be administered for onchocerciasis. Accordingly, in 2009 we conducted assessments to determine if onchocerciasis transmission has been interrupted and if IV could likewise be halted. Our skin snip study for microfilaridermia consisted of two elements 1) sampling of school-aged children resident in the five coendemic LGAs where LF transmission had been interrupted to determine if any recent Onchocerca infections had taken place, and 2) community-wide surveys conducted in six sentinel villages located in four other coendemic LGAs where 1992 baseline surveys showed a mean skin snip prevalence of 72%.

In the school surveys in five LGAs, we found only 1 skin snip positive among 2779 children (0.04%). In the six sentinel villages in four additional LGAs, we found 8 (0.4%) infections among 1919 persons. This represents a 98% decrease compared to the 1992 baseline. We believe that interruption of transmission of onchocerciasis throughout all or most of the two state area has likely been achieved, and that both IV and ALB can be stopped in the five LF/oncho coendemic LGAs. The next step should be the design and implementation of integrated post MDA surveillance for recrudescence of one or both filarial diseases.

799

DATA-CAPTURE AND DATA-MANAGEMENT FOR OPERATIONAL RESEARCH TO SUPPORT LARGE-SCALE NTD IMPLEMENTATION PROGRAMS

Alex L. Pavluck[1], Jeffrey Talbot[1], Kimberly Won[2], Dominique Kyelem[1], Brian Chu[1], Katherine Gass[1], Patrick Lammie[2], Eric Ottesen[1]

[1]Task Force for Global Health, Decatur, GA, United States, [2]Centers for Disease Control and Prevention, Decatur, GA, United States

To define the best diagnostic test for use in assessing end points in the elimination of Lymphatic Filariasis, an 8 country trial evaluating 8 different diagnostic tests was conducted. This study gathered data on more than 63,000 tests, from over 14,000 specimens, collected from nearly 10,000 individuals. To support the activities of this study, the Electronic Data Gathering and Evaluation (EDGE) system, a PDA based informatics solution, was developed. The EDGE system was designed to facilitate the collection of survey and gps data, to enable differential synchronization between in country and US based central servers, to utilize a bar code label tracking system, and to employ a specimen inventory management database. Additionally, the EDGE system produces on demand analysis datasets and provides secure online access to real-time reporting. Building on diagnostic knowledge gained from this initial study, 10 additional countries were designated as sites to evaluate a protocol for MDA stopping and post-MDA surveillance. The EDGE system was used to collect, manage, and report on these data. To date, activities from these studies have yielded an additional 15,000 test results. In anticipation of the need to expand collection of epidemiological data for monitoring and evaluation efforts to other NTD programs, a project to modify the EDGE system to utilize Wi-Fi and 3G communication technology is currently underway. This platform is designed to be cost effective, rapidly deployable, and flexible enough to support the operational needs of Lymphatic Filariasis, Schistosomiasis, STH, and Trachoma research projects.

800

EXPANSION OF THE UNITED STATES AGENCY FOR INTERNATIONAL DEVELOPMENT NTD CONTROL PROGRAM: 2010 AND BEYOND

Angela Weaver[1], Mary Linehan[2], Christy Hanson[1], Achille Kabore[3], Katie Zoerhoff[2], Ann Goldman[4], Scott Torres[2], Margaret Baker[5], Eric Ottesen[6]

[1]United States Agency for International Development, Washington, DC, United States, [2]RTI International, Washington, DC, United States, [3]Liverpool Associates in Tropical Health, Washington, DC, United States, [4]George Washington University/RTI International, Washington, DC, United States, [5]Georgetown University, Washington, DC, United States, [6]RTI International/Task Force for Global Health, Washington, DC, United States

In the 3½ years since the start of the U.S. Congressional and Presidential initiatives to support integrated efforts to control or eliminate 5 Neglected Tropical Diseases (NTDs), national programs in 12 countries have been enabled to progressively expand and provide more than 250 million (largely donated) drug treatments to 60 million individuals - most, multiple times - in yearly MDAs (mass drug administration) targeting lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminthiasis and trachoma. The success of this United States Agency for International Development NTD Control Program (managed by RTI International and its NGO partners) has resulted from a strategic design that demands full compliance with WHO guidelines and strict country selection criteria that include, most importantly, national government commitment to NTD control. With time a 'best practice' strategy for program implementation has evolved the following recommended, step-wise progression: 1) Country situation analysis - a standardized compilation of all existing NTD information; 2) Disease mapping - not prevalence maps, but action-maps defining where and what type of MDAs will be required; 3) Financial gap analysis - a standardized tool to identify the financial costs and needs for effective program implementation; 4) National strategy and plan of action (POA) - developed to meet WHO recommendations and guidelines, including integration of existing vertical NTD programs; 5) National stakeholders meeting - to agree to support the national POA and identify agreed roles and responsibilities; 6) Implementation of POA - with simple, but rigorous monitoring and evaluation; and 7) Advocacy within country - to ensure sustained support based on program success. Though challenges remain (e.g., policies for disease-specific mapping, treatment and surveillance; human capacity and training), this successfully developed model for effective program roll-out will serve as the foundation for the significantly expanded, new United States Agency for International Development support targeting the NTDs; details of this new program and its funding mechanisms will be available for presentation.

801

EFFECTS OF INTEGRATION ON FINANCING AND COVERAGE OF NEGLECTED TROPICAL DISEASE PROGRAMS

P.J. Hooper[1], Margaret Baker[2], Dominique Kyelem[1], Brian Chu[1], Mary Linehan[3], Katie Zoerhoff[3], Sanoussi Bamani[4], Roland Bougma[5], Massitan Dembele[4], Robert Dembele[4], Fiona Fleming[6], Ambrose Onapa[7], Alain Brice Pare[5], Seydou Toure[8], Scott Torres[3], Mamadou Traore[4], Bernadette Yoda[5], Eric Ottesen[1]

[1]Task Force for Global Health, Decatur, GA, United States, [2]Georgetown University, Washington, DC, United States, [3]RTI International, Washington, DC, United States, [4]Ministry of Health, Bamako, Mali, [5]Ministry of Health, Ouagadougou, Burkina Faso, [6]Schistosomiasis Control Initiative, London, United Kingdom, [7]RTI International, Kampala, Uganda, [8]Riseal, Ouagadougou, Burkina Faso

Neglected Tropical Disease (NTD) program activities are increasingly being integrated with the goal of improving resource availability and enhancing program reach. To evaluate the true impact of the United States Agency for International Development program for NTD control on program financing and coverage, data was collected by NTD program managers and non-governmental organization partners in Burkina Faso, Mali, and Uganda from 2 years prior to integration (2005, 2006) and 2 years post-integration (2007, 2008). Program coverage indicators included the number of people treated by district, geographic and therapeutic coverage, and program scale-up, by disease. Program financing indicators included financial support only and analyzed trends in governmental, internal non-governmental and external sources of funds, by disease. With United States Agency for International Development funding, overall geographical coverage and the number of persons treated for NTDs has increased, and high rates of therapeutic coverage of preexisting programs have been maintained. United States Agency for International Development funding has also enabled some countries to start treating persons for diseases not previously targeted (e.g., trachoma in Burkina Faso and Uganda) as well as to rapidly scale up existing programs (e.g., LF in Mali). When United States Agency for International Development funding began, NTD support from other sources in each country experienced post-integration changes that varied by disease; funding for those more established programs (i.e., LF and onchocerciasis) remained generally unchanged across study countries while for the more marginally supported programs (schistosomiasis, soil-transmitted helminths and trachoma) funding generally decreased. However, when the new United States Agency for International Development support is considered, every country experienced a significant increase in overall funding available for the integrated NTD programs. These findings suggest that significant decreases in external support might threaten program sustainability, so that seeking increased government commitment to establish NTD budget lines should be a top priority of these programs.

802

OVERCOMING THE CHALLENGES OF TARGETING THE NTDS IN THE URBAN SETTINGS OF AFRICA

Dominique Kyelem, Maureen Kimani, P.J. Hooper, Technical Working Group on Urban NTDs

Task Force for Global Health, Decatur, GA, United States

Neglected tropical diseases (NTDs) can be eliminated or controlled through effective Mass Drug Administration (MDA) to at risk populations. Urban settings, however, have proven to be a barrier to programmatic success, particularly because of challenges in mapping disease distribution and achieving satisfactory treatment coverage and compliance. In African NTD programs, the major challenges confronting *mapping* in urban settings are: (1) the large size of cities, (2) the lack of social and cultural homogeneity and (3) the rapid rural to urban migration contributing to slum development. The major challenges confronting the implementation of *MDA* in urban settings are related primarily to: (1) a complex urban social structure, (2) focal transmission in areas of low socioeconomic status resulting in an inappropriately low perception among decision-makers of the true disease threat and (3) inadequate advocacy and social mobilization leading to insufficient program resources and low population compliance. In-depth analysis of experiences of ongoing programs reported through detailed surveys and expert panels, has led to two sets of potential solutions to addressing NTD program success in urban settings. For the short term, recommendations include: (1) re-defining smaller implementation units and sampling frames that represent distinctly different levels of socioeconomic development and vector transmission foci; (2) implementing extensive social mobilization; (3) proper selection and in-depth training of drug distributors; and (4) strengthening pharmacovigilance. Longer term solutions will require operational research focused on (1) understanding the dynamics of LF transmission by different vectors in urban settings (which may impact both mapping and MDA costs); (2) use of hospital or clinic records to rapidly and effectively determine the endemicity status of NTDs; and (3) understanding the impact of imported cases on urban transmission.

803

ALLELE-SPECIFIC EFFICACY OF THE MONOVALENT APICAL MEMBRANE ANTIGEN 1 (AMA1) MALARIA VACCINE FMP2.1/AS02A

Amed Ouattara[1], Shannon Takala-Harrison[1], Drissa Coulibaly[2], Mahamadou A. Thera[2], Matthew B. Laurens[3], Amadou Niangaly[2], Renion Saye[2], Sheetij Dutta[4], D. Gray Heppner[4], Lorraine Soisson[5], Carter L. Diggs[5], Johan Vekemans[6], Joe Cohen[6], Amanda Leach[6], William C. Blackwelder[1], Ogobara K. Doumbo[2], Christopher V. Plowe[3]

[1]Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, [2]Malaria Research and Training Center, University of Bamako, Bamako, Mali, [3]Howard Hughes Medical Institute/Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, [4]Division of Malaria Vaccine Development, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [5]Malaria Vaccine Development Program, United States Agency for International Development, Washington, DC, United States, [6]GlaxoSmithKline Biologicals, Rixensart, Belgium

The limited efficacy to date of blood stage malaria vaccines may be explained by extreme genetic diversity in vaccine antigens resulting in allele-specific efficacy. Apical membrane antigen 1 (AMA1) gene sequences from clinical malaria episodes experienced by 400 children randomized 1:1 to receive either the FMP2.1/AS02A AMA1 malaria vaccine or rabies vaccine in a Phase 2 safety and efficacy trial conducted in Bandiagara, Mali were used to assess whether FMP2.1/AS02A vaccine is cross-protective against allelic variants of AMA1. Blood samples were collected at baseline, at days 90, 120, 150, 180, 210, 240, and whenever ill participants presented with malaria symptoms. For the primary analysis we defined homology to the vaccine strain 3D7 based on polymorphic codons in the cluster 1 loop (c1L) of Domain I of AMA1, a region previously shown to be immunologically important. For secondary analyses the definition of homology was extended to include polymorphic codons in Domain II and III. Time to first malaria episode with AMA1 c1L sequence identical to the five most frequent strains (3D7, Fab9, DD2, M5 and FVO) was assessed, the hazard of a clinical episode with parasites carrying AMA1 c1L homologous to any of these strains was measured, and FMP2.1/AS02A protective effect against malaria infection was evaluated. Vaccine efficacy against first malaria clinical episode with a 3D7-type c1L allele was 64% (p=0.03). The vaccine also showed significant efficacy against two strains that have c1L sequences identical to 3D7 but have differing amino acids in Domains II and III, but no significant efficacy against four strains with c1L different from 3D7. In conclusion, this first trial of the FMP2.1/AS02A malaria vaccine showed efficacy against strains with AMA1 c1L sequence identical to the vaccine strain 3D7, supporting the idea that polymorphism in this region of the molecule is an important determinant of allele-specific vaccine-induced immunity. A multi-allelic AMA1 vaccine is likely to be needed for broad efficacy against diverse parasites.

804

SAFETY, IMMUNOGENICITY AND IMPACT ON PARASITE MULTIPLICATION RATES OF THE CANDIDATE BLOOD-STAGE VACCINE AMA1-C1/ALHYDROGEL WITH THE NOVEL ADJUVANT CPG 7909 AGAINST BLOOD-STAGE MALARIA CHALLENGE IN HEALTHY MALARIA-NAIVE VOLUNTEERS

Christopher J. Duncan[1], Susanne H. Sheehy[1], Katie Ewer[2], Alexander D. Douglas[2], Fenella Halstead[2], Nick Edwards[2], Katharine Collins[2], Patrick J. Lillie[1], Ian Poulton[1], Angela Hunt-Cooke[1], Alison M. Lawrie[1], Simon J. Draper[2], Sarah C. Gilbert[2], Michael P. Fay[3], Kazutoyo Miura[3], Carol A. Long[3], Yimin Wu[3], Adrian V. Hill[1], Ruth D. Ellis[3]

[1]Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford, United Kingdom, [2]Jenner Institute, University of Oxford, United Kingdom, [3]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States

Human malaria challenges provide valuable evidence of candidate vaccine efficacy in non-immune populations prior to large-scale field trials. Growth-inhibitory assays (GIA) are widely used as a marker for biologic activity of the induced antibody, although there is no direct evidence that inhibitory activity in vitro relates to impact on parasite growth in humans. Inoculation of Plasmodium *falciparum* infected erythrocytes allows standardisation of the challenge dose, and may be more sensitive than sporozoite challenge for detecting subtle effects of blood-stage vaccines. We sought to compare in vivo and in vitro growth inhibitory activity of the bi-allelic AMA1 vaccine formulated on Alhydrogel, co-adjuvanted with the TLR agonist CPG 7909. We enrolled ten healthy malaria-naive volunteers aged 18-50 in the UK. Seven volunteers were immunised with two doses of AMA1-C1/Alhydrogel mixed with CPG 7909. Immunisations were safe, well tolerated and immunogenic, stimulating AMA1-specific antibodies and apparently higher levels of AMA1-specific T cells than have previously been reported with other protein-in-adjuvant blood-stage vaccines. Immune responses were boosted by the second dose of vaccine. Two vaccinated volunteers withdrew consent prior to challenge for non-clinical reasons. We inoculated the remaining five vaccinated volunteers and three unvaccinated controls with parasitised erythrocytes.

All challenged volunteers developed blood film patency at a similar level of parasitaemia to sporozoite challenged volunteers, but with a significant reduction in the frequency of symptoms. We observed no significant differences between the vaccinees and controls in time to first detectable PCR, time to positive blood film, magnitude of parasitaemia at blood film patency, or parasite growth rates. GIA is in progress to allow comparison with in vivo growth rates. We observed no evidence of an impact on in vivo parasite multiplication in this model.

805

PHASE 1 STUDY OF THE SAFETY AND IMMUNOGENICITY OF BSAM-2/ALHYDROGEL®+CPG 7909, AN ASEXUAL BLOOD STAGE VACCINE FOR *PLASMODIUM FALCIPARUM* MALARIA IN ADULTS IN MALI

Issaka Sagara[1], Ruth D. Ellis[2], Mahamadoun Hamady Assadou[1], Mamady Kone[1], Merepen A. Guindo[1], Renion Saye[1], Ousmane Guindo[1], Beh Kamate[1], Mohamed B. Niambele[1], Mahamadou S. Sissoko[1], Michael P. Fay[3], Kazutoyo Miura[2], Joan Aebig[2], Yimin Wu[2], Carole Long[4], Kelly Rausch[2], Alassane Dicko[1], Amagana Dolo[1], Dapa A. Diallo[1], Louis Miller[2], Ogobara K. Doumbo[1]

[1]Malaria Research and Training Center, Faculty of Medicine Pharmacy and Dentistry , University 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, [3]Biostatistics Research Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [4]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

A single blind, randomized, controlled Phase 1 clinical trial is being conducted to assess the safety and immunogenicity in malaria exposed adults of the *Plasmodium falciparum* blood stage vaccine BSAM-2, containing a four recombinant protein mixture of AMA1 (AMA1-FVO+AMA1-3D7) and MSP142 (MSP142-FVO+MSP142-3D7) / Alhydrogel® with the novel adjuvant CPG 7909. Participants are healthy adults 18-45 years old living in the village of Bancoumana, Mali. A total of 30 participants will receive up to 3 doses (Days 0, 56, and 120) of either BSAM-2 or Euvax® B/Hepatitis B vaccine. Enrollment and first vaccinations occurred in March and April of 2010. Initial vaccinations were well tolerated, with related adverse events being mostly mild or moderate injection site reactions. Adverse events and antibody responses up to two weeks after the third vaccination will be presented.

806

ANALYSIS OF CELL-MEDIATED IMMUNE RESPONSES IN VOLUNTEERS STERILELY PROTECTED AGAINST *PLASMODIUM FALCIPARUM* SPOROZOITE CHALLENGE FOLLOWING IMMUNIZATION WITH A GENE-BASED VACCINE

Martha Sedegah[1], Ilin Chuang[1], Shannon McGrath[2], Brent House[1], Harini Ganeshan[1], Jennylynn Lejano[1], Esteban Abot[1], Glenna Banania[1], Renato Sayo[1], Fouzia Farooq[1], Maria Belmonte[1], Nancy O. Richie[2], Chloe Wood[2], Carole A. Long[3], David Regis[1], Cindy Tamminga[1], Michele Spring[2], Keith Limbach[1], Noelle B. Patterson[1], Joe Bruder[4], Denise L. Doolan[5], Lorraine Soisson[6], Carter Diggs[6], Christian F. Ockenhouse[2], Thomas L. Richie[1]

[1]United States Military Malaria Vaccine Program, Naval Medical Research Center, Silver Spring, MD, United States, [2]United States Military Malaria Vaccine Program, Walter Reed Army Institute of Research, Silver Spring, 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]GenVec, Inc., Gaithersburg, MD, United States, [5]Queensland Institute for Medical Research, Brisbane, Australia, [6]United States Agency for International Development, Washington, DC, United States

We tested safety, immunogenicity and protective efficacy of a DNA prime / serotype 5 adenovirus boost *Plasmodium falciparum* (Pf) malaria vaccine in 15 malaria naïve adults. Both DNA and Ad5 constructs encoded the Pf circumsporozoite protein (CSP) and Pf apical membrane antigen-1 (AMA1). Three immunizations with DNA at four week intervals followed by a single immunization with Ad5 16 weeks later completely protected 4 of 15 volunteers against malaria sporozoite challenge. Antibody responses as measured by IFA, ELISA and growth inhibition assay were poor to moderate, and showed no relationship with protection. In contrast, *ex vivo* IFNg ELISpot assays performed at time of challenge using pools of synthetic 15mer peptides overlapping by 11 amino acids demonstrated that the two volunteers showing the strongest ELISpot responses to CSP and the three volunteers showing the strongest ELISpot responses to AMA1 were protected. Each of these individuals strongly recognized a single CSP and/or AMA1 peptide pool. In contrast, ELISpot responses of non-protected volunteers were more widely distributed among peptide pools and were not as robust. Significant recall responses were not observed in the fourth protected volunteer, to either antigen; we are currently synthesizing minimal HLA-restricted CD8+ T cell epitopes to see if the 15 mers used to stimulate the PBMC's from this volunteer may have been suboptimal for recalling responses. We are also conducting ELISpot assays following CD4+ and CD8+ T cell depletions as well as flow cytometry with ICS to delineate the role of different T-cell subsets in the protective responses observed in this trial. These encouraging results indicate the feasibility of protecting volunteers against Pf sporozoite challenge using a gene-based heterologous prime-boost approach designed to elicit strong cell-mediated immunity targeting the sporozoite and hepatic stages of the parasite.

807

THE FIRST PHASE 1/2A TRIAL OF THE METABOLICALLY-ACTIVE, WHOLE ORGANISM *PLASMODIUM FALCIPARUM* SPOROZOITE (PFSPZ) VACCINE

Judith E. Epstein[1], Kirsten E. Lyke[2], Matthew B. Laurens[3], Sharina Reyes[1], Cindy Tamminga[1], Martha Sedegah[1], Nicole Thomas[1], Jittawadee Murphy[4], Peter F. Billingsley[5], Eric James[5], Anusha Gunasekera[5], Charles Anderson[5], Sumana Chakravarty[5], Adam Richman[5], Ming Li[5], Adriana Ahumada[5], Jack Komisar[4], Jason Bennett[4], Walter Brandt[6], Christopher V. Plowe[3], Christian F. Ockenhouse[4], B. Kim Lee Sim[5], Robert Edelman[2], Thomas L. Richie[1], Stephen L. Hoffman[5]

[1]United States Military Malaria Vaccine Program, Naval Medical Research Center, Silver Spring, MD, United States, [2]Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, [3]Howard Hughes Medical Institute/Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, [4]United States Military Malaria Vaccine Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [5]Sanaria Inc., Rockville, MD, United States, [6]PATH Malaria Vaccine Initiative, Bethesda, MD, United States

It was shown in the 1970's that volunteers immunized by exposure to mosquitoes infected with radiation attenuated Pf sporozoites were protected when challenged by the bites of mosquitoes harboring fully infectious sporozoites, establishing a gold standard for the induction of sterile immunity to malaria. However, the technical difficulties of mass-producing a live sporozoite-based vaccine have, until recently, been considered insurmountable. Manufacturing obstacles have now been overcome and a metabolically-active (live), non-replicating (attenuated), cryopreserved Pf sporozoite (PfSPZ) vaccine suitable for parenteral injection has been produced (SanariaTM PfSPZ Vaccine). To evaluate the safety, tolerability, immunogenicity and protective efficacy of the SanariaTM PfSPZ Vaccine, we conducted a Phase 1/2a open-label, dose-escalation study in malaria-naïve, healthy adults aged 18 to 50 years. Eighty volunteers were randomized to receive four doses of intradermal or subcutaneous injection of the vaccine at 7,500, 30,000 or 135,000 sporozoites/ immunization. One group receiving high dose also received a fifth and sixth immunization. There were no vaccine-related severe or serious adverse events and reactogenicity to the study vaccine was mild in all dose groups, with approximately 85% of the reported adverse events (AEs) at an intensity level of Grade 1. There were no breakthrough infections, a finding supported by the absence of detectable antibodies to asexual stage parasites (IFAT) or to the blood stage antigens MSP-1 or EBA-175 (ELISA). In contrast, the vaccine induced antibody and T cell responses to whole sporozoites (IFAT, ELISpot) and antibody responses to the sporozoite

antigen PfCSP (ELISA) with a dose response observed. Protective efficacy was evaluated by challenging volunteers by the bites of 5 malaria-infected *Anopheles stephensi* mosquitoes. The full results of the safety, tolerability, immunogenicity, and protective efficacy of the vaccine and plans for subsequent clinical trials will be presented.

808

DEVELOPMENT OF A SAFE AND REPRODUCIBLE HUMAN SPOROZOITE CHALLENGE MODEL FOR *PLASMODIUM VIVAX* IN HEALTHY ADULTS IN THE UNITED STATES

Ilin Chuang[1], Jetsumon Sattabongkot[2], David Fryauff[1], Donna Tosh[3], Jittawadee Murphy[4], David Saunders[2], Jason Richardson[2], Jack William[4], Delia Bethel[2], Thomas Richie[1], Lisa Ware[5], Michele Spring[5], Mark Fukuda[6], Cindy Tamminga[1], Melanie Guerrero[3], Jason Bennett[5], James Cummings[3], Anjali Yadava[5], Jack Komisar[5], Mark Polhemus[1], Christian Ockenhouse[5]

[1]United States Military Malaria Vaccine Program, Naval Medical Research Center, Silver Spring, MD, United States, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]Clinical Trials Center, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [4]Department of Entomology, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [5]United States Military Malaria Vaccine Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [6]Walter Reed Army Institute of Research, Silver Spring, MD, United States

With ~70 to 80 million cases per year, *Plasmodium vivax* is the most widespread malarial infection of man, causing a highly debilitating disease characterized by multiple relapses. Increasing resistance to anti-malarials and to insecticides highlights the requirement for a *P vivax* vaccine and the urgency to develop a safe mosquito challenge model for vaccine and drug evaluation. This study is the first experimental challenge for *P vivax* conducted in the US under regulatory oversight. Objective: To conduct a proof-of-concept study to develop a safe and reproducible sporozoite challenge model for *P. vivax* in humans with a goal of 100% infectivity rate. Methods: Because *P. vivax* has proved difficult to culture *ex vivo*, *P. vivax*-infected blood from patients diagnosed with malaria in Mae Sot, Thailand was fed through membrane to laboratory-colonized *Anopheles dirus* mosquitoes from AFRIMS insectary. The blood was screened for potential confounding pathogens including other malaria species, filariasis, Japanese encephalitis, chikungunya, HIV, and hepatitis B & C viruses. Mosquito batches with highest oocyst infection and fed on pathogen negative blood were selected for an initial challenge of six healthy US volunteers, who subsequently received five infectious bites and were monitored for development of parasitemia by daily blood smears from day 5 until volunteers have 3 consecutive negative smears post treatment. All six developed parasitemia between days 12 to 14 post challenge and were treated with chloroquine @ 1500 mg base for 3 days and primaquine @ 30 mg/day for 14 days by direct observation therapy, with six month follow-up to document resolution of all symptoms and lack of recurrences. A second cohort will be conducted in summer 2010 to demonstrate reproducibility of the challenge procedure. Safety data, prepatent period, relapse rate, and parasite genotype data for both challenges will be presented.

809

ESTABLISHING THE MALARIA HUMAN CHALLENGE MODEL IN A NEW REGULATORY ENVIRONMENT

Angela K. Talley[1], Sara A. Healy[2], Olivia C. Finney[1], Sean C. Murphy[3], Ruobing Wang[1], Stefan H. Kappe[1], Jittawadee R. Murphy[4], Chris F. Ockenhouse[4], Donald G. Heppner[4], Patrick E. Duffy[1]

[1]Malaria Clinical Trials Center, Seattle Biomedical Research Institute, Seattle, WA, United States, [2]Department of Pediatrics, Division of Pediatric Infectious Diseases, University of Washington Medical Center, Seattle, WA, United States, [3]Department of Laboratory Medicine, University of Washington Medical Center, Seattle, WA, United States, [4]United States Military Malaria Vaccine Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States

Malaria human challenge studies are a critical assessment tool to refine leading vaccine candidates and ensure that only the most viable candidates advance to large-scale trials. However, the existing worldwide infrastructure for conducting these unique studies is inadequate to meet the projected global need over the next decade. In partnership with the Walter Reed Army Institute of Research (Walter Reed Army Institute of Research) and the PATH Malaria Vaccine Initiative (MVI), the malaria human challenge model was recently established at Seattle Biomedical Research Institute (Seattle BioMed). Activities at both centers were aligned to adapt to a new regulatory environment for conduct of the malaria human challenge model. Production of the challenge material was described in a Biologics Master File (BB-MF) and the challenge conducted under an Investigational New Drug Application (IND), both on file with the FDA. Likewise, efforts to harmonize the challenge model procedures with other centers worldwide are ongoing. In order to demonstrate establishment of the quality systems and procedures required to conduct the model, a demonstration challenge trial was conducted in six healthy malaria-naïve volunteers. Volunteers were challenged with wild-type NF54 strain *Plasmodium falciparum* by the bite of five infected *Anopheles stephensi* mosquitoes under controlled conditions. All participants developed a patent parasitemia and were treated upon first positive blood smear with standard doses of chloroquine by directly observed therapy. Immunologic assessments (IFN-γ EliSPOT and antibody ELISA) and qRT-PCR detection of subpatent parasitemia were performed. Prepatent and incubation periods and adverse event profile were consistent with published reviews of challenge cohorts at other centers. No serious adverse events occurred. Standardization of the human challenge model will ensure consistency in early-phase malaria vaccine efficacy assessment across centers and uniformly stringent safety monitoring of volunteers. Expanded infrastructure provides critical mutual backup for infected mosquito production between centers to reduce disruptions to product development and clinical testing.

810

TRENDS IN HOSPITALIZATION FOR CYSTICERCOSIS-UNITED STATES, 1981-2000

Peter M. Schantz[1], D.J. Thurman[2], Y-H Luo[2], V.C.W. Tsang[2]

[1]Department of Global Health, School of Public Health, Emory University, Atlanta, GA, United States, [2]School of Public Health, Emory University, Atlanta, GA, United States

Recent studies suggest that the occurrence of cysticercosis may be increasing in the United States. The purpose of this study was to examine secular trends in the diagnosis of cysticercosis among persons admitted to U.S. hospitals since 1980. Using data from the National Hospital Discharge Survey for 1981 through 2000, we estimated annual numbers and rates of cysticercosis-related hospitalization for the U.S. population. During this interval, an estimated 23,000 patients were hospitalized with this diagnosis; when averaged over 5-year periods, rates increased consecutively. The mean annual rate during 1996-2000 (6.6 per million population) was nearly five times the mean annual rate during 1981-1985

(1.3 per million population). Rates were highest in the western United States. Enhanced public health surveillance of this condition appears warranted to focus prevention programs with greater efficiency and reverse trends identified in this study.

811

CYSTIC ECHINOCOCCOSIS IN SOUTHERN AFRICA: MANY OPEN QUESTIONS

Kerstin Wahlers[1], Colin N. Menezes[2], Michelle Wong[3], Peter Kern[1], Martin P. Grobusch[4]

[1]Comprehensive Infectious Diseases Center, University Hospitals Ulm, Ulm, Germany, [2]Helen-Joseph-Hospital, University of Witwatersrand, Johannesburg, South Africa, [3]Chris-Hani-Baragwanath-Hospital, University of Witwatersrand, Johannesburg, South Africa, [4]Department of Internal Medicine, University of Witwatersrand, Johannesburg, South Africa

Cystic Echinococcosis (CE) is a zoonotic infection caused by the cestode Echinococcus granulosus. The life cycle of the parasite involves herbivores like sheep, goats, cattle and others as intermediate hosts and carnivores as the definite host. CE is highly endemic in Northern and Eastern Africa, but few epidemiological data on hydatid disease from Sub-Saharan Africa exist. Transhumant pastoralist populations such as in the Turkana region of Kenya carry the highest burden of disease. To date no published epidemiological data of note on hydatid disease exist from Southern Africa; however, there are a number of case reports and the disease is considered to be common, particularly in rural communities. In addition, little is known about co-infections with CE and either HIV or Tuberculosis (TB), or viral hepatitides; whilst it has been observed that the clinical course and treatment outcome can be significantly altered, as there is evidence that the host immune response to the larval cyst greatly influences the clinical course of CE. Unusual cases of disseminated CE have been documented in patients co-infected with HIV and also the Tb-specific response in patients co-infected with echinococcosis and TB is diminished, but improves with successful treatment of echinococcal infection. Apart from more severe clinical courses of CE in the presence of HIV and/or TB, treatment of all three conditions poses a great problem due to significant drug interactions and the possibility of Immune Reconstitution Inflammatory Syndrome (IRIS). We present a retrospective analysis of patients with CE treated between 1995 and 2009 at two large urban hospitals in Johannesburg with a large population of migrant workers from rural areas. The analysis is conducted with particular reference to geographical distribution of cases, risk factors for acquiring CE and presentation and course of disease in the presence of co-infections with HIV and/or TB.

812

HUMAN CYSTIC ECHINOCOCCOSIS IN PERUVIAN ENDEMIC AREAS

Saul J. Santivanez[1], Cesar G. Naquira[2], Cesar M. Gavidia[3], Armando E. Gonzalez[3], Silvia Rodriguez[4], Luis Tello[1], Eddy Hernandez[1], Manuela R. Verastegui[2], Hector H. Garcia[5]

[1]Instituto Peruano de Parasitologia Clinica y Experimental, Lima, Peru, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Facultad de Medicina Veterinaria, Universidad Nacional Mayor de San Marcos, Lima, Peru, [4]Cysticersosis Unit, Instituto de Ciencias Neurologicas, Lima, Peru, [5]Department of Microbiology, Universidad Peruana Cayetano Heredia, Lima, Peru

Cystic Echinococcosis (CE) is a zoonosis widely distributed around the world; CE affects principally to livestock and accidentally to humans; CE is acquired by the ingestion of *Echinococcus granulosus* eggs that are transmitted by infected dogs. Clinical presentation is characterized by the formation of cystic lesion in the infected host; liver is the principal organ involved (52 to 77%), followed by the lungs (9 to 44%); signs and symptoms of CE depend on the organ involved, the size of the cyst and the presence of any complication, it is asymptomatic during the first years of the infection. Diagnoses is based on imaging techniques (ultrasound-US, chest X Ray, CT scan), serological evaluation is used to support the presumptive diagnose. Peru is considered an endemic country, previous studies in endemic regions showed a CE prevalence of ~7%; nevertheless there is not any current control program. The aim of the present study is to determine the current human CE prevalence in 3 endemic regions. We performed an imagenological (abdominal US, and Chest X-Ray) and serological evaluation (Electro immune transfer blotting) in the population of 9 districts located in 3 Peruvian endemic regions (Junín, Huancavelica y Puno); A total of 1456 participants (Junin), 1675 participants (Puno) and 953 (Huancavelica) were evaluated. In Junin, we found a liver compromise in 4.7% (52 of 1106), lung involvement in 1.96 % (20 of 1018), and a serological positive result in 4.8% (57 of 1188); In Huancavelica, we found a liver involvement in 1.47% (12 of 690), lung involvement in 0.72 % (5 of 691), and a serological positive result in 3.1% (15 of 498); In Puno, we found a liver involvement in 2.33% (26 of 1114), lung involvement in 0.93% (11 of 1185), and a serological positive result in 2.86% (29 of 1014). Human CE is still a public health problem in Peruvian endemic areas, furthermore despite there is not any control program in endemic regions, these results showed a decrease in the last 10 years, (from ~7% to ~5%); nevertheless it is important to recognize the necessity of a local control program to reduce the prevalence found in our study.

813

THE IMPACT OF CYSTIC ECHINOCOCCOSIS ON HUMAN HEALTH AND QUALITY OF LIFE IN RIO NEGRO PROVINCE, ARGENTINA

Glenda M. Bingham[1], Christine M. Budke[1], Edmundo Larrieu[2], G. Mujica[3]

[1]Texas A&M University, College Station, TX, United States, [2]Coordinador Provincial Salud Ambiental, Ministerio de Salud, Provincia de Rio Negro, Argentina, [3]Veterinario Privado, Ingeniero Jacobacci, Provincia de Río Negro, Argentina

Cystic echinococcosis (CE), a zoonosis which results in substantial morbidity and economic losses for affected individuals, is highly endemic in Rio Negro Province, Argentina (RNP). A cross-sectional study, which incorporated abdominal ultrasound screening and a questionnaire, was conducted in a rural community in RNP in order to evaluate potential risk factors for CE as well as the health and financial impacts of the disease. A total of 341 adult participants were examined, 33 (9.7%) of whom were identified as being CE positive. The participants, 78% of whom were female, ranged in age from 18 to 89 years, with a mean age of 42 years. Neither age, nor gender, was significantly associated with being CE positive. However, CE positive participants had a significantly ($p = .02$) higher unemployment rate compared to CE negative participants, with rates of 75% and 56%, respectively. In order to examine the impact of CE on the physical and mental health and quality of life of previously undiagnosed individuals, the short form 12 v2 (SF-12v2) health survey was administered to all participants prior to abdominal ultrasound examination. This study found that individuals with a positive diagnosis for CE had a significantly lower mean score for five (physical functioning, role physical, bodily pain, general health, and role-emotional) of the eight domains measured by the health survey. The CE positive participants' mean scores for vitality, social functioning, and mental health, did not differ significantly from those of CE negative individuals. These results suggest that CE is associated with a decrease in the overall health and quality of life of patients prior to formal diagnosis, which is likely to be associated with decreased productivity and a diminished ability to earn income. The results of the questionnaire and the SF-12 v2 health survey will be used in the evaluation of indirect costs associated with human CE among previously undiagnosed individuals. These data will then be incorporated into a larger project examining the total economic impact of CE in this region.

814

G1 STRAIN OF *ECHINOCOCCUS GRANULOSUS* INFECTS SOUTH AMERICAN CAMELIDS IN PERU

Saul J. Santivanez[1], Mara Rosenzvit[2], Cesar M. Gavidia[3], Mara Martínez[4], Mary L. Rodriguez[5], Marcela Cucher[2], Nelly Huamani[6], Hector H. Garcia[7]

[1]Instituto Peruano de Parasitologia Clinica y Experimental, Lima, Peru, [2]Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina, [3]Facultad de Medicina Veterinaria, Universidad Nacional Mayor de San Marcos, Lima, Peru, [4]Instituto de Patobiología, Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina, [5]Universidad Peruana Cayetano Heredia, Lima, Peru, [6]Dirección Regional de Salud Ayacucho, Ayacucho, Peru, [7]Department of Microbiology, Universidad Peruana Cayetano Heredia, Lima, Peru

Cystic echinococcosis (CE) is a zoonosis widely distributed throughout the world. CE is caused by the taenia *Echinococcus granulosus* that lodges in the intestines of dogs. Herbivores and, accidentally, humans could be infected by the ingestion of *E. granulosus* eggs, resulting in cystic larvae in their liver, lungs, or other tissues. Molecular identification of Echinococcus strains were described as important information to be considered for implementing local control measures in endemic countries. Previous studies performed in Peru and other South American countries described the presence of G6 strain (camel strain) in human and animal population, in addition to the knowledge of CE infection in South American camelids. In order to assess if South American camelids behave as a natural reservoir of the G6 strain (present in goats in the region), we determined the strains of *E. granulosus* in samples from infected South American camelids. First we carried out a survey using PCR and CO1 sequencing of *E. granulosus* isolates collected from llamas located in Peruvian Andes; macroscopic information on the appearance, size, and status of the larvae was collected; the nature and fertility of the sample were confirmed by microscopic observation; total *E. granulosus* DNA was extracted using the DNeasy Tissue kit (QIAGEN, Hilden, Germany); using PCR reactions, and *E. granulosus* genotype was determined by mitochondrial cytochrome c oxidase subunit 1 (CO1) sequencing; sequences were compared using Macrogen kit (Korea). DNA was amplified from 12 isolates; 3 isolates (25%) were identified as G1 and 9 (75%) did not correlate with known strains but had 81% identity to CO1 of Taenia hydatigena, leaving their origin unclear. In this small series, G1 was present and G6 was absent in South American camelids.

815

IN VITRO SCREENING FOR NEW COMPOUNDS AGAINST *ECHINOCOCCUS MULTILOCULARIS* METACESTODES IDENTIFIES ANTI-ECHINOCOCCAL ACTIVITY OF MEFLOQUINE

Britta Stadelmann, Tatiana Küster, Corina Herrmann, Sabrina Scholl, Joachim Müller, **Andrew Hemphill**

University of Berne, Berne, Switzerland

Alveolar echinococcosis is caused by the metacestode stage of the fox tapeworm *Echinococcus multilocularis* and causes severe disease in the human liver and occasionally other organs, which is fatal if treatment is unsuccessful. The present chemotherapy of AE is based on mebendazole and albendazole, which has been found to be ineffective in some instances, parasitostatic rather than parasiticidal, and usually involves the lifelong uptake of massive doses of drugs. Thus, new treatment options are urgently needed. Within this study, a recently validated parasite viability assay was applied, based on the release of phosphoglucose isomerase (PGI) by dying parasites. A range of 30 thiazolides, 19 pentamidine- and 12 artemisinin-derivatives, and of mefloquine and its (+) and (-) erythro-enantiomers, were tested for their efficacy against E. multilocularis metacestodes *in vitro*. Initial screening of compounds was performed at 40 µM, and those compounds exhibiting considerable anti-parasitic activity were assessed also at lower concentrations. Mefloquine was chosen for subsequent studies. *In vitro* mefloquine treatment at 24 µM resulted in rapid and complete detachment of large parts of the germinal layer from the inner surface of the laminated layer within a few hours, and prolonged treatment for a period of 10 days was parasiticidal as determined by bioassay in mice. Interestingly, as determined by the PGI-assay, the (-) erythro-enantiomer of mefloquine was more active than the (+) enantiomer or a mixture of both erythro-enantiomers. Affinity chromatography employing epoxy-agarose-coupled mefloquine and E. multilocularis extracts identified fructose-bi-phosphate-aldolase and malate-dehydrogenase as mefloquin-binding proteins. In conclusion, mefloquine represents an interesting drug candidate, and is currently followed in appropriate *in vivo* studies on alveolar echinococcosis in the mouse model.

816

LONG-LASTING EFFECT OF OXFENDAZOLE AGAINST CYSTIC ECHINOCOCCOSIS IN NATURALLY INFECTED SHEEP

Cesar M. Gavidia[1], Eduardo A. Barron[1], Carlos Angulo[1], Manuela R. Verastegui[2], Beatriz Truel[1], Diego Diaz[1], Jean M. Cerna[1], Saul Santivañez[2], Hector H. Garcia[2], Robert H. Gilman[3], Armando E. Gonzalez[1]

[1]San Marcos University, Veterinary School, San Borja, Peru, [2]Universidad Peruana Cayetano Heredia, San Martin de Porres, Peru, [3]Bloomberg School of Public Health, The Johns Hopkins University, Baltimore, MD, United States

Cystic Echinococosis (CE) is a zoonotic disease caused by larval stage *Echinococcus granulosus*. It is a public health problem with considerable economical losses in both humans and animals. Treatment options in animal intermediate hosts might have two objectives: animal model for drug use in humans, or eliminate / reduce the parasite burden in animals as potential control strategy. We determined the long-lasting effect of high dose of Oxfendazole (OXF) against CE in naturally infected sheep. A randomized placebo-controlled trial was carried out on 10 randomly selected ewes. Liver ultrasound was performed in order to select those infected sheep. They were assigned to one of the following groups: 1) placebo (n=3); 2) OXF 60mg/Kg of body weight (BW) weekly for four weeks (n=7). Necropsies were performed 7 month after finishing the treatment. Treated animals had significantly smaller cysts in both lung and liver than the controls (approximately 22mm smaller) ($p<0.05$). Percent protoscolex (PSC) viability, evaluated using a 0.1% aqueous eosin vital stain for each cyst, demonstrated a significant decrease in the treatment group as compared to the control ones for both organs lungs and livers (more than 50% reduction) ($p<0.05$). Follow-up ultrasound examination also exhibited a progressive degeneration stages in treated sheep. We demonstrate that Oxfendazole at 60mg weekly is a successful schema that can be added to control measures in animals and merits further study for the treatment of animal CE. Apparently, Oxfendazole has a long-lasting effect on cyst degeneration at least for 7 months after stopping the treatment. Further investigations on different schedules of monotherapy or combined chemotherapy are needed, as well as studies to evaluate the safety and efficacy of Oxfendazole in humans.

817

BABESIA SPP. IN WHITE-TAILED DEER AND EASTERN COTTONTAIL RABBITS IN TENNESSEE

Charissa Fritzen[1], Junjun Huang[1], Kathleen Westby[1], Roger D. Applegate[2], Sam R. Telford, III[3], Michael Yabsley[4], L. Rand Carpenter[1], Emily Mosites[1], John Dunn[1], Abelardo Moncayo[1]

[1]Tennessee Department of Health, Vector-Borne Diseases Section, Communicable and Environmental Diseases, Nashville, TN, United States, [2]Tennessee Wildlife Resources Agency, Nashville, TN, United States, [3]Tufts University Cummings School of Veterinary Medicine, North Grafton, MA, United States, [4]University of Georgia, Southeastern Cooperative Wildlife Disease Study, Athens, GA, United States

Babesiosis is an emerging zoonotic disease in the United States caused by protozoan parasites of the genus *Babesia*. *Babesia spp.* parasites are transmitted by hard ticks (Ixodidae). Endemic human disease in the United States occurs in the northeast and upper Midwest. Most human cases in the United States are caused by *B. microti*. Recently there have been human cases in Missouri, Washington and Kentucky attributed to a *B. divergens*-like protozoan termed "MO-1". The first confirmed human case of babesiosis in Tennessee occurred in 2008. To investigate the potential public health risks of babesiosis in Tennessee we conducted an environmental survey in the area where the Tennessee babesiosis case reported exposure to wildlife and ticks. To assess potential reservoirs for common North American *Babesia spp.* we collected blood samples from 5 white tailed deer (*Odocoileus virginianus*) and from 7 eastern cottontail rabbits (*Sylvilagus floridanus*). Spleens from 8 rabbits were collected and stored. Additionally, 167 *Ixodes scapularis* and 1 *Amblyomma americanum* were collected from wildlife and flannel tick drags and stored for *Babesia* parasite screening using conventional PCR. Serum was separated and detection of antibodies to *B. odocoilei*, *B. microti*, and *B. divergens*-like MO-1 was determined by indirect immunofluorescence. Serum samples were considered reactive with an IgG titer ≥1:64. Evidence of exposure to a specific *Babesia spp.* was determined by comparing the titers of the 3 *Babesia spp.* tested. A four-fold higher titer was considered evidence of exposure to a specific *Babesia spp.* Four deer serum samples were reactive to *B. odocoilei* antigen, with the last deer serum sample being non-reactive. Two of the rabbit serum samples were non-reactive, 2 were reactive to *B. odocoilei* antigen, and 3 were reactive to MO-1 antigen. We described wildlife with serologic evidence of exposure to *B. odocoilei* and MO-1 parasites in the environment most likely associated with transmission of *Babesia* in Tennessee. Since *B. MO-1* has been attributed to human cases in the United States, including nearby Kentucky, babesiosis should be considered in the differential diagnosis of patients in Tennessee with febrile illness and hemolytic anemia.

818

IDENTIFICATION OF *BARTONELLA* SPECIES IN SMALL MAMMALS FROM AN URBAN AND A RURAL LOCATION IN KENYA

Michael Kosoy[1], Jennifer Iverson[1], Ying Bai[1], Darryn Knobel[2], Jo Halliday[3], Bernard Agwanda[4], Robert Breiman[5]

[1]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [2]University of Glasgow, Glasgow, United Kingdom, [3]University of Edinburgh, Edinburgh, United Kingdom, [4]National Museum of Kenya, Nairobi, Kenya, [5]Centers for Disease Control and Prevention - KEMRI, Nairobi, Kenya

Bacteriological and ecological investigations of small mammals from rural homesteads within Nyanza Province in western Kenya, and from Kibera, an urban slum in Nairobi were conducted in order to characterize the distribution and diversity of *Bartonella* spp. in East Africa. Small mammals were trapped using Sherman live traps placed in and around human dwellings. Whole blood from mammals was placed on blood-enriched agar to culture *Bartonella* bacteria. Additionally, blood samples were screened by PCR using citrate synthase gene (*gltA*) primers for identification of non-cultivable bacteria. *Bartonella* was identified in 13/53 (24.5%) small mammals of 4 species from Nyanza Province: *Rattus rattus* (3), *Mastomys natalensis* (6), *Crocidura olivieri* (3), and *Lemniscomys striatus* (1). In Kibera, 20/33 (60.6%) peri-domestic rats but 0/146 house mice were *Bartonella* positive. Sequence analyses of the *gltA* revealed that Kenyan rodents harbored 4 *Bartonella* spp.: *B. tribocorum* (16), *B. elizabethae* (12), *B. queenslandensis* (5), and a novel strain, which is different from all known *Bartonella* species with the highest percent of identity to *B. birtlesii*. The most prevalent *Bartonella* spp. (*B. elizabethae* and *B. tribocorum*) found in Kenyan rats are highly specific for *Rattus* and *Bandicota* rats in Asian countries. In America and Europe, both *Bartonella* spp. were detected only in urban settings from two domesticated rats, which were introduced within historic times to these continents from Asia. By contrast, in Kenya, these *Bartonella* species were observed not only among peri-domestic rats in Kibera, but also in wild rodents (*M. natalensis* and *L. striatus*) from the rural area. Surprisingly, domestic rats in Kibera also carried *B. queenslandensis*, the bacterium previously identified in Queensland, Australia. The genetic diversity of *Bartonella* spp. found in Kenya is significantly lower than in Asia. The novel strain was detected in the African giant shrew (*C. olivieri*) and additional genetic and phenotypical characterizations are required for description of the new bacterial species. The isolation of the human pathogenic *B. elizabethae* from rodents trapped in close proximity to humans, in an area of high HIV prevalence, raises the possibility of spillover of this pathogen into humans.

819

MOLECULAR CHARACTERIZATION OF *BARTONELLA* SPECIES ASSOCIATED WITH DOGS AND THEIR FLEAS AND TICKS IN THE COASTAL LOWLANDS OF MANABI, ECUADOR

Shari Lydy[1], Ruben Aleman[2], Andrea Enriquez[2], Allison Earon[3], Josselyn Garcia[2], Michael Levin[1], Mario J. Grijalva[4]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Catholic University of Ecuador, Quito, Ecuador, [3]Emory University, Atlanta, GA, United States, [4]Ohio University, Athens, OH, United States

Bartonella cause both zoonotic and vector-borne diseases and infect a wide variety of vertebrate hosts. Thirteen species of *Bartonella* have been associated with human disease while eight species are known to be pathogenic for dogs. In Ecuador, we investigated *Bartonella* species in the blood of domestic dogs and in ectoparasites found on those dogs. Fleas, ticks, and blood samples were collected from 22 dogs residing at twenty-one households in the rural communities of El Bejuco and San Francisco, Manabi Province, Ecuador during June-July 2009. Ectoparasites were collected in 70% ethanol, washed in water, processed by freezing in liquid nitrogen and crushing, and their DNA was extracted. Genetic analysis included PCR amplification of *Bartonella* RibC or GltA gene fragments and sequencing. Fleas and ticks were identified using taxonomic keys along with molecular analyses of mitochondrial 12S and 16S rDNA gene sequences. Matched blood samples to ectoparasite collections were obtained from 17 dogs by venipuncture using EDTA-vacutainers, and DNA was extracted and analyzed as above. The number of fleas collected from single dogs ranged from 1-24, with multiple flea genera being found on single dogs in many cases. Fleas were identified as *Ctenocephalides felis*, *C. canis*, *Pulex irritans*, and *Xenopus cheopis*. In addition, ticks (engorged and flat) were collected from 12/17 dogs, with 1-7 ticks collected per dog. Ticks were identified as *Amblyomma triste*, *A. ovale*, one *Rhipicephalus* spp, and one *Dermacentor* spp. In blood samples taken directly from dogs, only *B. henselae* was detected by PCR in 5/17 dogs. This is in contrast to *Bartonella* spp detected by PCR in both dog-associated fleas and ticks that included *B. vinsonii* subsp. *berkhoffii*, *B. quintana*, *B. henselae*, *B. tribocorum, and B. elizabethae*. Excluding *B. tribocorum*, these *Bartonella* spp are all pathogenic for dogs. Our findings suggest that further investigations of the ecology and epidemiology of *Bartonella* in Ecuador are warranted.

820

SOCIOECONOMIC AND ENVIRONMENTAL FACTORS: INCREASING THE POTENTIAL RISK OF TICK-BORNE DISEASES IN THE REPUBLIC OF KOREA

Terry A. Klein[1], Heung-Chul Kim[2], Jin-Won Song[3], Joon-Seok Chae[4], Won-Jong Jang[5], Kyung-Hee Park[5], William J. Sames[6], Allen L. Richards[7]

[1]65th Medical Brigade, APO, AE, United States, [2]5th Medical Detachment, 168th Multifunctional Medical Battalion, APO, AE, United States, [3]College of Medicine, Korea University, Seoul, Republic of Korea, [4]College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea, [5]College of Medicine, Konkuk University, Seoul, Republic of Korea, [6]Armed Forces Pest Management Board, Washington, DC, United States, [7]Rickettsial Diseases Research Program, Naval Medical Research Center, Silver Spring, MD, United States

Man-made and natural ecological events have changed the landscape of the Republic of Korea (ROK) since the 1900's. During the Japanese occupation (1910-1945), forested hillsides were cleared, leaving hills and mountains largely covered by grasses and shrub vegetation. Following WWII (>1945), local populations scavenged for wood for heating during the cold winters and cooking. Just as the ROK was recovering, North Korea attacked, beginning a long drawn-out conflict (1950-1953), which ended in an uneasy cease fire between the two countries. In the 1960's, a tree planting policy was instituted that reestablished the long-ago lavish forested mountains and hillsides that make up >70% of the ROKs landscape. Today, mountains and hillsides are completely forested, which has resulted in increased protection and habitat for large and small mammals, birds, and associated tick populations, all of which increase the establishment and maintenance of zoonotic tick-borne pathogens in these populations and potential transmission to man. A tick-borne disease surveillance program established by the Eighth US Army (2001) has resulted in an increased knowledge of the prevalence of known tick-borne pathogens and identification of new pathogens in small and large mammals, their ectoparasites, and man. The increased use of permethrin-treated uniforms and repellents, reduce the risk of potential for transmission of tick-borne pathogens to US military, but not US civilians and family members while conducting outdoor activities. In addition, training environments of unmanaged forests and grasslands with abundant small and large mammal populations at some field training sites remain largely unchanged, with personnel training in tall grass/forested areas and sleeping in tents that abut forested areas where animals and their ectoparasites are present. Increased surveillance by military personnel and civilian agencies has expanded our knowledge of the prevalence tick populations and tick-borne pathogens, including several spotted fever group rickettsial pathogens previously unreported in the ROK.

821

DETECTION OF *RICKETTSIA* SPECIES IN ECTOPARASITES FROM AREAS OF COSTA RICA ENDEMIC FOR SPOTTED FEVERS

Adriana Troyo, Lizeth Taylor, Ólger Calderón-Arguedas, Carlos Mata, Jusara Ortíz, Adrián Avendaño, Laya Hun-Opfer

Centro de Investigación en Enfermedades Tropicales, Universidad de Costa Rica, San Jose, Costa Rica

The ecology of spotted fevers in Costa Rica is not well known: even though outbreaks have been reported since the 1950s, the most recent studies date back to the 1980s and the vectors responsible for transmission to humans have not been directly identified. Moreover, rickettsial diseases in several regions of the world have been associated recently with species of *Rickettsia* that had not been described or were considered non-pathogenic to humans. In this study, species of *Rickettsia* were detected in ectoparasites from five areas of Costa Rica where cases of spotted fevers have been reported. In each area, ectoparasites were collected using drag cloths or directly from domestic and wild animals, identified, and organized into lots according to species, host, and location. One pooled sample of 1 to 10 specimens was prepared from each lot for PCR analysis. Specific *Rickettsia* DNA fragments of the *gltA* (citrate synthase), *htrA* (17 kDa protein), and *ompA* (190 kDa protein) genes were detected by PCR in each of the pools, and pools were considered positive when fragments of at least two of the genes were evidenced. Several of the *gltA* gene fragments were sequenced to confirm the presence of *Rickettsia* and identify species. A total 205 pools of ectoparasites were analyzed, and 29% were considered positive for *Rickettsia* DNA by evidence of at least two of the gene fragments. These included 62% of *Amblyomma cajennense,* 60% of *Ctenocephalides felis,* 44% of *Amblyomma ovale*, 18% of *Boophilus microplus*, 10% of *Dermacentor nitens*, and 4% of *Pulex simulans* pools analyzed. No *Rickettsia* DNA was detected in *Rhipicephalus sanguineus* (39 pools). Positivity also varied between the areas evaluated. Sequencing of gene fragments confirmed the presence *Rickettsia felis* as well as species from the typical spotted fever group (SFG). Results show the presence of rickettsiae in vectors that may be responsible for transmission to humans in Costa Rica, and evidence suggests rickettsial infection in the human environment may be common. This is the first study to report different species of *Rickettsia* in various species of ticks and fleas in Costa Rica.

822

A COMPARATIVE META-ANALYSIS OF TICK PARALYSIS IN NORTH AMERICA AND AUSTRALIA: EPIDEMIOLOGY, CLINICAL AND ELECTRODIAGNOSTIC MANIFESTATIONS AND OUTCOMES

James H. Diaz

Louisiana State University Health Sciences Center, New Orleans, LA, United States

Tick paralysis (TP) is a neurotoxic poisoning that mimics polio and primarily afflicts young girls worldwide, especially in hyperendemic regions of the North American Pacific Northwest and Eastern Australia. A comparative meta-analysis of the scientific literature was conducted using Internet search engines to assess the epidemiology, clinical and electrodiagnostic manifestations, and outcomes of TP in North America versus Australia. Well-documented cases of TP were collected in North America and Australia. Continuous data including age, time to tick removal, and duration of paralysis were compared for statistically significant differences by unpaired t-tests; categorical demographic data including sex, geographic distribution, tick vector, and misdiagnosis were compared for statistically significant differences by chi-squares. TP following gravid female ixodid tick bites occurred seasonally and sporadically in individuals and in more clusters of children than in adults of both sexes in urban and rural locations in North America and Australia. The case fatality rate for TP was low, and the proportion of misdiagnoses of TP as the Guillain-Barré syndrome (GBS) was greater in North American than in Australia. Although electrodiagnostic manifestations were similar in North America and Australia, neurotoxidromes differed significantly with prolonged weakness and even recurrent neuromuscular paralysis following tick removal in Australian cases compared to North American cases. TP was a potentially lethal poisoning that occurred in children and adults in a seasonally and regionally predictable fashion. TP was increasingly misdiagnosed as GBS during more recent reporting periods in North America. Such misdiagnoses often directed unnecessary therapies such as central venous plasmapheresis with intravenous immunoglobulin G, and delayed correct diagnosis and tick removal. TP should be added to and quickly excluded from the differential diagnoses of acute ataxia with ascending flaccid paralysis, especially in children living in TP-endemic regions worldwide.

823

MEADOW VOLES SERVE AS COMMON BLOODMEAL SOURCES FOR SUBADULT DOG TICKS IN SITES WITH ACTIVE TULAREMIA TRANSMISSION

Heidi Goethert, Sam R. Telford

Tufts University School of Veterinary Medicine, N. Grafton, MA, United States

The enzootic cycle of *Francisella tularensis tularensis*, the agent of Type A tularemia, remains poorly described. Dogma suggests that the cottontail rabbit, *Sylvilagus floridanus*, is the main reservoir for Type A infection, but the evidence for this remains largely circumstantial. Martha's Vineyard, Massachusetts has continuously reported human tularemia cases each year for the last 10 years. We have implicated the American dog tick, *Dermacentor variabilis*, as critical to perpetuation but have yet been unable to define the role of rabbits in Type A tularemia ecology there. To determine the identity of the animals that serve as the main hosts for feeding subadult dog ticks and thereby serve as the likely Type A reservoir, we identified bloodmeal residues in host seeking adult dog ticks by the use of a reverse line blot (RLB) assay. Host seeking dog ticks were collected from well characterized tularemia natural foci on Martha's Vineyard. DNA was extracted from individual ticks, and mammalian DNA was amplified by PCR using primers targeting a conserved region of the 12S rRNA gene. The amplicons were then assayed by RLB using species-specific probes, and probe binding was detected using chemiluminescence. The probes included the major reservoir host candidates such as meadow voles, white footed mice, and cottontail rabbits, as well as other animals found on the island. Of 106 ticks that have been tested to date, 58 (55%) did not have amplifiable mammalian DNA. Of the ticks that yielded an amplicon, the vast majority were from voles (46%). Only 6% of the ticks appeared to have fed as a nymph on rabbits, with a similar proportion from white footed mice. Interestingly, skunk and raccoon also appeared to serve as hosts for subadult dog ticks, an unexpected finding inasmuch as carnivores are only known to feed adult dog ticks. A third of the amplicons are as yet unidentified. We conclude that voles, not rabbits, are the main hosts for subadult dog ticks and are more likely to serve as reservoir for *F. tularensis tularensis* on Martha's Vineyard.

824

WOLBACHIA ENDOSYMBIONTS INVADE THE GERMLINE OF YOUNG ADULT *BRUGIA MALAYI* FROM THE LATERAL CHORDS

Kerstin Fischer, Wandy L. Beatty, Gary J. Weil, Peter U. Fischer

Washington University School of Medicine, St. Louis, MO, United States

The lymphatic filarial parasite *Brugia malayi* contains *Wolbachia,* α proteobacteria,that are crucial for filarial development and reproduction. In filarial larvae, *Wolbachia* mainly reside in the hypodermis and in hypodermal precursor cells. Developing reproductive tissue is free of endobacteria. In mature adult female worms *Wolbachia* can be observed in the ovaries, oocytes and developing microfilariae. We used immunohistology, in situ hybridization and transmission electron microscopy to study the germline invasion process of *Wolbachia.* We found massive multiplication of *Wolbachia* in the lateral chords of immature adult stage female worms (5 weeks pi) in cells that border the ovaries. *Wolbachia* enter the pseudocoelomic cavity in the median region of the developing ovaries and invade germ cells. In inseminated females (8 weeks pi) *Wolbachia* were detected in embryos and in decreasing numbers in the lateral chords. In young adult stage males (5 weeks pi) *Wolbachia* were found in distinct zones of the developing testis and in large numbers in the lateral chords in the vicinity of testicular tissue. *Wolbachia* were never detected in mature spermatids or spermatozoa in older male worms. The invasion of *Wolbachia* into the reproductive tissue in young adult worms is essential for transovarial propagation of *Wolbachia.* This may be an Achilles heel in the filarial life cycle that could be explored for further intervention.

825

THE INVOLVEMENT OF THE *WOLBACHIA* WSP-LIKE PROTEINS IN THE ENDOSYMBIOTIC RELATIONSHIP WITH *BRUGIA MALAYI*

Sara Lustigman[1], Jing Liu[1], Shulin Xu[2], Lin Li[1], Elena Melnikow[1], Elodie Ghedin[3], Thomas R. Unnasch[2]

[1]New York Blood Center, New York, NY, United States, [2]University of South Florida, Tampa, FL, United States, [3]University of Pittsburgh School of Medicine, Pittsburgh, PA, United States

The parasite *Brugia malayi* is a lymphatic dwelling filarial nematode that infects over 138 million individuals worldwide, primarily in the tropics, and causes Lymphatic Filariasis. *B. malayi* harbors endosymbiotic intracellular bacteria, *Wolbachia* (*w*Bm), that are required for the development and reproduction of this worm. The crucial role of the endosymbiont for *B. malayi* survival suggested novel anti-*Wolbachia* chemotherapeutic approaches for the treatment of human filarial infections. Our study aims to identify proteins that have potentially an essential function in this endosymbiotic relationship. The major *Wolbachia* surface protein (WSP) contains transmembrane domains and a standard signal peptide for secretion. In previous studies, this protein was shown to act as an inducer of the innate immune system and was also implicated in the pathogenesis of the filarial infections in the host. The *Wolbachia* genome contains 6 WSP-like proteins (Wbm0100, Wbm0152, Wbm0284, Wbm0432, Wbm0506 and Wbm0575). Immunoelectron microscopy analyses using anti-WSP (Wbm0284) antibodies confirmed that the protein is not only present on the surface of *w*Bm within the hypodermal region of the worm and inside the oocytes, embryos and microfilaria, but that it is also found in the body cavity within the uterine wall of the adult worms, eggshells surrounding the developing microfilaria and in the hypodermal region of the cuticle. This points to the possibility that WSP plays a role in the symbiotic relationship. To further explore whether the WSP-like proteins interact with proteins produced by the filarial host, we expressed them as GST or HIS tagged recombinant proteins. Each recombinant protein was found to bind specifically to *B. malayi* crude extracts using a modified ELISA assay. The putative *B. malayi* target protein for WSP (Wbm0284) was identified by the panning of a *B. malayi* cDNA phage library, and was named WSP/G2. The *B. malayi* WSP/G2 protein contains a BTB/POZ-like domain proven experimentally in other systems to be involved in Protein:Protein interactions. The specific interactions between the *B. malayi* WSP/G2 protein, WSP and the other WSP-like *Wolbachia* proteins will be presented. Our studies further improve our understanding of this particular symbiotic relationship.

826

FUNCTIONAL ANALYSIS OF POLYMORPHISMS IN THE SL CORE PROMOTER DOMAINS OF RIBOSOMAL PROTEIN GENES OF *BRUGIA MALAYI*

Michelle Bailey, Chitra Chauhan, Canhui Liu, **Thomas R. Unnasch**

University of South Florida, Tampa, FL, United States

Previous studies have indicated that the promoters of *Brugia malayi* are unusual in that they do not exhibit the CAAT or TATAA sequences usually found in the core domains of promoters of most eukaryotic organisms. Analysis of the promoters of the ribosomal proteins (RPs) showed that the 22nt region flanking the splice leader (SL) addition site plays an important role in transcription and may function as the core promoter domain in *B. malayi*. Interestingly, the promoters of the *B. malayi* RPs demonstrated a wide variation in activity when evaluated using a homologous transfection assay. To evaluate the relative contribution that polymorphisms in the SL core domains made to observed variation seen among the RP promoters, the SL addition site of the BmRPL13 gene was replaced with the SL

addition domains derived from six other RP genes. The promoter activity of the replacement constructs was found to be intermediate between that of BmRPL13 and the corresponding wild type RPs. Comparison of the activity of the replacement constructs to the wild type promoters indicated that on average 85% of the variation in activity observed among the RP promoters could be ascribed to variation in the SL core domain. The activity of replacement mutants containing the 10nt upstream of each RP promoter produced activity levels that were similar to those produced with the substitution mutants containing the entire 22nt SL domain. These data suggest that the majority of the variation in promoter activity seen among the *B. malayi* RP promoters is a result of polymorphisms in the SL core domain, and that the 10nt upstream of the SL addition site represents the primary determinant of promoter activity.

827

THE TRANSCRIPTOME OF THE *WOLBACHIA*-FREE FILARIAL NEMATODE *ONCHOCERCA FLEXUOSA*

Samantha N. McNulty, Sahar Abubuckar, Kerstin Fischer, Makedonka Mitreva, Gary J. Weil, Peter U. Fischer

Washington University School of Medicine, St. Louis, MO, United States

We have recently reported the presence of *Wolbachia* -like sequences in DNA from filarial nematode species that do not contain *Wolbachia* endosymbionts. These results suggest that ancestors of these parasites contained *Wolbachia,* acquired *Wolbachia* DNA by horizontal gene transfer, and later eliminated the bacteria. RT-PCR studies showed that some of the *Wolbachia* -like sequences were transcribed. We now report studies of the transcriptome of *Onchocerca* flexuosa. One objective of this work was to identify the most abundantly transcribed *Wolbachia* sequences in this *Wolbachia* -free parasite. The transcriptome was sequenced using GS-FLX 454 Titanium technology. 575,491 reads were assembled with 2,124 EST sequences derived from a conventional cDNA library to produce 13,129 isogroups and 30,861 singletons. 48,372 peptides were predicted based on transcript sequences, and deduced amino acid sequences were analyzed by blastp. 49% of predicted proteins had significant homology to known filarial nematode proteins, 3% had homology to proteins in other nematodes, and 4% had homology to proteins in other organisms. KEGG analysis showed that the most highly represented metabolic pathways were purine metabolism, oxidative phosphorylation, pyrimidine metabolism, and glycolysis. At the nucleotide level, 144 isogroups and 175 singletons showed homology to *Wolbachia* sequences. 42 of these isogroups and 19 of these singletons also contained sequences with homology to filarial genes on other regions of the transcript. 66 of the transcribed *Wolbachia* -like sequences contained putative open reading frames. These results confirm the presence of *Wolbachia* -like sequences in O. flexuosa transcripts and show that these sequences are often found on transcripts that also contain typical filarial sequences. Studies in progress will determine whether any of these *Wolbachia* -like sequences are expressed at the protein level. This may explain the ability of a minority of filarial species to live and reproduce without an endobacterial partner.

828

A RELIABLE *IN VIVO* APPROACH TO RNA INTERFERENCE IN MOSQUITO-BORNE FILARIAL WORMS

Lyric C. Bartholomay, Chuanzhe Song, Timothy A. Day, Michael J. Kimber

Iowa State University, Ames, IA, United States

Diseases caused by parasitic nematodes perpetuate socioeconomic instability in developing countries by inflicting crippling morbidity and significant mortality. One reason for the persistence of these diseases is the limited portfolio of effective drugs available to combat parasitic nematodes. A major obstacle to the rational development of new anthelmintics is the experimental intractability of parasitic nematodes; they simply are not amenable to many of the techniques commonly used to develop drugs for other diseases. Illustrative of this is RNA interference (RNAi) - a reverse genetic tool that allows researchers to rapidly and specifically 'turn off' genes of interest in an organism or cell line. RNAi has become a standard tool in rational drug development and validation of potential new drug targets for many diseases, but a reliable and reproducible protocol has yet to be established for animal parasitic nematodes. We describe an innovative strategy for the application of RNAi to study gene function and validate drug targets in animal parasitic nematodes. Our approach uses the filarial nematode *Brugia malayi* as a model and targets developing parasites in the mosquito host. We can profoundly suppress expression of a cathepsin-L-like gene using and RNAi trigger injected directly into infected mosquitoes. RT-qPCR confirms that suppression is specific and results in an 83% decrease in transcript abundance. Most importantly, cathepsin L-like suppression stunts parasite growth and development and elicits profound motility defects that effectively abolish transmission potential. Finally we present evidence that other genes are susceptible to this approach; we have successfully now used this method to suppress genes encoding a beta-tubulin (Bm-tub-1), G-protein linked acetylcholine receptor 2 (Bm-gar-2), prohomone convertase (Bm-pc-2), and a transcript with homology to a nicotinic acetylcholine receptor alpha subunit.

829

BEYOND FILARIAL GENOMICS: PROTEOMIC ANALYSES PROVIDE INSIGHTS INTO BOTH THE FILARIAL HOST AND ITS *WOLBACHIA* ENDOSYMBIONT

Sasisekhar Bennuru[1], Zhaojing Meng[2], Roshanak T. Semnani[1], Elodie Ghedin[3], King Chan[2], David Lucas[2], Jose M. Ribeiro[4], Timothy D. Veenstra[2], Thomas B. Nutman[1]

[1]Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [2]Laboratory of Proteomics and Analytical Technologies, SAIC-Frederick, Inc., NCI-Frederick, National Institutes of Health, Frederick, MD, United States, [3]Center for Vaccine Research, Department of Computational and Systems Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA, United States, [4]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Global proteomic analyses of pathogens have thus far been limited to unicellular organisms (e.g. protozoa and bacteria). Proteomics of most eukaryotic pathogens (e.g. helminths) have been restricted to specific organs, specific stages or secretomes. We report here a large-scale proteomic characterization of almost all the major mammalian stages of *Brugia malayi* (Bm), a causative agent of lymphatic filariasis and its endosymbiont *Wolbachia* (wBm). Proteomes of both the *B. malayi* and *Wolbachia* of multiple stages were analysed by nanobore reverse-phase liquid chromatography-tandem-MS (nanoRPLC-MS/MS). The obtained spectra were searched against *B. malayi* and *Wolbachia* databases using SEQUEST. Methionine oxidation and phosphorylations on serine, threonine and tyrosine were included as dynamic modifications in the database search. A total of 7267 proteins (~62% of the 11610 genes predicted from the *Brugia* genome) from adult male, adult female, microfilariae, L3 larvae and uterine immature microfilariae (UTMF) were identified. Genomic analysis predicted that 4956 (42.4%) of the total number of genes as being hypothetical proteins; the present study was able to confirm 2336 (47.1%) as *bonafide* proteins. Among the identified proteins in each stage studied (except UTMF [13%]), 4-5% were determined to have 'stage-specific' protein expression. Gene set enrichment analysis demonstrated that extracellular matrix related and immunologically related proteins are enriched in the microfilarial and L3 stages compared to the other stages. Proteomic analysis of *Wolbachia* resulted in the identification of 557 of the 805 predicted wBm proteins, some of which appeared to be expressed stage-specifically. Parallel analysis of Bm and wBm protein families and domains in concert with each of their stage-specific expression highlight important pathways (both parasite and endosymbiont) that benefit the parasite during its development in the host.

830

IVERMECTIN DISRUPTS THE FUNCTION OF THE EXCRETORY-SECRETORY APPARATUS IN MICROFILARIAE OF *BRUGIA MALAYI*

Yovany Moreno[1], Joseph F. Nabhan[1], Jonathan Solomon[1], Charles D. Mackenzie[2], Timothy G. Geary[1]

[1]McGill University, Sainte Anne de Bellevue, QC, Canada, [2]Michigan State University, East Lansing, MI, United States

In vivo, ivermectin (IVM) treatment of filarial infections is characterized by a rapid drop in the levels of circulating microfilariae (Mf) followed by the long term suppression of their production. Nevertheless, the direct effect of this drug on the Mf of most filarial nematodes is still debatable as there is no clear *in vitro* evidence of its microfilaricidal action. IVM acts upon binding to nematode Glutamate-gated Chloride Channels (GluCl), resulting in disruption of the neurotransmission processes that are regulated by the activity of these channels. To identify the physiological effects of IVM on Mf, we cloned and localized two AVR-14 subunits from *Brugia malayi*, which constitute their only putative IVM-sensitive GluCl subunits. Bma-AVR-14 subunits co-localized with a muscle structure surrounding the Mf- Excretory-Secretory (ES) vesicle. It suggests that under the control of GluCl, protein release in the ES apparatus is driven by the contraction of this vesicle. Consistently, *in vitro* IVM treatment led to a decrease in total protein released from Mf. Protein release decreased in 0.1 µM IVM up to 58, 68 and 42 % the amount released by the control at 24, 48 and 72 h, respectively. To understand how IVM can affect secretion of proteins released by the parasite, we identified 3 different localization patterns among a group of 5 known Mf- ES products; suggesting that either the parasite surface or the ES apparatus are probable anatomical pathways for physiological protein release. Nevertheless, the presence of muscle association with the ES-vesicle and the low permeability characteristic of the Mf sheath points to the ES-apparatus as the main source of Mf protein delivery to the mammalian host. Mf treatment with IVM targets the ES apparatus, which constitutes the main source of parasite protein release to the mammalian host. This leads to the inference that rapid Mf clearance related to IVM treatment *in vivo* reduces the secretion of immunomodulatory parasite proteins and thus induces a parasiticidal effect of the host immune system.

831

AN ALPHAVIRUS REPLICON BASED DENGUE VACCINE IS IMMUNOGENIC AND PROTECTIVE IN RHESUS MACAQUES AND INDUCES PREDOMINANTLY DOMAIN III REACTIVE NEUTRALIZING ANTIBODIES

Laura J. White[1], Vorraphun Yingsiwaphat[1], Wahala Wahala[1], Melissa Parsons[1], Lauren Fuller[1], Martha Collier[1], Carlos Sariol[2], Aravinda de Silva[1]

[1]University of North Carolina, Chapel Hill, NC, United States, [2]University of Puerto Rico, San Juan, PR, United States

Domain III of flavivirus E protein (EDIII) has been identified as a major target of strongly neutralizing monoclonal antibodies. However, natural infection of humans with DENV and WNV results in only a small proportion of antibodies directed to EDIII, representing a minor contribution to the neutralization potency of the DEN and WNV immune sera. Therefore, it is likely that tetravalent live attenuated dengue vaccines, which mimic natural infection and are currently in clinical trials, will induce a small proportion of EDIII neutralizing Abs. We have developed a vaccine modality based on an alphavirus replicon vector (VRP) expressing soluble DENV E protein (Es) that induces neutralizing antibodies directed predominantly to EDIII and complete protection in macaques. Interestingly, we found that neutralizing antibodies induced in macaques infected with live DENV, mostly recognize epitopes outside EDIII, as seen in humans. Rhesus macaques (n=6) were immunized by s.c. inoculation with 10e8 IU DEN3 Es-VRP at weeks 0, 7 and 18. All animals produced neutralizing antibodies to DEN3 after the first immunization, and maintained them throughout the study. To determine protective efficacy, vaccinated macaques (at 15 wks-post 3rd dose) along with 4 unvaccinated controls, were challenged with 10e5 pfu of DEN3 live virus. All VRP-immunized animals showed complete protection from viremia, as measured by focus assay on Vero cells. The contribution of EDIII reactive antibodies to the neutralizing response was determined by depleting DEN3 EDIII binding antibodies from the sera of DEN3 Es-VRP vaccinated and DEN3 live virus infected monkeys. EDIII adsorbed sera from VRP immunized macaques lost most of its neutralizing activity; while EDIII adsorbed sera from live virus infected animals retained most of its neutralizing activity. We propose that VRP delivery of soluble DENV E protein allows EDIII to become visible to the immune system, in the presence of EDI and EDII. This may represent an advantage over other vaccine platforms. A tetravalent Es-VRP has been constructed and will be tested in macaques.

832

TOWARDS DEVELOPING A NON-INVASIVE INTRADERMAL VACCINATION STRATEGY WITH A TETRAVALENT DENGUE VACCINE (DENVAX): IMMUNOGENICITY AND EFFICACY STUDIES IN MICE AND NON-HUMAN PRIMATES

Jorge E. Osorio[1], Charalambos D. Partidos[2], Joe Brewoo[2], Michael A. Royals[3], John J. Arguello[4], Shawn J. Silengo[4], Tim D. Powell[4], Richard M. Kinney[5], Claire Y-H Huang[5], Dan T. Stinchcomb[4]

[1]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI, United States, [2]Inviragen, Madison, WI, United States, [3]PharmaJet, Inc., Golden, CO, United States, [4]Inviragen, Fort Collins, CO, United States, [5]Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO, United States

Among the mosquito-borne viruses, dengue (DEN) viruses are notable for their global distribution and the frequency of large scale epidemics that they cause. To date, there is no effective vaccine to prevent against DEN infections. DENVax is a tetravalent DEN vaccine based on using the DEN-2 vaccine, strain PDK-53, as a vector. Three chimeric recombinant viruses were constructed, each bearing the vector`s capsid and non-structural gene backbone while expressing DEN-1,-3 or -4 prM and E structural genes.

In a series of experiments we examined the immunogenicity and protective efficacy of DENVax administered via the ID route in mice and non-human primates. Comparison of the immunogenicity of DENVax-4 vaccine via the ID vs. subcutaneous (SC) route in AG129 mice clearly indicated the superiority of the ID route over the SC. The neutralizing anti-DEN-4 antibody responses were 5-fold higher. Additional groups of AG129 mice were immunized ID or SC with a tetravalent DENVax formulation bearing the ratio of 105: 104: 105:105 PFU of DENVax-1, DENVax-2, DENVax-3 and DENVax-4, respectively. The neutralizing titers against DEN-1, -3 and -4 were higher when DENVax was given ID. However, responses to DEN-2 were unaffected by the route of immunization. Non-human primates (Cynomologous macaques) were immunized with a tetravalent DENVax formulation containing 105 PFU of each vaccine virus by SC administration with needle and syringe or ID administration using a needle-free device. Needle-free ID administration induced neutralizing antibody titers against all four DEN serotypes greater than those elicited after SC injection. All ID vaccinated animals challenged SC with 105 PFU of wild-type DEN-1 West Pacific (WP) or DEN-2 New Guinea C (NGC) viruses were shown to be free of viremia. Needle-free ID DENVax delivery has the advantage of making vaccine administration simple and eliminating concerns about needle reuse and disposal. These preclinical studies set the stage for human clinical testing of DENVax formulations in dengue naïve adult volunteers.

833

CLINICAL DEVELOPMENT OF A RECOMBINANT SUBUNIT VACCINE FOR DENGUE

Beth-Ann Coller, David Clements, Steven Ogata, Timothy Martyak, Mike Thorne, Michele Yelmene, Elliot Parks

Hawaii Biotech, Inc., Aiea, HI, United States

Dengue viruses are a major cause of morbidity and mortality throughout the tropics and subtropics with an estimated 50-100 million cases of dengue annually. To date no specific vaccine or therapy has been licensed to combat this important disease. To address this unmet need, Hawaii Biotech, Inc. is developing a tetravalent recombinant subunit vaccine to protect individuals against dengue virus induced disease. GMP manufacture of the four truncated dengue envelope proteins to support human clinical trials has been completed. Preclinical studies conducted in mice and rabbits have demonstrated the immunogenicity of both monovalent and tetravalent formulations adjuvanted with alum, and formal toxicology studies have demonstrated acceptable safety. An alum-based monovalent DEN1 formulation was shown to be immunogenic and protective in non-human primates. A Phase 1 clinical study of monovalent DEN1 has been conducted in healthy volunteers. Currently, final preparations are ongoing for a Phase 1 clinical study of the tetravalent subunit vaccine. The current status of preclinical and clinical development will be presented.

834

EVALUATION OF THE SAFETY AND IMMUNOGENICITY OF TETRAVAX-DV, A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE

Anna P. Durbin[1], Beth Kirkpatrick[2], Kristen Pierce[2], Daniel Elwood[1], Cecilia Tibery[1], Robbyn Lewis[1], Kimberli Wanionek[1], Bhavin Thumar[1], Catherine Luke[3], Brian Murphy[3], Kanta Subbarao[3], Stephen Whitehead[3]

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

Dengue virus (DEN) has become the most important arbovirus worldwide with a 30-fold increase in DEN infections and hundreds-fold increase in the more severe form, dengue hemorrhagic fever/shock syndrome. Because a secondary DEN infection with a serotype different from that which caused the primary infection is a significant risk factor for DHF/DSS, a DEN vaccine must induce a long-lived immune response to all four DENV serotypes. The goal of the National Institutes of Health (NIH) DEN vaccine program is to produce a minimally reactogenic, highly immunogenic, genetically stable, live attenuated DEN vaccine that is cost-effective and safe for the community. Over the past ten years, the NIH has developed numerous live attenuated candidate vaccines against the four individual DEN serotypes. We have tested 8 monovalent vaccines in 15 Phase I clinical trials to identify DEN1, DEN2, DEN3, and DEN4 candidate vaccine viruses that are safe and maintain the optimal infectivity and immunogenicity profiles for inclusion in a tetravalent formulation. Each monovalent candidate was well tolerated by volunteers with no volunteer experiencing a dengue-like illness. Following a single subcutaneous injection of 1,000 PFU, each of the candidate vaccines induced seroconversion rates of 80 - 100% to its parent wild-type virus. Factors such as infectivity, immunogenicity, and reactogenicity were used to determine which vaccine candidates to include in the tetravalent formulation, and this selection process will be discussed. A Phase I Clinical trial evaluating 3 different tetravalent admixtures in flavivirus naïve subjects was initiated in 2010. Preliminary safety and immunogenicity results from this Phase I clinical trial will be discussed.

835

DIFFERENT INNATE SIGNATURES INDUCED IN HUMAN MDCS BY WILD-TYPE DENGUE 3 VIRUS, ATTENUATED BUT REACTOGENIC DENGUE 3 VIRUS, OR NON-REACTOGENIC ATTENUATED DENGUE 1-4 VACCINE VIRUSES

Bruno Guy, Claire Balas, Audrey Kennel, Florence Deauvieau, Regis Sodoyer, Nadege Arnaud-Barbe, Jean Lang

sanofi pasteur, Marcy l'Etoile, France

Dengue infection is a major and growing public health issue worldwide. Different vaccine candidates are being developed, including YFV 17D vaccine-based chimeric dengue virus vaccines (CYDs). Dendritic cells (DCs) play a key role in initiating immune responses and are primary targets of dengue infection. The consequence of human monocyte-derived DCs (mDCs) infection by various wild type (wt) dengue strains has been investigated by several authors. We addressed the innate profile induced in mDCs upon infection by each of the 4 CYDs and their tetravalent combination. In a first study, a limited set of activation markers, cytokines and chemokines was assessed by ELISA, flow cytometry and qRT-PCR. This first study showed that CYDs induced DC maturation, a controlled immune response, limited inflammatory cytokine production, and consistent expression of anti-viral interferons, confirming clinical observations of safety and immunogenicity. A second study used 22K and 44K Agilent DNA microarrays to assess mDCs infected by the 4 CYDs alone or in combination, or by a wt serotype 3 virus, or a classically attenuated serotype 3 virus (VDV3) shown to be reactogenic in a clinical trial. The results of this second study confirmed and expanded upon the first: we observed a very reproducible signature for each of the 4 CYDs, involving stimulation of Type I IFN genes and associated ISGs, together with genes encoding chemokines and other mediators involved in the initiation of adaptive responses. In contrast, the wt virus induced a predominantly inflammatory profile, while VDV3 appeared to induce a blunted response, which may have been insufficient to trigger early immune responses and prevent initial viral replication. This could have contributed to VDV3 symptomatic outcome in clinical trials. These studies contributed to documenting the safety and immunogenicity of the 4 CYD candidate vaccine viruses, which are currently in evaluation in large scale efficacy trials.

836

PRIMING EFFECT OF PREVIOUS JAPANESE ENCEPHALITIS VACCINATION ON HUMORAL IMMUNE RESPONSE TO TETRAVALENT DENGUE VACCINE

Francisco Galan[1], Remi Forrat[2], Betzana Zambrano[3], Alain Bouckenooghe[4], Jean Lang[2], **Gustavo Dayan**[5]

[1]CIF BIOTEC Medicasur Hospital, Mexico DF, Mexico, [2]sanofi pasteur, Marcy l'Etoile, France, [3]sanofi pasteur, Montevideo, Uruguay, [4]sanofi pasteur, Singapore, Singapore, [5]sanofi pasteur, Swiftwater, PA, United States

The potential priming effect of prior Japanese encephalitis vaccination (JEV) on antibody responses to tetravalent live-attenuated chimeric dengue virus vaccine (TDV) was assessed. The TDV vaccine viruses were constructed by replacing genes for non-structural pre-membrane (PrM) and envelope (E) proteins of the attenuated YF 17D vaccine with wild-type dengue serotypes sequences. In a randomized, controlled, multicenter, Phase II study in Mexico City, 18-45 year-old participants received two injections of TDV at days 0 and 105 (Group 1) or 3 injections of JE-VAX® at days -14, -7, and 0 followed by one dose of TDV at day 105 (Group 2). Dengue antibody levels were determined by microneutralization assay before, 28 and 60 days after each TDV vaccination and at day 365. Viremia was assessed by PCR methods 7-14 and 21 days after each TDV vaccination. Safety was documented after vaccination through day 28. Seropositivity rates (antibody level ≥10 1/dil) 28 days after 2 doses of TDV (Group 1, n=31) were 61%, 36%, 71% and 75% respectively against dengue serotypes 1-4. After 1 dose of TDV among JEV primed (Group 2,

n=30), these rates were 85.2%, 85.2%, 85.2%, 92.6%. GMTs against dengue serotypes 1-4 for Group 1 / Group 2 were: 18.4/37.4, 12.6/27.4, 16.7/42.4, and 56.5/219. The percentage of seropositive subjects for at least 3 serotypes was 46% for Group 1 and 78% for Group 2. JEV priming did not increase viremia, which remained low and infrequent in both groups. Serotype 4 was the most frequently detected followed by serotype 3. Both groups had acceptable and similar safety profiles. Injection site pain and headache were the most common solicited reactions. In conclusion, seropositivity rates and GMTs of subjects primed with JEV vaccine receiving 1 dose of TDV were higher than those who received 2 doses of TDV, suggesting that pre-existing JE immunity may boost antibody responses to the TDV without modifying the safety profile.

837

PRE-EXISTING IMMUNITY TO JAPANESE ENCEPHALITIS VIRUS IS ASSOCIATED WITH AN INCREASED RISK OF SYMPTOMATIC ILLNESS FOLLOWING A DENGUE VIRUS INFECTION

Kathryn B. Anderson[1], Stephen J. Thomas[2], Robert V. Gibbons[2], Alan L. Rothman[3], Ananda Nisalak[2], Ruth L. Berkelman[1], Daniel H. Libraty[3], Timothy P. Endy[4]

[1]Rollins School of Public Health, Emory University, Atlanta, GA, United States, [2]Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]Center for Infectious Disease and Vaccine Research, University of Massachusetts Medical School, Worcester, MA, United States, [4]Infectious Disease Division, Department of Medicine, State University of New York, Upstate Medical University, Syracuse, NY, United States

Dengue viruses (DENVs) and Japanese Encephalitis virus (JEV) co-circulate in Southeast Asia, where they are both important causes of human morbidity and mortality. They have significant cross-reactivity in serological assays, but the possible clinical implications of this remain poorly defined. Previous studies have suggested a protective effect of JEV immunization against dengue hemorrhagic fever (DHF). An improved understanding of whether and how JEV immunity modulates the clinical outcome of DENV infection is important as large-scale DENV vaccine trials will commence in areas where JEV is co-endemic and/or JEV immunization is routine. The association between pre-existing JEV neutralizing antibodies (NAbs) and the clinical severity of subsequent DENV infection was evaluated in a cohort of school children in Northern Thailand. Covariates considered included age, baseline DENV antibody status, school of attendance, epidemic year, and infecting DENV serotype. The presence of JEV NAbs in serum collected prior to DENV infection was associated with an increased probability of symptomatic versus subclinical infection (OR=2.02, p<0.01). This association was strongest in children with negative DENV serology (DENV-naive) (OR=3.13, p<0.01); the OR declined with seropositivity to an increasing number of DENV serotypes. Significant differences in the association were observed by infecting DENV serotype in multivariate analysis. There was no significant effect of JEV NAb on the probability of DHF (OR=1.40, p=0.30). The prior existence of JEV NAbs was associated with an increased probability of symptomatic as compared to subclinical DENV illness. JEV seropositivity was not associated with the occurrence of DHF, but the small number of DHF cases limited the power of this comparison. These findings are in contrast to previous studies suggesting a protective effect of heterologous flavivirus immunity on DENV disease severity. Further evaluations of possible clinical and immunological interactions between flaviviruses are warranted.

838

ROLE OF RAPID DIAGNOSTIC TESTING (RDT) IN THE CONTEXT OF HOME MANAGEMENT OF CHILDHOOD FEVER (HMCF) WITH DISPERSIBLE ARTEMETHER-LUMEFANTRINE: AN OPEN LABEL RANDOMIZED CONTROLLED TRIAL IN A RURAL AND SEASONAL MALARIA TRANSMISSION AREA OF BURKINA FASO

Tiono B. Alfred[1], Konate T. Amadou[1], Convelbo Nathalie[1], Convelbo Edmond[1], Pagnoni Franco[2], Sirima Sodiomon Bienvenu[1]

[1]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [2]World Health Organization-TDR, Geneva, Switzerland

Delivery of prompt and adequate treatment (currently ACTs) for uncomplicated malaria at community level remains a key strategy to reduce the burden of malaria in sub-Saharan Africa. Rapid Diagnosis Tests (RDT) could help improve diagnosis at home level by trained community health workers (CHW) and therefore rationalize the use of ACTs. This study aimed to assess fever management based on RDT or presumptive ACT treatment and RDT use by CHW. In an open label, cluster randomized study, 12 villages were randomized (1:1) ratio into an intervention and a control arm. Children aged 6-59 months (mo) with a history of fever (last 24 hours) or axillary T°> 37.5°C received presumptive treatment with antimalarial drug (dispersible artemether-lumefantrine, DAL) in the control arm. In the intervention arm, RDT and Respiratory rate counting was performed so that febrile children received DAL and/or antibiotic based on an algorithm followed by the CHW. In both arms, a blood smear was obtained at baseline (Day 0). Participants were assessed at Day 3 and Day 7 and febrile children were referred to a community clinic. A total of 732 children (354 in the control arm; mean age 29.4±15.4 mo vs. 378 in the intervention arm; mean age of 27.6±15.1 mo) were enrolled during the malaria high transmission period. Prevalence of malaria infection was comparable at baseline across arms (76.2% in the control vs. 76.4% in the intervention arm, P= 0.97). Specificity and sensitivity of RDT as compared to microscopy were respectively 28.7% and 98.6%. On day 3, fever clearance rate (FCR) was 96.3% in the control vs. 93.3% in the intervention arm (P = 0.08), and on Day 7, 99.0% in the control vs. 97.3% in the intervention arm (P=0.1). Data for the dry season are being collected, and an analysis by season (Low vs. High transmission season) will be presented. A high fever clearance rate was achieved with DAL in both arms. The study has shown that the use of RDT by community Health Workers is feasible in our context. However a more specific test is needed for use at community level to be recommended.

839

MANAGEMENT OF FEBRILE YOUNG CHILDREN IN MALARIA MIX-ENDEMIC AREAS (*PLASMODIUM FALCIPARUM* AND *P. VIVAX*). QUICK ATTENDANCE, RAPID TESTING AND EFFECTIVE TREATMENT: A SAFE ATTITUDE IN PAPUA NEW GUINEA

Nicolas Senn[1], Ivo Mueller[2], Patricia Rarau[3], Blaise Genton[1]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea, [3]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea

The management of febrile children in malaria endemic areas has evolved and the following approach has been proposed: febrile children should attend quickly to get tested [rapid diagnostic test (RDT) or microscopy] and receive efficient malaria treatment if positive. There are safety concerns about withholding antimalaria drugs from children with negative test. More generally, we have no data on the accuracy of this strategy, especially in areas with mixed endemicity (Pf and Pv). The present study explores the feasibility of this approach in Papua New Guinea (PNG), a highly endemic country for both Pf and Pv. Alongside a malaria preventive drug trial (IPTi), a morbidity surveillance was set up to record all illness

episodes. When presenting fever, study participants were screened by health workers for malaria using RDT's (ICT combo®) and treated with Coartem® when tested positive. Blood slides (BS) were also collected. From 2006 to 2009, 1605 infants 3 months old were enrolled and followed-up for 2 years. A total of 7004 febrile episodes were recorded. The median symptoms' duration was 2 days. 3807 (54%) had a negative RDT. Among them, 146 (3.8%) re-attended the clinic within 7 days for fever, 1 died (negative RDT & BS) and 24 (0.6%) presented a serious adverse event: 13 had a negative RDT, 3 had a positive RDT or quick read (but negative BS) and 8 had no RDT's results (2 had negative BS, 1 positive BS, 4 without BS were treated without antimalarial drugs for alternative diagnostics, 1 without BS received Coartem®).There were 1677 positive RDT's. All treated with Coartem®, 39 (2.3%) re-attended within 7 days for fever, none died and 5 (0.3%) presented a serious adverse event (2 possible severe malaria, 1 possible meningitis, 1 severe pneumonia and 1 gastroenteritis). This study provides good evidence that the approach "quick attendance, rapid testing and effective treatment" is safe and feasible in infants in countries with limited resources and a high level of Pv infections.

840

HOW CAN MALARIA RAPID DIAGNOSTIC TESTS ACHIEVE THEIR POTENTIAL? A QUALITATIVE STUDY OF A TRIAL AT HEALTH FACILITIES IN GHANA

Clare I. Chandler[1], Chris J. Whitty[1], Evelyn Ansah[2]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Ghana Health Services, Dodowa, Ghana

Rapid diagnostic tests (RDTs) for malaria are at the early stages of introduction across malaria endemic countries in an effort to improve case management. Evidence of the effect of introducing RDTs on the prescription of antimalarials is mixed. A recent trial of RDTs in rural health facilities in Ghana reduced overprescription of antimalarials, but found that 45.5% patients who tested negative were still prescribed an antimalarial. We conducted a qualitative study of the implementation of RDTs in this trial. We interviewed health workers who had been shown to either continue to prescribe anti-malarials to most patients with negative RDT results or who largely restricted anti-malarials to patients with positive RDT results. Interviews explored the experiences of using RDTs and their results amongst trial participants.

Meanings of RDTs were constructed by health workers through participation with the tests themselves as well as through interactions with colleagues, patients and the research team. These different modes of participation with the tests and their results led to a change in practice for some health workers, and reinforced existing practice for others. Many of the characteristics of RDTs were found to be inherently conducive to change, but limited support from purveyors, lack of system antecedents for change and limited system readiness for change were apparent in the analysis. When introduced with a limited supporting package, health workers had learnt to use RDTs differently. To build confidence of health workers in the face of negative RDT results, a supporting package should include local preparation for the innovation; unambiguous guidelines; training in alternative causes of disease; regular support for health workers to meet as communities of practice; interventions that address negotiation of health worker-patient relationships and encourage self-reflection of practice; feedback systems for results of quality control of RDTs and of prescribing practices; and RDT augmentation such as a technical and/or clinical troubleshooting resource.

841

A LARGE PROPORTION OF ASYMPTOMATIC MALARIA INFECTIONS WITH LOW PARASITE DENSITIES IN TEMOTU PROVINCE, SOLOMON ISLANDS: CHALLENGES FOR MALARIA DIAGNOSTICS IN AN ELIMINATION SETTING

Ivor Harris[1], Wesley Sharrock[1], Lisa Bain[1], Karen-ann Gray[1], Albino Bobogare[2], Ken Lilley[1], Darren Krause[1], Andrew Vallely[3], Michelle Gatton[4], Dennis Shanks[1], **Qin Cheng**[1]

[1]Australian Army Malaria Institute, Brisbane, Australia, [2]Vector Borne Disease Control Programme, Ministry of Health Solomon Islands, Honiaria, Solomon Islands, [3]Pacific Malaria Initiative Support Centre, School of Population Health, University of Queensland, Brisbane, Australia, [4]Queensland Institute of Medical Research, Brisbane, Australia

Many countries are scaling up malaria interventions towards elimination. This transition changes demands on malaria diagnostics from detecting parasites in ill patients to detecting asymptomatic reservoirs. The selection of diagnostic methods must be solved prior to transitioning a malaria control program to elimination. A baseline malaria parasitological survey was conducted in Temotu Province, Solomon Islands, in late 2008, as the first step in a provincial malaria elimination program. The survey provided opportunities to obtain point prevalence and epidemiological characteristics of malaria infections on the island, as well as to assess how well different diagnostic methods performed in this particular setting.

During the survey, 9491 blood samples (~50% population) were collected and examined by microscopy with a subset also examined by PCR and RDTs. A total of 256 samples were determined positive by microscopy, of which 17.5% and 82.4% had Plasmodium *falciparum* and P. vivax, respectively. Interestingly in this low transmission setting, only 17.8% of the P. *falciparum* and 2.9% of P. vivax infected subjects were febrile (≥38oC) at the time of survey with 50% and 66.7% of the P. *falciparum* and P. vivax fever observed in the 5-14 age group. Overall, 40% of the P. *falciparum* and 65.6% of the P. vivax infected subjects had parasite density below 100/µL. There was increase in the proportion of parasite density below 100/µL with age for P. vivax infections, but no such a correlation was observed for P. *falciparum* infections. The observed large proportion of infections with densities below 100/µL presents a major challenge to microscopy, RDT and PCR. In general, there is a reasonable agreement between microscopy and PCR in detecting P. vivax, but poor agreement in detecting P. *falciparum* and mixed infections, particularly in samples below 100/µL where 85.7% of the discrepancies occur. The results suggest a combination of methods, or new diagnostics, may be required to detect infections in asymptomatic parasite reservoirs, the prevalence of which is high even in low transmission settings.

842

DOES THIS PATIENT HAVE MALARIA? A META-ANALYSIS OF THE DIAGNOSTIC UTILITY OF CLINICAL FACTORS FOR ENDEMIC AND IMPORTED MALARIA

Steve M. Taylor[1], Malcolm E. Molyneux[2], Steven R. Meshnick[3], Jonathan J. Juliano[4]

[1]Duke University Medical Center, Durham, NC, United States, [2]Malawi–Liverpool–Wellcome Trust Clinical Research Programme, Blantyre, Malawi, [3]University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC, United States, [4]University of North Carolina, Chapel Hill, NC, United States

Malaria commonly infects residents of and travelers to tropical regions, and the clinical features, if any, are notoriously nonspecific. We endeavored to systematically review and synthesize data regarding the predictive value of clinical findings for the diagnosis of malaria in endemic areas and in returning travelers. We searched MEDLINE (1950-July 2009) to identify English-language studies of endemic malaria and "imported malaria," and additional studies were identified from reference lists. We included studies that compared the presence or absence of pre-defined

clinical findings with blood smear confirmation of parasitemia in patients suspected of acute malaria. Two authors independently identified studies, appraised study quality, and extracted data. We identified over 500 studies for endemic malaria, but only 15 met review criteria. Individual symptoms are of limited diagnostic utility, but splenomegaly (summary positive likelihood ratio [LR] 3.3; 95% confidence interval [CI] 2.0-4.7) and hepatomegaly (summary positive LR 2.7; 95% CI 1.9-3.5) make malaria infection more likely. Combinations of findings can impact the likelihood of malaria, but their performance varies by setting. We identified over 900 studies for imported malaria, but only 28 met criteria. The presence of fever (positive LR 5.1; 95% CI 4.9-5.3), splenomegaly (summary positive LR 6.5; 95% CI 3.9-11), hyperbilirubinemia (positive LR 7.3; 95% CI 5.5-9.6), or thrombocytopenia (summary positive LR 4.9; 95% CI 2.4-10) make malaria much more likely. In endemic areas, the likelihood of finding parasitemia is increased by the presence of splenomegaly and hepatomegaly, but individual findings are overall of limited utility; clinical algorithms may be useful to risk-stratify patients but their performance is variable between settings. In returning travelers, the clinical assessment can provide substantial diagnostic benefit, but all patients still require laboratory testing.

843

PHRP-2 PLASMA CONCENTRATIONS DISTINGUISH BETWEEN MALAWIAN CHILDREN WITH RETINOPATHY-POSITIVE AND RETINOPATHY-NEGATIVE CEREBRAL MALARIA

Karl B. Seydel[1], Lindsay L. Fox[2], Simon J. Glover[3], Paul Pensulo[4], Alice Muiruri[4], Ashley C. Mpakiza[4], Terrie E. Taylor[1]

[1]Michigan State University, East Lansing, MI, United States, [2]University of Washington, Seattle, WA, United States, [3]College of Medicine, Blantyre, Malawi, [4]Blantyre Malaria Project, Blantyre, Malawi

As malaria control and eradication efforts expand, it will be increasingly important to identify cases of severe malaria. The standard clinical case definition of cerebral malaria incorrectly identifies 25% of children as having cerebral malaria when compared with post-mortem histology. The best clinical predictor, malarial retinopathy, is >95% sensitive and specific, but well-trained personnel and expensive equipment are required. We investigated plasma concentrations of pHRP-2, a *Plasmodium*-specific protein released mainly on schizont rupture, as an indicator of "true" cerebral malaria in patients fulfilling the standard clinical case definition. In a group of 64 patients with clinically defined cerebral malaria who died and underwent autopsy, 47 patients had histological evidence of sequestration in the cerebral microvasculature. The sensitivity and specificity of pHRP-2 concentrations for distinguishing the patients with true CM were 98% and 94%, respectively (area under the ROC, AUROC, was 0.98). In a larger group of children with clinically defined cerebral malaria (n=260), a cut-off concentration of pHRP-2 distinguished retinopathy-positive children from those without retinopathy with a sensitivity and specificity of 0.90 (AUROC = 0.90). In contrast, the AUROC associated with peripheral parasitemia was 0.53. pHRP-2 is a measure of both circulating and sequestered parasites, and the association of higher concentrations with histologically- and fundoscopically-confirmed cerebral malaria suggests that parasite burden is important in disease pathogenesis. Plasma concentrations of pHRP-2 may be a more field friendly approach to identifying patients with retinopathy-positive (ie, "true") cerebral malaria, and if so, this would facilitate appropriate treatment and simplify disease surveillance.

844

REAL-TIME QUANTITATIVE RT-PCR FOR MONITORING PARASITEMIA IN MALARIA HUMAN CHALLENGE TRIALS

Sean C. Murphy[1], Jennifer L. Prentice[1], Kathryn S. Williamson[2], Michal Fried[2], Angela K. Talley[2], Ruobing Wang[2], Patrick E. Duffy[3], Brad T. Cookson[1]

[1]University of Washington Medical Center, Seattle, WA, United States, [2]Seattle Biomedical Research Institute, Seattle, WA, United States, [3]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Malaria vaccine clinical trials using the malaria human challenge model require accurate and sensitive methods for detection of parasitemia. Currently the gold standard is peripheral blood smear, although development of highly sensitive and reproducible quantitative assays that detect pre-patent parasitemia is desirable. We developed a real-time quantitative reverse transcription PCR (qRT-PCR) for the A-type 18S rRNAs of *Plasmodium falciparum*. Methods: Total nucleic acids were extracted from frozen whole blood samples spiked with an exogenous competitive control RNA. Quantitative RT-PCR with dual hybridization probes for the malaria 18S rRNA was performed. The standard curve generated from *in vitro*-transcribed RNA standards was correlated against a parasite-containing whole blood standard to determine the number of 18S rRNA molecules per parasite. Results: The analytical sensitivity of the assay is 20 parasites/mL. The assay correctly identified all parasite-containing samples (ranging from 40 parasites/mL to 4x107 parasites/mL) and all negative samples (clinical sensitivity and specificity 100%). The reportable range is ≥20 parasites/mL. The observed values for >98.7% of samples tested were within 0.5 log10 units of the nominal values. At 80 parasites/mL, the within-run coefficient of variation was 1.8% and between-runs 4.0%; at 4x107 parasites/mL, the within- and between-run variation was 0.6% and 1.6%, respectively. This method was used to assess A-type 18S rRNA expression throughout the blood-stage lifecycle of highly synchronized *P. falciparum* parasites. We also used the assay to monitor parasitemia in a *P. falciparum* human challenge trial. Conclusions: This method offers advantages over comparably-sensitive DNA-based PCR assays because specimen handling requirements are reduced (filtering of leukocytes is not required) and a small blood volume can be used. This qRT-PCR format can be adapted for other *Plasmodium* species. The assay will be an important tool for monitoring malaria clinical trials and also may be adapted for diagnostic purposes in the clinical laboratory.

845

COMMON RED BLOOD CELL POLYMORPHISMS CONFER DIFFERENT LEVELS OF PROTECTION AGAINST *PLASMODIUM FALCIPARUM* MALARIA: RESULTS FROM A PEDIATRIC COHORT IN MALI, WEST AFRICA

Drissa Konaté[1], Tatiana M. Lopera-Mesa[2], Karim Traoré[1], Seidina A. S. Diakité[1], Saibou Doumbia[1], Mory Doumbia[1], Michael A. Krause[2], Greg Tullo[2], Kazutoyo Miura[2], Jennifer M. Anderson[2], Michael P. Fay[3], Carole A. Long[2], Mahamadou Diakite[1], Rick M. Fairhurst[2]

[1]University of Bamako, Bamako, Mali, [2]Laboratory of Malaria and Vector Research/National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States, [3]Biostatistics Research Branch, National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States

In Mali, 5 red blood cell (RBC) polymorphisms are extremely common, are frequently co-inherited, and are associated with reduced risk of severe *falciparum* malaria. These include sickle hemoglobin S (HbS), HbC, alpha-thalassemia, G6PD deficiency, and type O blood group antigen. To determine whether and to what degree each of these RBC polymorphisms protect against uncomplicated *falciparum* malaria in a single study population, we initiated a 5-year longitudinal cohort study in three villages

in rural Mali. From June 2008 to December 2009, we enrolled 1419 children aged 6 months to 17 years. We found that the vast majority of children carry at least 1 RBC polymorphism: 15% HbS, 8% HbC, 28% alpha-thalassemia, 15% G6PD deficiency, and 40% type O blood group antigen. During the 2008 and 2009 transmission seasons (June-December), we diagnosed 1277 children with 1980 episodes of *falciparum* malaria (92% uncomplicated, 8% severe - mostly prostration, repetitive vomiting, and cessation of eating and drinking). To estimate the relative risk (RR) of malaria by RBC polymorphism, we compared malaria incidence rate ratios using a Poisson regression model that took into account age (a surrogate of acquired immunity), sex, ethnicity, and village. Since only 11 episodes of cerebral malaria or severe malarial anemia were diagnosed, we combined all cases of malaria for ease of analysis. Compared to HbA, HbS reduced the incidence of malaria by 39% (RR 0.61, 95%CI 0.51-0.73, p <0.0001). This reduction was comparable to that associated with age 6-10 years (RR 0.77, 95%CI 0.68-0.88, p <0.0001) and 11-17 years (RR 0.35, 95%CI 0.29-0.42, p <0.0001), when compared to age 0.5-5 years. Other RBC polymorphisms were not associated with statistically significant reductions in malaria incidence. The malaria protective effect of HbS was not associated with reduced parasite density. In contrast, the protective effect of age was strongly associated with reduced parasite density. Relative to children aged 0.5-5 years, children aged 6-10 years and 11-17 years showed 63% and 83% reductions in mean parasite density. Contrasting the protective effects of HbS and age on the development of malaria may yield important clues about the mechanism of protection by the sickle-cell trait.

846

IRON DEFICIENCY DECREASES THE RISK OF *PLASMODIUM FALCIPARUM* MALARIA AND DEATH IN CHILDREN

Moses M. Gwamaka[1], Jonathan D. Kurtis[2], Bess E. Sorensen[3], Theonest K. Mutabingwa[4], Michal Fried[3], Patrick E. Duffy[5]

[1]Mother-Offspring Malaria Studies Project, Muheza Designated District Hospital, Muheza; Ifakara Health Institute, Morogoro, United Republic of Tanzania, [2]Brown University Medical School, Providence, RI, United States, [3]Seattle Biomedical Research Institute, Seattle, WA, United States, [4]National Institute of Medical Research, Dar Es Salaam, United Republic of Tanzania, [5]National Institutes of Health, Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States

Iron supplementation in malaria endemic areas may increase malaria morbidity and mortality. We explored whether iron status alters malaria risk in Tanzanian children (N=785) living in an area of intense malaria transmission. Children were enrolled at birth, and monitored for malaria and iron status for up to 3 yrs with an average of 47 blood smears and 3 iron status determinations/child. We evaluated the impact of iron deficiency (ID) on malaria outcomes and mortality using multivariate models accounting for repeated measures and potential confounders. Compared to iron-replete children, children with ID had reduced prevalence of concurrent parasitemia (6.6-fold lower), hyperparasitemia (24.0-fold lower) and severe malaria (4.0-fold lower), and, if infected, 3.9 fold lower parasite density (all P < 0.001). ID predicted significant decreases in the odds of subsequent parasitemia (23% decrease, P<0.001) and subsequent severe malaria (38% decrease, P=0.04). Children with ID on half or more of their iron status measurements (N=407) had 63 % lower all cause mortality (P = 0.04) and 73% lower malaria-associated mortality (P = 0.07) compared to children with ID on fewer than half of their iron status measurements (N=378). Together with published data, our results indicate that malaria risk is influenced by physiologic iron status as well as iron supplementation. Future interventional studies should assess whether ID and malaria control measures can mitigate the risks of iron supplementation for children in areas of malaria transmission.

847

THE ROLE OF VARIANT ANTIGEN SWITCHING IN THE DEVELOPMENT OF SYMPTOMATIC MALARIA

Mark A. Travassos[1], Amadou Niangaly[2], Drissa Coulibaly[2], Abdoulaye K. Kone[2], Karim Traoré[2], Youssouf Tolo[2], Issa Diarra[2], Bouréma Kouriba[2], Ahmadou Dembélé[2], Matthew B. Laurens[1], Mahamadou A. Thera[2], Ogobara K. Doumbo[2], Christopher V. Plowe[1]

[1]University of Maryland School of Medicine, Baltimore, MD, United States, [2]Malaria Research and Training Center, University of Bamako, Bamako, Mali

Var genes encode proteins that mediate adherence of erythrocytes infected by the malaria parasite *Plasmodium falciparum* to human tissue and comprise 50 to 60 genes within each parasite's genome. The parasite's ability to switch *var* gene expression may permit it to sequester in different sites in the body and potentially avoid the immune system, thereby contributing to virulence. Previous work in Bandiagara, Mali has shown that cerebral malaria is associated with the expression of a group of structurally distinct *var* genes. To date, there have been no longitudinal studies evaluating how *var* gene expression changes in a population. We aimed to compare *var* gene expression changes in individuals who progress from asymptomatic to symptomatic malaria infection compared to individuals who remain asymptomatic over time. We enrolled 300 children in an ongoing longitudinal cohort study of malaria incidence in Bandiagara, Mali. Blood samples obtained quarterly and during episodes of clinical malaria were evaluated for parasite RNA expression of *var* genes with reverse transcription PCR using degenerate primers specific to this gene family. During the first year of follow-up, study subjects experienced on average at least one clinical malaria episode each, and six subjects were diagnosed with cerebral malaria. The parasite RNA collected during symptomatic and asymptomatic time points is being evaluated for *var* gene expression, and results will be presented describing association of expression of particular *var* gene groupings with clinical illness and the extent to which a change in *var* gene expression increases an individual's risk of developing symptomatic malaria.

848

VARIATION WITHIN THE TOLL-LIKE RECEPTOR-9 (TLR-9) GENE PROMOTER (-1237C/T) IS ASSOCIATED WITH PROTECTION AGAINST PEDIATRIC SEVERE MALARIAL ANEMIA AND FUNCTIONAL CHANGES IN CIRCULATING IFN-Γ

Sammy K. Kiplagat[1], Gregory C. Davenport[2], Tom Were[3], Samuel Anyona[3], Evans O. Raballah[3], James B. Hittner[4], John M. Vulule[5], John-Michael Ong'echa[3], Douglas Perkins[6], Collins Ouma[1]

[1]Maseno University, Kisumu, Kenya, [2]University of Pittsburgh, Pittsburgh, PA, United States, [3]University of New Mexico/KEMRI, Kisumu, Kenya, [4]College of Charleston, Charleston, NC, United States, [5]KEMRI, Kisumu, Kenya, [6]University of New Mexico, Albuquerque, NM, United States

Plasmodium falciparum malaria still remains one of the leading global causes of infectious disease burden. In holoendemic *P. falciparum* transmission areas, such as western Kenya, severe malarial anemia (SMA) results in high rates of pediatric morbidity and mortality. Since Toll-like receptors (TLRs) affect innate and adaptive immune responses, the roles of polymorphic variants within TLR-9 in conditioning susceptibility to SMA were investigated. The relationship between the TLR-9 variant (-1237C/T, rs5743836) and susceptibility to SMA (Hb<6.0 g/dL, any density parasitemia) was investigated in children (n=277) with *falciparum* malaria from a holoendemic *P. falciparum* transmission region in western Kenya. Hematological and parasitological profiles were determined in all study participants. TLR-9 -1237C/T genotypes were determined using a bi-directional allele-specific PCR amplification. Circulating interferon (IFN)-γ levels were determined using BiosourceTM hu multiplex inflammatory profile. Frequencies of the -1237CC, CT and TT were 6.8%, 44.4%, and

48.7%, respectively. Multivariate logistic regression analyses controlling for potential confounders demonstrated that homozygous C individuals (OR; 0.31, 95% CI, 0.10-0.95; *P*=0.040) and heterozygous (TC) individuals (OR; 0.57, 95%CI, 0.34-0.95; *P*=0.030) were protected against SMA relative to TT carriers. In addition, carriers of the CC genotype had significantly lower circulating IFN-γ levels relative to TC (*P*=0.040) and TT individuals (*P*=0.098). Results presented here demonstrate that variation in TLR-9 at -1237 is associated with protection against SMA and functional changes in circulating IFN-γ levels.

849

C5A IS ELEVATED IN AFRICAN CHILDREN WITH CEREBRAL MALARIA (CM) AND C5A RECEPTOR DEFICIENCY IMPROVES SURVIVAL IN EXPERIMENTAL CEREBRAL MALARIA (ECM)

Hani Kim[1], Laura Erdman[1], Aggrey Dhabangi[2], Charles Musoke[2], Craig Gerard[3], Christine Cserti-Gazdewich[4], Kevin C. Kain[1]

[1]McLaughlin Rotman Centre for Global Health, Toronto General Hospital, University of Toronto, Toronto, ON, Canada, [2]Makerere University College of Health Sciences, Kampala, Uganda, [3]Pulmonary Division, Department of Pediatrics, Children's Hospital, Boston, MA, United States, [4]University Health Network, University of Toronto, Toronto, ON, Canada

Cerebral malaria (CM) in humans and in animal models (ECM) is associated with a dysregulated host innate immune response to infection. For example, high levels of inflammatory cytokines contribute to endothelial cell activation and increased expression of adhesion molecules resulting in the sequestration of parasitized erythrocytes in the cerebral microvasculature. The complement system is an essential component of host innate immunity, and its activation culminates in the generation of pro-inflammatory anaphylatoxins, C3a and C5a. Using a panel of inbred and congenic mice, our laboratory has recently demonstrated that susceptibility to ECM is associated with the generation of C5a, which contributes to dysregulated inflammatory and angiogenic responses to parasite products. C5a can bind two receptors, C5aR and C5L2. The pro-inflammatory effects of C5a are thought to occur via its interaction with C5aR. Based on the hypothesis that excessive complement activation contributes to severe malaria, we have further investigated the role of C5a in the pathogenesis of CM by: i.) examining the levels of C5a in Ugandan children with CM versus those with uncomplicated malaria (UM); and ii.) determining if mice deficient in C5aR or C5L2 are protected from ECM. In a case-control study of Ugandan children, we show that children with CM had significantly elevated plasma levels of C5a compared to children with UM (median(range); CM, 45.4(10.0, 126.2) vs. UM, 22.4(3.3, 226.7); p =0.0003, Mann-Whitney). In ECM studies we show that C57BL/6 mice deficient in C5aR (C5aR KO), had significantly improved survival following challenge with *Plasmodium berghei* ANKA (PbA) compared to wild-type controls. Survival was associated with increased levels of angiopoietin-1, an angiogenic factor linked to endothelial cell quiescence. These results are consistent with a role for C5a in the pathogenesis of ECM and in CM in human infection. Additional studies examining the role of the alternate receptor, C5L2, in ECM are underway.

850

ELEVATED LEVELS OF CSF TETRAHYDROBIOPTERIN AND NEOPTERIN DISTINGUISH NON-MALARIAL COMA FROM CEREBRAL MALARIA WITH HIGH SPECIFICITY AND SENSITIVITY

Jackson P. Mukemba[1], Salvatory Florence[1], Willy Sangu[2], Frances Mchomvu[3], Matthew P. Rubach[4], Bert K. Lopansri[5], Tsin W. Yeo[6], Nicholas M. Anstey[6], J. Brice Weinberg[7], Donald L. Granger[4], Esther D. Mwaikambo[1]

[1]Hubert Kairuki Memorial University, Dar es Salaam, United Republic of Tanzania, [2]Hubert Kairuki Memorial University and Amana Hospital, Ilala District, Dar es Salaam, United Republic of Tanzania, [3]Hubert Kairuki Memorial University and Mwananyamala Hospital, Kinondoni District, Dar es Salaam, United Republic of Tanzania, [4]University of Utah and Veterans Affairs Hospital, Salt Lake City, UT, United States, [5]Loyola University and Veterans Affairs Hospital, Chicago, IL, United States, [6]Menzies School for Health Research, Darwin, Australia, [7]Duke University and Veterans Affairs Hospital, Durham, NC, United States

Based on finding abnormal aromatic amino acid (AAA) metabolism in children with cerebral malaria (CM), we conducted a prospective observational cohort study to test the hypothesis that CNS products of AAA, biogenic amine neurotransmitters (BAN), i.e. catecholamines and serotonin, were deficient. BAN metabolites were quantified in CSF of CM cases and compared to: a) children with coma from non-malarial conditions (NMC) and b) a reference database of normals (Biodef). AAA metabolism requires a pterin cofactor, tetrahydrobiopterin (BH4), for BAN synthesis. We hypothesized that BH4 was deficient in CM. We measured BH4 levels in CSF of children with CM and NMC and compared the results to Biodef. CSF was collected per protocol and analyzed by Medical Neurogenetics, Atlanta, GA, for BAN metabolites and pterins. Study groups were defined by WHO criteria. Excluded were children in whom LP was contraindicated and those with severe anemia. All participants (6 months to 6 years) were entered with IRB-approved guardian consent. We enrolled 33 with CM and 43 with NMC. CSF levels of BAN metabolites were normal and indistinguishable between CM and NMC participants. CSF BH4 was elevated in 70% of children with CM but it was normal in all children with NMC (p = 0.002; 100% specificity for CM). Neopterin (another CSF pterin) was elevated in all with CM but in only 28% of NMC subjects (p = 0.003; 100% sensitivity for CM). Elevated CSF neopterin was reported in CM, encephalitis and hemophagocytic syndrome. Elevated CSF BH4 is very unusual; it was found in a rare genetic disorder, Aicardi-Goutières syndrome, caused by mutations of *RNASEH2B* or *TREX1* or *SAMHD1* genes. The common feature of this inflammatory brain disease is uninhibited signaling through TLR-9 by non-degraded nucleic acid polymers. The recent finding of TLR-9 signaling by *Plasmodium falciparum* DNA bound to hemazoin (as reported previously) may relate to increased CNS pterin synthesis as a manifestation of a brain-specific inflammatory pathway leading to malarial coma.

851

IN VIVO EVIDENCE THAT MICROCIRCULATORY OBSTRUCTION IS A CENTRAL PATHOLOGICAL PROCESS IN SEVERE *FALCIPARUM* MALARIA

Richard J. Maude, Prakaykaew Charunwatthana, Nicholas P. Day, Nicholas J. White, Arjen M. Dondorp

Wellcome Trust Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand

First identified more than a century ago by Marchiafava and Bignami, the quantitative contribution of microcirculatory obstruction to the pathogenesis of severe *falciparum* malaria is still disputed. We have studied the different factors compromising the microcirculation and their relationships with disease severity. Direct microscopic observation of the rectal microcirculation in living patients reveals obstructed capillaries in

patients with severe malaria. Red cell deformability (RCD) is decreased in relation to disease severity, and is likely to contribute to reduced perfusion in capillaries already partly obstructed by sequestered erythrocytes. The sequestered parasite biomass, estimated from parasite derived plasma PfHRP2 concentrations, correlates strongly with disease severity. Marked sequestration is evident in the brain microvasculature of patients who died from cerebral malaria (CM) and cytoadhesion of mature parasitized red blood cells to the venular and capillary endothelium is readily seen on electron microscopy. In living patients evidence of microcirculatory obstruction in the brain in CM is difficult to obtain. Observation of the retinal vasculature has the advantage that it is the only central nervous system vascular bed easily accessible for visualisation and provides a unique opportunity to observe vascular pathology and its effect on neurological tissue. A specific retinopathy has been described in African children with CM and its severity correlates with outcome. Detailed fluorescein angiography suggests this results from microcirculatory obstruction. Recent studies have found the same retinopathy in adults. This is most common (around 80%) in cerebral and fatal malaria and its severity correlates with disease severity. Markers and causes of microvascular obstruction (RCD, rectal microvascular blood flow, blood lactate and PfHRP2) correlate strongly with the severity of retinopathy. These data all point to obstruction of microcirculatory flow as a central pathophysiological mechanism causing coma and death in CM.

852

CHARACTERISTICS AND ETIOLOGY OF MODERATE-TO-SEVERE DIARRHEA OF PROLONGED OR PERSISTENT DURATION AMONG CHILDREN LESS THAN FIVE YEARS OLD IN RURAL WESTERN KENYA, 2008-2009

Katharine A. Schilling[1], Richard Omore[2], Tracy Ayers[1], Tamer H. Farag[3], Dilruba Nasrin[3], Lynette Berkeley[3], Sandra Panchalingam[3], James P. Nataro[3], Karen Kotloff[3], Mike M. Levine[3], Joseph Oundo[2], Michele B. Parsons[1], Cheryl Bopp[1], John Vulule[4], Kayla Laserson[5], Eric Mintz[1], Ciara E. O'Reilly[1], Robert F. Breiman[6]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Kenya Medical Research Institute/Centers for Disease Control and Prevention, Kisumu, Kenya, Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya, [3]University of Maryland, School of Medicine, Center for Vaccine Development, Baltimore, MD, United States, [4]Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya, [5]Kenya Medical Research Institute/Centers for Disease Control and Prevention, Kisumu, Kenya, [6]Kenya Medical Research Institute/Centers for Disease Control and Prevention, Nairobi, Kenya

Diarrheal disease is a leading cause of illness and death among children <5 years old in sub-Saharan Africa. Data on diarrhea of extended duration is limited. We examined diarrhea duration in Kenyan children <5 years old participating in the Global Enterics Multicenter Study. Children presenting at a clinic were enrolled if they met the case definition for acute moderate-to-severe diarrhea defined as ≥3 loose stools in the last 24 hrs, within 7 days of illness onset, with ≥1 of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization. To determine diarrhea duration, the child's caretaker was asked to recall the number of days the child had diarrhea in the 7 days pre-enrollment, and to record each day of diarrhea post-enrollment on a form for 14 days. Stool specimens were collected at enrollment, and the post-enrollment form was collected during a home visit. We defined acute diarrhea (AD) as ≤6 days duration, prolonged diarrhea (ProD) as 7-13 days, and persistent diarrhea (PD) as ≥14 days. From January 31, 2008 to January 30, 2009, 485 children with acute moderate-to-severe diarrhea were enrolled. Of these, 47% (n=226) had AD, 46% (n=221) had ProD, and 8% (n=38) had PD. For males (n=271) and females (n=214) respectively, 42% and 52% had AD, 49% and 41% had ProD, and 8% and 7% had PD. Infants (n=219), toddlers (n=138), and older children (n=128) had AD (40%, 46%, and 59%); ProD (50%, 45%, and 38%); and PD (10%, 9%, and 3%), respectively. Children with ProD or PD respectively, had enteroaggregative *E. coli* (EAEC) identified in 21% and 32% of their stool specimens, *Giardia* in 19% and 11%, enterotoxigenic *E. coli* (ETEC) *in* 14% and 11%, rotavirus in 13% and 11%, enteropathogenic *E. coli* in 13% and 5%, *Campylobacter* in 12% and 16%, *Cryptosporidium* in 10% and 26%, norovirus in 9% and 8%, *Shigella* in 7% and 3%, and *Salmonella* 5% and 5%. A high proportion of moderate-to-severe diarrheal illness was of prolonged duration, and the most common etiologies of ProD were EAEC, *Giardia* and ETEC, and of PD were EAEC, *Cryptosporidium* and *Campylobacter*.

853

A PROSPECTIVE AETIOLOGICAL, EPIDEMIOLOGICAL AND CLINICAL STUDY ON DIARRHOEAL DISEASE IN CHILDREN UNDER FIVE YEARS OF AGE IN HO CHI MINH CITY, VIETNAM

My V. Phan[1], James I. Campbell[1], Hoang V. Nguyen[1], Minh V. Pham[1], Vinh T. Nguyen[1], Thuy T. Cao[1], Nga T. Nguyen[1], Thuong C. Tang[2], Phuc L. Hoang[2], Tuan M. Ha[3], Tuyet T. Pham[3], Chinh T. Nguyen[4], Vinh Ha[4], Jeremy Farrar[1], Stephen Baker[1]

[1]Oxford University Clinical Research Unit and Wellcome Trust Asia Research Programme, Ho Chi Minh, Vietnam, [2]Children's Hospital 1, Ho Chi Minh, Vietnam, [3]Children's Hospital 2, Ho Chi Minh, Vietnam, [4]Hospital for Tropical Diseases, Ho Chi Minh, Vietnam

Childhood diarrhoea remains an important public health challenge in developing countries, where the disease represents the second leading cause of mortality in children under the age of five. Routine identification of the aetiological agents causing diarrhoea is seldom performed in resource limited countries. Vietnam is typical of a country undergoing economic transition where the spectrum of infectious diseases is changing rapidly. We conducted a prospective study to investigate the aetiology, epidemiology and clinical features of acute diarrhoea in children under the age of five in Ho Chi Minh City, in southern Vietnam. The study was designed to enroll 1,500 patients admitted to three referral hospitals over a period of one year. Our preliminary data demonstrated that viral pathogens were responsible for more than 60% of diarrhoeal infections with rotavirus being the most prevalent cause followed by norovirus. Rotavirus G1P[8] and norovirus genogroup II were the two most common viral genotypes isolated. The results also demonstrated that more than 80% of these diarrhoea episodes were treated with antimicrobials. This unnecessary use of antimicrobials may contribute to the dramatic level of antimicrobial resistance seen in the bacterial pathogens isolated, including common resistance to fluoroquinolones and 3rd generation cephalosporins. Bacterial pathogens accounted for approximately 20% of all cases, of which *Shigella spp*, *Salmonella spp*, *Campylobacter jejuni* and *Campylobacter coli* were the major agents identified. We were also able to identify specific symptoms, which may help in developing clinical algorithms, hence aiding diagnosis and guide appropriate treatment regimens. Our findings highlight a change in the aetiology and epidemiology of childhood diarrhoea in Ho Chi Minh City. This change is concurrent with socio-economic development and will help inform public health policy for the prevention and treatment of childhood diarrhoea.

854

SPATIAL AND TEMPORAL RELATIONSHIP OF CIRCULATING *SALMONELLA* TYPHI IN KATHMANDU, NEPAL

Stephen Baker[1], Kathryn Holt[2], Archie Clements[3], Abhilasha Karkey[4], Amit Aryjal[4], Buddha Basnyat[4], Jeremy Farrar[1]

[1]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, [2]The University of Melbourne, Melbourne, Australia, [3]The University of Queensland, Brisbane, Australia, [4]Oxford University Clinical Research Unit, Kathmandu, Nepal

Enteric fever, the disease caused by invasive Salmonella serovars Typhi and Paratyphi A is still a considerable public health problem in some setting. Enteric fever is mainly isolated to parts of Asia, South America and Africa, and is limited to densely populated areas with poor sanitation, which facilitates ongoing transmission of the organisms. The disease is restricted to humans, therefore, understanding the structure and

dynamics of Salmonella Typhi is essential to study ongoing interactions between the pathogen and host. The amalgamation of genetic, phenotypic, spatial and temporal data can provide an extensive view of the epidemiology and evolution of this bacterial pathogen. However, the lack of a suitable technique has made it difficult to identify changes in Salmonella Typhi populations through time and space. New generation sequencing technologies have been applied to identify ~2,000 SNPs within the Salmonella Typhi population, providing loci for refined single nucleotide polymorphism (SNP) typing of clinical isolates. Four hundred and fifty Salmonella Typhi strains isolated over a four year period from a an urban area in Kathmandu were genotyped and analyzed along with GPS, epidemiological and geospatial data. We find the circulation of a dominant genotype, which we describe as the epidemic strain, which co-localizes with the areas surrounding the municipal water spouts. We additionally find evidence of microevolution and specific localities in which less common genotypes circulate. Our findings demonstrate that, contrary to popular belief, acute transmission within the household is highly limited and that specific genotypes show integral spatial relationships. These data add insight into the dissemination of these organisms in this community and will provide the framework for molecular epidemiological studies of other bacterial pathogens.

855

CHARACTERIZATION OF ANTI-*SALMONELLA ENTERICA* SEROTYPE TYPHI ANTIBODY RESPONSES IN BACTEREMIC BANGLADESHI PATIENTS USING IMMUNO-AFFINITY PROTEOMIC-BASED TECHNOLOGY (IPT)

Richelle C. Charles[1], Alaullah Sheikh[2], Bryan Krastins[3], Jason B. Harris[1], Md. Saruar Bhuiyan[2], Regina C. LaRocque[1], Tanya Logvinenko[4], David A. Sarracino[3], Indira T. Kudva[5], Jana Eisenstein[1], Michael Podolsky[1], Anuj Kalsy[1], Albrecht Ludwig[6], Manohar John[7], Stephen B. Calderwood[1], Firdausi Qadri[2], Edward T. Ryan[1]

[1]Massachusetts General Hospital, Boston, MA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]Thermo Fisher Scientific, Cambridge, MA, United States, [4]Tufts University Medical Center, Boston, MA, United States, [5]United States Department of Agriculture, Ames, IA, United States, [6]Klinikum der Johann Wolfgang Goethe-Universitat, Frankfurt am Main, Germany, [7]Pathovacs Incorporated, Ames, IA, United States

Salmonella enterica serotype Typhi (S. Typhi) is the cause of typhoid fever and a human-restricted pathogen. Currently available typhoid vaccines provide only 50-75% protection for 2-5 years, and available diagnostic assays to identify individuals with typhoid fever lack both sensitivity and specificity. Identifying immunogenic S. Typhi antigens expressed during human infection could lead to improved diagnostic assays and vaccines. Here we describe a platform Immuno-affinity Proteomic-based Technology (IPT) that involves the use of columns charged with IgG, IgM or IgA antibody fractions recovered from humans bacteremic with S. Typhi to capture S. Typhi proteins subsequently identified by mass spectrometry. This screening tool identifies immunogenic proteins recognized by antibodies from infected hosts. Using this technology and the plasma of patients with S. Typhi bacteremia in Bangladesh, we identified 57 proteins of S. Typhi, including proteins known to be immunogenic (PagC, HlyE, OmpA, and GroEL), and a number of proteins present in the human-restricted serotypes S. Typhi and S. Paratyphi A but rarely found in broader host-range Salmonella spp. (HlyE, CdtB, PltA, and STY1364). We categorized identified proteins into a number of major groupings, including those involved in energy metabolism, protein synthesis, iron homeostasis, biosynthetic and metabolic functions, and those predicted to localize to the outer membrane. We assessed systemic and mucosal anti-HlyE responses in S. Typhi infected patients, and detected anti-HlyE responses at the time of clinical presentation in patients but not in controls. These findings could assist in the development of improved diagnostic assays.

856

LEPTOSPIROSIS AMONG HOSPITALIZED PATIENTS WITH FEBRILE ILLNESS IN NORTHERN TANZANIA

Holly M. Biggs[1], Duy M. Bui[2], Renee L. Galloway[2], Robyn A. Stoddard[2], Sean V. Shadomy[2], Annie B. Morrissey[1], John A. Bartlett[1], Jecinta J. Onyango[3], Venance P. Maro[3], Grace D. Kinabo[3], Wilbrod Saganda[4], John F. Shao[5], John A. Crump[1]

[1]Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Durham, NC, United States, [2]Bacterial Zoonoses Branch, Division of Foodborne, Bacterial, and Mycotic Diseases, National Center for Zoonotic, Vectorborne, and Enteric Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Kilimanjaro Christian Medical Center, Moshi, United Republic of Tanzania, [4]Mawenzi Regional Hospital, Moshi, United Republic of Tanzania, [5]Kilimanjaro Christian Medical College, Tumaini University, Moshi, United Republic of Tanzania

The epidemiology of human leptospirosis in Tanzania is not well described. We examined leptospirosis as a cause of febrile illness among inpatients in northern Tanzania to determine its importance and inform control measures. We identified febrile patients among consecutive admissions to two hospitals in Moshi, Tanzania, from September 2007 through August 2008, recorded standardized demographic and clinical information, and collected acute and convalescent sera. Confirmed leptospirosis was defined as a ≥4-fold increase in MAT titer; probable leptospirosis as any reciprocal MAT titer ≥ 800; and exposure to leptospirosis as any titer ≥100. Among 870 patients enrolled in the study, 453 (52.1%) had paired sera available, and 40 (8.8%) of these met the definition for confirmed leptospirosis. Of 831 patients with ≥1 serum sample available, 30 (3.6%) had probable leptospirosis and an additional 277 (33.3%) had evidence of leptospirosis exposure. Of 70 persons with confirmed or probable leptospirosis 39 (55.7%) were male, the median age was 23 (range <1-78) years, and none were diagnosed clinically. Among those subsequently found to have confirmed or probable leptospirosis the most common clinical diagnoses were pneumonia in 18 (25.7%) and malaria in 31 (44.3%); 14 (20.0%) were treated with antimalarials alone. Among adults and adolescents, leptospirosis was associated with thrombocytopenia (OR 2.1, p=0.019). Leptospirosis was associated with living in a rural area (OR 3.4, p<0.001). Among 40 patients with confirmed leptospirosis, the predominant reactive serogroups were Mini and Australis, and 16 (40.0%) had evidence of co-infection with ≥1 additional pathogen. Leptospirosis is underdiagnosed yet accounts for a substantial proportion of febrile illness in northern Tanzania where it appears to be endemic. Clinicians should suspect leptospirosis in febrile patients, particularly those with thrombocytopenia or rural residence. Based on the pattern of serogroup reactivity, livestock are likely reservoirs. Further research to inform control measures in Tanzania is warranted.

857

ALTERNATIVE COMMUNITY-BASED SCREENING FOR RAT INFESTATION TO IDENTIFY HIGH-RISK HOUSEHOLDS FOR LEPTOSIROSIS IN URBAN SLUM SETTLEMENTS

Federico Costa[1], Guilherme S. Ribeiro[2], Norlan Santos[1], Nilzelene Santos[1], Renato B. Reis[1], Maria Gorete Rodrigues[3], Helena MC de Farias[3], Isabel Guimarães[3], Jonas Brant[4], Mitermayer G. Reis[1], Albert I. Ko[5]

[1]Oswaldo Cruz Foundation, Brazilian Ministry of Health, Salvador, Brazil, [2]Institute of Collective Health, Salvador, Brazil, [3]Zoonosis Control Center, Salvador, Brazil, [4]Field Epidemiology Training Program, Brazilian Ministry of Health, Brasilia, Brazil, [5]Department of Epidemiology and Public Health, Yale School of Medicine, New Haven, CT, United States

The domestic rat is believed to be the principal reservoir for urban leptospirosis. However, few if any studies have identified markers for rodent infestation in slum environments and evaluated their use in predicting the risk for leptospirosis. We performed a case-control study,

which enrolled households of leptospirosis cases identified between 2007 and 2009 in Salvador, Brazil and neighboring control households in the same slum communities. Households were surveyed for signs of rodent infestation and environmental characteristics. We used conditional logistic regression modeling to identify risk factors and develop a predictive score for leptospirosis with data collected from 2007 to 2008. We used receiver operating characteristic (ROC) curve analysis to evaluate the performance of the prediction score with an independent data set collected in 2009. We identified signs of rodent infestation in 63% (60/95) and 35% (64/184) of the cases and control households, respectively. Independent risk factors for acquiring leptospirosis in a household were rodent burrows (OR, 3.30; 95% CI, 1.50-7.26), *Rattus norvegicus* feces (2.86; 1.24-6.59), rodent runs (2.57; 1.06-6.22), household bordering an abandoned house (2.48; 1.04-6.02), and unplastered walls (2.22; 1.02-6.02). A prediction score was developed by assigning points (3, 3, 2, 2 and 2 respectively) to each risk factor. The area under the ROC curve for the scoring system was 0.70 (95% CI, 0.64-0.76) and 0.71 (0.65-0.79) for the development and validation datasets. In conclusion, our study indicates that high proportions (>44%) of urban slum households are infested with *R. norvegicus*. A simple prediction score demonstrated good performance in identifying high-risk households for leptospirosis within slum communities. These findings need to be confirmed in other urban centers. Yet, they suggest that community-based screening for rodent infestation may be a feasible strategy to target rodent and environmental control measures to populations at highest risk for leptospirosis.

858

Q FEVER AMONG HOSPITALIZED FEBRILE PATIENTS IN NORTHERN TANZANIA, 2007-2008

Malavika Prabhu[1], William L. Nicholson[2], Aubree J. Roche[2], Gilbert J. Kersh[2], Kelly A. Fitzpatrick[2], Lindsay D. Oliver[2], Robert F. Massung[2], Anne B. Morrissey[1], John A. Bartlett[3], Jecinta J. Onyango[4], Venance P. Maro[4], Grace D. Kinabo[5], Wilbrod Saganda[6], John A. Crump[1]

[1]Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Durham, NC, United States, [2]Rickettsial Zoonoses Branch, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Duke Global Health Institute, Duke University, Durham, NC, United States, [4]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania, [5]Kilimanjaro Christian Medical College, Tumaini University, Moshi, United Republic of Tanzania, [6]Mawenzi Regional Hospital, Moshi, United Republic of Tanzania

Little is known about *Coxiella burnetii* as a cause of febrile illness in sub-Saharan Africa, and its putative role as an HIV coinfection is unclear. We identified febrile patients among consecutive admissions to two hospitals in Moshi, Tanzania, from September 2007 to August 2008, recorded standardized clinical data, and collected acute and convalescent sera. After *C. burnetii* phase II antigen ELISA screening, positive samples with paired sera were tested by IgG immunfluorescence assay. A >=4-fold increase in titer to *C. burnetii* phase II antigen defined acute Q fever; a titer >=1/1,000 to *C. burnetii* phase I antigen defined probable chronic Q fever. Predictors of Q fever and patient management were examined. Among 870 febrile patients, 483 (55.5%) had sera screened; results suggested acute and chronic Q fever among 24 (5.0%) and 7 (1.4%) patients, respectively. The median (range) age for acute and chronic cases was 26 (1, 73) years and 29 (1, 49) years, respectively. Clinical features of acute Q fever included headache (82.4%), cough (75.0%), rigors (58.8%), and anemia (60.9%). Acute Q fever was associated with hepato- or splenomegaly (OR 3.1, p=0.026), anemia (OR 3.5, p=0.003), leukopenia (OR 4.0, p=0.012), jaundice (OR 7.0, p=0.008), and livestock parturient season (OR 3.2, p=0.004). HIV infection was not associated with acute Q fever (OR 1.7, p=0.217). Q fever was never clinically diagnosed; the most common diagnoses among those subsequently found to have acute Q fever were malaria in 8 (33.3%) and pneumonia in 5 (20.8%); 2 (8.3%) patients received antimicrobials active against *C. burnetii*. No chronic cases had clinical endocarditis and no patients with Q fever died. Despite being an important cause of febrile illness in northern Tanzania, Q fever is not considered in the differential diagnosis and patients are seldom treated with an agent active against *C. burnetii*. Increased awareness, access to Q fever diagnostic tests and more frequent use of tetracyclines may improve patient outcomes. *C. burnetii* does not appear to be an HIV-related coinfection.

859

DISEASE ASSOCIATION MAPPING IN *ANOPHELES GAMBIAE*: WHAT IS THE EFFECT OF SINGLE NUCLEOTIDE POLYMORPHISMS ON MALARIA INFECTION?

Ashley A. Horton[1], Lattha Souvannasang[1], Yoosook Lee[1], Cheick A. Coulibaly[2], Anthony J. Cornel[1], Gregory C. Lanzaro[1], Shirley Luckhart[1]

[1]University of California Davis, Davis, CA, United States, [2]University of Bamako, Bamako, Mali

There are over one million deaths and 500 million cases annually of malaria, a disease caused by parasites of the genus *Plasmodium* that are vectored by anopheline mosquitoes. We have adopted a "disease association mapping" approach, commonly used in humans, in a novel way to determine whether single nucleotide polymorphisms (SNPs) in immune signaling genes of *Anopheles gambiae* are associated with *Plasmodium falciparum* infection. We examined the encoded conserved domains of more than 30 mosquito immune signaling genes to determine SNP presence and predicted effects on protein function. For preliminary functional analyses, we have developed a "designer SNP" approach to analyze SNPs that are predicted to inhibit the interaction of the *A. gambiae* signaling proteins MEK and ERK. These proteins are components of a MAPK signaling cascade that has been shown to regulate *P. falciparum* development in the mosquito host. Our data can be used not only to answer important basic science questions about susceptibility under natural conditions, but also to discover genetic markers that can be used for surveillance and to develop genetically engineered mosquitoes that are refractory to malaria parasite transmission.

860

SMALL RNA PROFILE ANALYSIS PROVIDES NEW INSIGHT INTO MOSQUITO-DENGUE INTERACTIONS

Ann Hess[1], Andre Ptitsyn[1], Ken Olson[1], Greg Ebel[2], Abhishek Prasad[2], **Corey Campbell**[1]

[1]Colorado State University, Fort Collins, CO, United States, [2]University of New Mexico School of Medicine, Albuquerque, NM, United States

In vector mosquitoes, such as Aedes aegypti, arboviruses hijack host cell processes to establish a successful infection, evade the immune response, and support transmission to mammalian hosts. RNA interference (RNAi) is one way that mosquitoes defend themselves against Dengue virus (DENV). This pathway is an important regulator of host cell gene expression and also cleaves viral genomes. Small RNA (sRNA) regulatory pathways (SRRPs) control host gene expression in a variety of ways. A major product of this control mechanism is the production of small RNAs in different size classes. RNAi and PIWI pathway-produced sRNAs are 20-23 nts and 24-30 nts, respectively. We show that one component of the Ae. aegypti RNAi pathway, Argonaute-2, binds to small RNAs in the expected size range and is associated with a large molecular weight complex (RNA-induced Silencing Complex). These complexes are present in mosquitoes prior to a bloodmeal and are depleted by 1 day after bloodfeeding, suggesting that available RISC complexes may be rate-limiting in anti-viral defense. We used deep sequencing technology to analyze changes to small RNA profiles during DENV infection. Enrichment of sRNAs for a given target mRNA indicates depletion of the target by an SRRP. This method allows us to assess biological pathways that are altered during arbovirus infection in an RNAi-dependent manner. We found that transcript levels in the following categories are altered during DENV infection at 2 and 4 dpi:

immunity, transport, transcription factors, and energy metabolism. Each of these functional categories is important to establishing a successful DENV infection. In addition, viral sRNA profiles change over the course of infection. The data presented will provide insight into the cellular processes that are affected during DENV infection.

861

A PEROXIDASE/DUAL OXIDASE SYSTEM MODULATES MIDGUT EPITHELIAL IMMUNITY IN *ANOPHELES GAMBIAE*

Alvaro Molina-Cruz, Sanjeev Kumar, Lalita Gupta, Janneth Rodrigues, Carolina Barillas-Mury

National Institutes of Health, Bethesda, MD, United States

A large number of commensal bacteria reside in the gut of insects, like in most metazoa. Gut epithelial cells need to protect the host from pathogenic organisms but must do so without mounting immune responses against the normal microbiota. This is especially challenging in blood-feeding insects because commensal bacteria proliferate extensively during blood digestion. We have found that a heme peroxidase (Immuno modulatory peroxidase, IMPer), secreted by the mosquito *Anopheles gambiae* midgut, and dual oxidase (DUOX) form a dityrosine network that decreases gut permeability to immune elicitors. This network protects the microbiota by preventing activation of epithelial immunity. IMPer midgut expression is upregulated around 12h after a blood meal and its activity is localized to the ectoperitrophic space. IMPer dsRNA mediated knock down leads to a decrease in gut bacteria and to an up regulation of mosquito immune related genes, suggesting that during normal digestion IMPer activity prevents a midgut immune response against bacteria. IMPEr or DUOX dsRNA mediated knock down decrease infection of the midgut by *Plasmodium berghei* or *Plasmodium falciparum* by up regulating nitric oxide synthetase (NOS) expression. This indicates that IMPer and DUOX normal activity prevent the induction of NOS expression in response to *Plasmodium*. Peroxidases are known to form protein netwoks by formation of dityrosine covalent bonds, for which DUOX can generate a required substrate (H2O2). Immunostaining of the mosquito midgut detected dityrosine bonds in the luminal side of the midgut epithelia, which decreased upon dsRNA mediated knock down of IMPer or DUOX. Midgut permeability to fluorescent dextran increases upon dsRNA mediated knock down of IMPer or DUOX. The results indicated that IMPer and DUOX prevent immune activation of the midgut by forming a dityrosine protein network that decreases permeability to immune elicitors, a novel function for a Peroxidase/oxidase system.

862

THE ROLE OF AUTOPHAGY IN FOLLICULAR ATRESIA DURING OOGENESIS IN THE MOSQUITO *AEDES AEGYPTI*

Lyric C. Bartholomay, Susan R. Hodgkins, Alex O. Osanya

Iowa State University, Ames, IA, United States

Autophagy is a process for degrading and recycling cytoplasmic contents via formation of an autophagosome that fuses with a lysosome. Autophagy is triggered by diverse physiological stressors including starvation and infection; indeed recently published and preliminary data indicate that autophagy is an innate immune response in arbovirus-infected flies. To explore the importance of autophagy in oogenesis in the mosquito, autophagy-related genes (Atg) were identified in the *Ae. aegypti* genome and profiled for spatial and temporal transcript production by RT-PCR. Autophagy-related gene 1 (Atg1) was of particular interest because it is a protein kinase that regulates induction of autophagy directly downstream of the target of rapamycin (TOR), and we observed an increase in Atg1 transcript in *Ae. aegypti* ovaries post-blood feeding. Adult *Ae. aegypti* were subjected to Atg1 suppression by injection with double-stranded RNA. At 20 and 48 hours post blood meal (hpbm), ovaries were dissected and subjected to neutral red staining, and gene suppression was confirmed by RT-PCR. In Atg1 suppressed mosquitoes, significantly more resorbing, atretic follicles are observed at 48 hpbm as compared to those in normally progressing ovaries in control mosquitoes. This distinct phenotype is consistent with Atg1 Drosophila mutants that show decreased apoptotic cell death in mid-stage oogenesis when follicles are undergoing atresia, suggesting that autophagy functions upstream of apoptosis in clearing atretic follicles from the ovariole. These data reveal similar and significant interplay between apoptosis and autophagy in the process of follicular atresia in a mosquito, and highlight the importance of autophagy in mosquito oogenesis. Given that autophagy is detrimental to arbovirus persistence, but critical for oogenesis, vertically transmitted mosquito-borne viruses must strike a careful balance in the ovary to avoid and not suppress autophagic events.

863

MOLECULAR CLONING OF SLIMFAST FROM THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI* REVEALS A HISTIDINE-SPECIFIC TRANSCEPTOR

Dmitri Y. Boudko[1], Immo Hansen[2], Lisa Drake[2], Dmitry Voronov[3], Ella Meleshkevitch[1], Sarah Aguirre[2], Geoffrey Attardo[2], Shin-Hong Shiao[4], Alexander Raikhel[5]

[1]Rosalind Franklin University, North Chicago, IL, United States, [2]New Mexico State University, Las Cruces, NM, United States, [3]Moscow State University, Moscow, Russian Federation, [4]National Taiwan University, Taipei, Taiwan, [5]University of California, Riverside, CA, United States

Anautogenous mosquitoes are vectors of numerous diseases because they require blood feeding for their egg maturation and as a consequence they transmit pathogens. Following blood feeding, the surge of amino acids in the hemolymph of female mosquitoes induces multiple events including yolk protein precursor (YPP) gene expression in the fat bodies via direct activation of the TOR (target of rapamycin) signal transduction pathway controlling the activation of ovarian development and egg maturation in conjunction with the ecdysone-coupled signaling. Principle mediators and plasma membrane molecular components controlling those nutritional signaling changes remain to be identified in vectors insects. Two members of the cationic amino acid transporter (CAT) subfamily of the solute carrier family 7 (SLC7) named slimfast (slif) and iCAT2 were previously identified as essential nutrient signaling mechanisms in the fruit fly and the mosquito *Aedes aegypti*. The cloned mammalian CAT-SLC7 members are y+ system exchangers for cationic L-amino acids; however, functions of CAT-SLC7 in other organisms were enigmatic. We determine the expression and transport function of slif from Ae. aegypti. AeSlif gene transcription increases during postembryonic development and peaks in the 3rd and 4th larval stages, but the corresponding protein was detected only in pupae and emerging adult mosquitoes. Functional expression of AeSlif in Xenopus oocytes reveals a sodium-independent cationic amino acid transporter with a preference for transporting L-histidine. Therefore, AeSlif represents the first CAT-SLC7 member acting as a L-His transceptor. This protein can execute two functions: absorption of L-histidine with K 0.5 L-His ~0.4 mM, and transport of L-histidine messengers across cell membranes of fat bodies' trophocytes for signaling nutrient availability in the female mosquitoes. This study has contributed to our understanding of the role of nutritional blood meal mediated signaling of egg development in vector mosquitoes.

864

A NEWLY DESCRIBED NATURAL POPULATION SUBGROUP OF THE MOSQUITO *ANOPHELES GAMBIAE* IS EXCEPTIONALLY SUSCEPTIBLE TO HUMAN MALARIA

Michelle M. Riehle[1], Moussa Guelbeogo[2], Awa Gneme[2], Karin Eiglmeier[3], Inge Holm[3], Emmanuel Bischoff[3], Thierry Garnier[3], Greg Snyder[1], Kyriacos Markianos[4], N'Fale Sagnon[2], Kenneth D. Vernick[3]

[1]University of Minnesota, St. Paul, MN, United States, [2]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [3]Institut Pasteur, Paris, France, [4]Children's Hospital, Boston, MA, United States

Capture of indoor-resting mosquitoes via aspirator or pyrethroid spray is a commonly used approach for *Anopheles* population sampling. However, indoor collections do not efficiently recover behaviorally distinct compartments of the population, and larval sampling followed by genotyping and genetic analysis may represent a comprehensive and less biased alternative. When this approach was used in the Sudan Savannah region of Burkina Faso, a novel group of outdoor-resting *A. gambiae* was found at high frequency in larval captured *A. gambiae*. The newly described exophilic subgroup freely segregates for both molecular form markers (>35% M/S hybrids) and the 2La chromosome inversion (>50% 2L+ chromosomes). In contrast, contemporaneous site-matched indoor collections confirmed the canonical description of the *A. gambiae* population in this region of West Africa: namely M and S molecular forms speciating and near fixation of the inverted form of the 2La inversion. Most striking is the larger population abundance and the greater susceptibility to *Plasmodium falciparum* infections of the exophilic subgroup of *A. gam*biae. The existence of a group of *A. gambiae* that is both highly susceptible to *P. falciparum* infections and not indoor resting could have significance for the efficiency of malaria control efforts, particular those targeting indoor resting mosquitoes.

865

CYTOGENETIC MAP FOR *ANOPHELES NILI*: APPLICATION FOR POPULATION GENETICS AND COMPARATIVE PHYSICAL MAPPING

Maria Sharakhova[1], Christophe Antonio-Nkondjio[2], Ai Xia[1], Cyrille Ndo[2], Parfait Awono-Ambene[2], Frederic Simard[3], Igor Sharakhov[1]

[1]Virginia Tech, Blacksburg, VA, United States, [2]Malaria Research Laboratory OCEAC, Yaounde, Cameroon, [3]Institut de Recherche pour le Développement (IRD), Montpellier, France

Anopheles nili is one of the major malaria vectors in Africa with wide geographic distribution. However, the taxonomic and population genetic studies on this species are scarce. New research tools are urgently needed to genetically characterize this important malaria vector. In this study, a high-resolution cytogenetic map was developed for *An. nili* polytene chromosomes. Chromosomes were straightened and subdivided into 46 numbered divisions according to the banding pattern. Population analysis of *An. nili* females collected in Burkina Faso revealed the presence of two highly polymorphic inversions on the 2R chromosomal arm. To determine chromosome homologies and gene order conservation between *An. nili* and other major malaria vectors, PCR probes based on the *An. gambiae* coding sequences were mapped to An. nili chromosomes. Comparative mapping demonstrated that An. nili chromosomes have an An. stephensi-like arm association and that whole arm translocations and paracentric inversions were the major types of rearrangement in evolution of these mosquitoes. The minimum number of fixed inversions among An. nili, An. gambiae, and An. stephensi was calculated using the Multiple Genome Rearrangements (MGR), Genome Rearrangements In Man and Mouse (GRIMM), and Sorting Permutation by Reversals and block-INterchanGes (SPRING) programs. The data suggest that the An. nili is, at least, as diverged from An. gambiae as An. stephensi. We provided evidence that 2La/a arrangement of An. gambiae is present in outgroup species An. nili and An. stephensi confirming the ancestral status of the 2La inversion in the An. gambiae complex. Availability of the new polytene chromosome map, polymorphic inversions, and physically mapped DNA markers for An. nili will further stimulate population genetic, taxonomic, and genomic studies of this neglected malaria vector.

866

TRANSDUCTION OF *SCHISTOSOMA MANSONI* WITH VESICULAR STOMATITIS VIRUS GLYCOPROTEIN PSEUDOTYPED LENTIVIRUS

Sutas Suttiprapa, Sergey Iordanskiy, Victoria H. Mann, Gabriel Rinaldi, Michael I. Bukrinsky, Paul J. Brindley

The George Washington University, Washington, DC, United States

Retrovirus-mediated transduction offers a means to insert reporter transgenes into the schistosome genome, to elucidate schistosome gene function and expression through vector-based RNA interference, and to establish transgenic lines of schistosomes. Previously we have reported that murine leukemia virus (MLV) pseudotyped with vesicular stomatitis virus glycoprotein (VSVG) can transduce developmental stages of Schistosoma mansoni. In addition, we have been investigating whether human immunodeficiency virus (HIV-1) lentivirus (a complex retrovirus) might likewise be utilized for transgenesis of schistosomes. We constructed lentiviral vectors using the ViraPower Gateway (Invitrogen) system; we modified pLenti6/R4R2/V5-DEST by insertion of an endogenous schistosome gene promoter; from the spliced leader (SL) RNA gene, upstream of the reporter gene encoding jellyfish green fluorescent protein (GFP). 293 FT producer cells were transformed with this construct and viral packaging plasmids to produce replication incompetent lentivirus virions pseudotyped with VSVG. We investigated early steps of lentivirus infection of schistosomes including attachment of virions to the schistosome tegument, reverse transcription to synthesize proviral DNA, and integration of the provirus into the schistosome genome. Schistosomes were incubated with HIV virions in the presence of the cationic polymer polybrene. At several times from 0 minutes to four hours thereafter, schistosomes were washed and the surface cross-linked with formalin. Using a VSVG specific antibody as the probe, time course dependent immunolocalization was evident to both schistosomules and adult worms, with increasing fluorescence signals from 0 to 180 min after exposure. Downstream events were investigated at one day post infection. Genomic DNAs (gDNA) were extracted from infected worms and used as the template for quantitative real time PCR (qPCR). qPCR targeting linear viral cDNA and integrated viral genome were performed with single step PCR and two step anchored PCR approaches, respectively, which revealed the presence of linear viral cDNA and integrated proviruses. We are now investigating integration junctions and reporter gene activity, with the aim of establishing the potential of VSVG-HIV-1 lentivirus as a vector for genetic manipulation of schistosomes.

867

MOLECULAR MIMICRY BETWEEN ALLERGENS AND HELMINTH PROTEINS UNDERLIES RESPONSES AT THE HELMINTH-ALLERGY INTERFACE

Helton C. Santiago[1], Mark Wilson[1], Sasisehkhar Bennuru[1], Flavia Ribeiro-Gomes[1], John Grainger[1], Thomas Wynn[1], Joe Urban[2], Thomas Nutman[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]United States Department of Agriculture, Beltsville, MD, United States

Although chronic helminth infection can diminish allergic skin test reactivity, many helminth infections are associated with allergic symptoms, possibly mediated by cross-reactivity between allergens and helminth proteins. To investigate the extent of this cross-reactivity, we performed in silico comparisons of 410 common (and molecularly defined) allergens

against the predicted proteins of the entire *Brugia malayi* genome. Among the 410 allergens, we found 170 that had at least one filarial orthologue (identities ranging from 22% to 92%). To investigate the implications of this finding, we assessed IgE levels specific to common allergens in 132 filarial-infected subjects and in 165 uninfected controls. When compared to uninfected individuals, filarial infection was associated with increased IgE prevalence to extracts of both house dust mite (HDM, Der p) (71% x 51%, P=0.02) and cockroach (Bla g) (60% x 16%, P<0.001) both of which contain several orthologues in filariae. In contrast, the IgE prevalence to timothy grass extract (Phl p), containing virtually no orthologues, was no different between the filarial-infected and -uninfected individuals. To extend these findings *in vivo*, mice were infected twice with H. polygyrus and the IgE response to helminth homologous and non-homologous allergens of cockroach and HDM were analyzed. Infected animals developed cross-reactive IgE only to allergens with helminth homologues (Der p10 and Bla g5), but not to those without homologues (Der p7 and Phl p6). Furthermore, cross-reactive IgE was functional as infected animals developed immediate hypersentivity skin test reactions to recombinant allergens (Der p1, Der p10 and Bla g5) structurally related to parasite antigens but not to structurally unrelated allergens (Der p7). These data suggest that molecular mimicry between helminth proteins and aeroallergens may cause the development of cross-reactive IgE and allergic sensitization and provide new insights into the allergy-helminth interface.

868

TRANSFECTION OF DEVELOPMENTALLY COMPETENT *BRUGIA MALAYI*

Canhui Liu[1], Shulin Xu[1], George Tzertzinis[2], Ray Kaplan[3], Chris Evans[3], **Thomas R. Unnasch**[1]

[1]University of South Florida, Tampa, FL, United States, [2]New England Biolabs, Ipswich, MA, United States, [3]University of Georgia, Athens, GA, United States

Transient transfection of isolated *Brugia malayi* embryos by biolistic bombardment has proven to be useful in defining promoter structure and function in this human filarial parasite. However, isolated transfected embryos are developmentally incompetent. A method of producing developmentally competent transfected parasites is therefore needed. We have found that L3 parasites can be chemically transfected in situ in the peritoneal cavity of a gerbil. The in situ chemically transfected parasites are developmentally competent, producing adult parasites with an efficiency similar to that obtained from implanted untreated L3. To refine this system, a reporter plasmid was constructed consisting of the a secreted gaussia luciferase reporter gene (gLUC) sequence flanked by the 5' and 3' untranslated domain of the *B. malayi* HSP70 gene, and containing the first intron derived from the *B. malayi* HSP70 gene inserted into the gLUC ORF. Cultured adult parasites and progeny mf derived from L3 transfected with this construct secreted gLUC into the culture medium. When the transfected mf were mixed with blood, fed to mosquitoes and the resulting L3 collected, the L3 also secreted gLUC into the culture medium. Transfected adults and progeny mf contained transgenic DNA, and the transgenic mRNA produced in these parasites was found to be correctly cis- and trans-spliced. These data suggest that it is possible to produce developmentally competent transfected *B. malayi* and that the transgenic sequences are inherited remain transcriptionally active in all lifecycle stages. Furthermore, the data demonstrate that gLUC may be employed as a selectable marker to identify transfected parasites. These studies open the way to using transgenesis to study all lifecycle stages of *B. malayi*.

869

TBEIF4E-3 IS A PUTATIVE AND DIVERGENT TRANSLATION INITIATION FACTOR EIF4E ORTHOLOGUE IN *TRYPANOSOMA BRUCEI* THAT IS ESSENTIAL FOR CELL CYCLE PROGRESSION AND A POTENTIAL CANDIDATE FOR DRUG TARGETING

Salvador Z. Tarun, Jr.

University of Pittsburgh, Pittsburgh, PA, United States

Trypanosoma brucei is a unicellular, flagellated protozoan of the kinetoplastid group of eukaryotes causing 'African sleeping sickness' in humans. Current drugs have limited efficacy and high toxicity. Pathogenesis of *T. brucei* and related parasites rely on a complex life cycle involving proliferation, differentiation and transmission between polymorphic insect and human stages. Remarkably, mRNA transcription and processing are generally constitutive in trypanosomes, and thus, gene expression control driving their life cycle may instead rely predominantly upon regulated mRNA turn-over and translation. In most eukaryotes, the mRNA 5' cap binding protein eIF4E plays a central role in both the process and regulation of mRNA translation and turn-over. To explore translational regulation in *T. brucei*, we studied unique and essential features of TbeIF4E-3, one of several putative, divergent orthologues of eIF4E. First, unlike canonical eIF4E, it does not appear to bind well with 5' cap analogue 7mGTP, consistent with *ab initio* X-ray structure modeling of its cap-binding pocket suggesting only partially conserved, cap-interacting amino acid residues. Second, down-regulation of its expression by inducible RNAi caused multiple defects in cell cycle progression in both procyclic and bloodform stages of the parasite, inevitably leading to cell death. Finally, down-regulation of TbeIF4E-3 in bloodforms caused remarkable hypersensitivity to Rapamycin, a drug also causing cell cycle defects in *T. brucei*, and inhibiting TOR complexes central to cytoskeletal remodeling and/or translational regulation. These findings indicate that TbeIF4E-3 is involved in crucial cellular pathways regulating normal cell cycle and survival in *T. brucei*. The potential presence of a divergent cap-binding pocket in TbeIF4E-3 according both to its predicted structure and biochemical behavior, as well as its synergistic interaction with a known growth inhibitor drug, provides strong rationale for exploring TbeIF4E-3 and the pathway it controls as potential target for drug therapy against this pathogen.

870

BACTERIA-MEDIATED RESISTANCE TO *PLASMODIUM* INFECTION IN *ANOPHELES* MOSQUITOES

Chris M. Cirimotich[1], Yuemei Dong[1], April M. Clayton[1], Jayme A. Souza-Neto[1], Musapa Mulenga[2], George Dimopoulos[1]

[1]Johns Hopkins School of Public Health, Baltimore, MD, United States, [2]Malaria Institute at Macha, Choma, Zambia

Before transmission to a vertebrate host occurs, *Plasmodium* parasites must transition through discrete developmental stages in the mosquito. During the earliest stages of development in the midgut lumen, the parasite encounters a diverse environment of mosquito-derived factors, host blood-derived factors, and the resident microbiota. Our lab and others have shown that bacteria have a profound effect on the ability of *Plasmodium* to infect the mosquito. Removal of resident bacteria increases mosquito susceptibility to infection more than two-fold. However, data generated on bacteria-mediated resistance to infection is in large part limited to lab isolates of bacteria and no mechanism has been resolved. To further examine the interaction between bacteria and *Plasmodium*, we first assessed microbial exposure of *Anopheles* mosquitoes collected in Zambia and then performed molecular and phenotypic analyses in regards to *Plasmodium* infection. Similar to previous reports, Gram-negative bacteria inhibited oocyst development. However, we identified a Gram-negative bacterium that potently inhibits infection during early stages of parasite development. Characterization of the bacteria-parasite-mosquito interaction shows that the antimicrobial immune response does not play

a major role in the observed microbe-mediated *Plasmodium* refractoriness but large populations of replicating bacteria are required. Physical interaction between bacteria and parasite were not observed following oral co-introduction in mosquitoes and supplementing nutrients required for parasite development do not rescue infection. We are currently using biochemical and continued phenotypic analyses to elucidate the mechanism of inhibition, which could lead to the identification of novel anti-*Plasmodium* molecules.

871

ANTIBODIES PRODUCED AGAINST RECOMBINANT SIX-CYSTEINE GAMETE SURFACE HOMOLOGY FRAGMENTS FROM *PLASMODIUM FALCIPARUM* PFS48/45 AND PFS230 RECOGNIZE SEXUAL STAGES AND MAY BLOCK TRANSMISSION

Raul Herrera, Marian Ortiz, Christopher Rowe, Vu Nguyen, Richard L. Shimp, Karine Reiter, Lynn Lambert, Louis H. Miller, Patrick E. Duffy, Nicholas J. MacDonald, Yimin Wu, David L. Narum

Laboratory of Malaria Immunology and Vaccinology, Rockville, MD, United States

The development of a *falciparum* malaria transmission blocking vaccine is being evaluated as a complement to a pre-erythrocytic vaccine. Antibodies against the sexual stage protein, Pfs25, significantly inhibit oocyst development when assessed by a membrane feeding assay, though it requires higher antibody titers in humans to achieve complete blocking in transmission. In order to evaluate whether the transmission blocking activity of Pfs25 may be enhanced by the inclusion of additional sexual stage antigens, we aimed to produce two recombinant forms of the cysteine-rich *Plasmodium* gamete surface homology fragments derived from Pfs48/45 and Pfs230. Using a modified *Pichia pastoris* host that overexpresses protein disulfide isomerase, multiple forms of Pfs48/45 have been expressed and purified that contain 8 to 10 cysteines, which form a "double domain". An amino-terminal region of Pfs230 containing a single "double domain" was expressed and refolded from inclusion bodies derived from *Escherichia coli*. Antibodies generated in rabbits against both of these recombinant forms of Pfs48/45 and Pfs230 recognized unfixed gametes by indirect immunofluorescence. In contrast, only rabbit antisera against Pfs230 inhibited oocyst development by greater than 97% (reduction in oocyst prevelance was 50 to 90%) using neat sera in the presence of complement. The failure of the Pfs48/45 antisera to inhibit oocyst development may be the result of a poorly folded immunogen or that the Pfs48/45 "double domain" is not a significant biological target. Now that a recombinant form of Pfs230 has been produced using a scalable system that induces inhibitory antibodies, pre-clinical studies evaluating Pfs25 and Pfs230 may be performed.

872

HUMAN ANTIBODY RESPONSE TO *ANOPHELES* SALIVARY GSG6-P1 PEPTIDE: NEW IMMUNO-EPIDEMIOLOGICAL TOOL FOR EVALUATING THE EFFICACY OF INSECTICIDES TREATED NETS (ITNS) IN MALARIA VECTOR CONTROL

Papa M. Drame[1], Anne Poinsignon[2], Patrick Besnard[3], Sylvie Cornelie[2], Jacques Le Mire[3], Filomeno Fortes[4], Jean-Claude Toto[5], Mbacke Sembene[6], Francois Simondon[7], Pierre Carnevale[2], Franck Remoue[1]

[1]Institut de recherche pour le Developpement, Cotonou, Benin, [2]Institut de recherche pour le Developpement, Montpellier, France, [3]Malaria Control Program, Medical Service of ACERGY-SONAMET, Lobito, Angola, [4]National Malaria Control Program, Ministry of Health, Luanda, Angola, [5]Laboratoire de Recherche sur le Paludisme, OCEAC, Yaoundé, Cameroon, [6]Department of Animal Biology, Faculty of Sciences and Technics, Cheikh Anta Diop University, Dakar, Senegal, [7]Epidemiology Department of Sanofis-Aventis, Lyon, France

To optimize malaria control, WHO has emphasized the need for new indicators to evaluate the efficacy of vector control strategies. Previous studies have shown that the quantification of human antibody (Ab) response to *Anopheles* salivary proteins represent an epidemiological biomarker of exposure to *Anopheles* bites and malaria risk. In particular, only one salivary peptide, the gSG6-P1, is one clear candidate to evaluate the level of exposure to *An. gambiae* and *An. funestus* bites. The aim of the study was then to validate this peptide as a new tool to evaluate the efficacy of ITNs use. One longitudinal study, concerning individuals (n=108) living in malaria endemic area was performed from March 2005 to January 2007 (Angola). The cohort was followed for parasitological, entomological and immunological data, before and after the well-controlled use of ITNs (installation in Feb. 2006). Significant decrease of the percentage of immune responders and of anti-gSG6-P1 IgG Ab level was observed just after the ITNs use and was correlated with the decrease of malaria parasitemia, the current and referent criteria of ITNs efficacy. Interestingly, the decrease of specific IgG level was observed in all age groups (0-6; 7-14 and >14 years-old) and for the majority of ITNs-protected individuals, suggesting its potentiality as an individual biomarker. However, in concordance with the considerable loss of ITNs and lack of ITNs use, specific IgG response increased only four months after ITNs introduction in this studied population. It suggests that this salivary tool could be also an indicator to the time-dependent incorrect use of ITNs. This study shows that the assessment of IgG response to gSG6-P1 salivary peptide could be a pertinent tool to evaluate the ITNs efficacy, whatever age, and potentially a biomarker of efficacy at an individual level. This study represents a first approach to elaborate new tools of evaluation of malaria vector control and future studies are needed to confirm this hypothesis in other areas and using different vector control strategies.

873

TREATMENT OF ASYMPTOMATIC CARRIERS OF *PLASMODIUM FALCIPARUM* MALARIA WITH ARTEMETHER-LUMEFANTRINE TO REDUCE DISEASE TRANSMISSION: A MODELING AND SIMULATION STUDY

Bernhards Ogutu[1], Alfred B. Tiono[2], Michael Makanga[3], Adama Dodji Gbadoé[4], Zulfiqarali Premji[5], Issaka Sagara[6], David Ubben[7], Marc Cousin[8], Oliver Sander[8], Fiyinfolu Oladiran[9], Steven E. Kern[10]

[1]Centre for Clinical Research-Kenya Medical Research Institute and Malaria Clinical Trials Alliance – INDEPTH Network, Nairobi, Kenya, [2]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [3]European and Developing Countries Clinical Trials Partnership, Cape Town, South Africa, [4]University of Lome, Lome, Togo, [5]Muhimbili University of Health and Allied Sciences, Dar-es-Salaam, United Republic of Tanzania, [6]Malaria Research and Training Center, University of Bamako, Bamako, Mali, [7]Medicines for Malaria Venture, Geneva, Switzerland, [8]Novartis Pharma, Basel, Switzerland, [9]Novartis Pharma, Kempton Park, South Africa, [10]University of Utah, Salt Lake City, UT, United States

Asymptomatic carriers (AC) of *Plasmodium falciparum* serve as a reservoir for malaria transmission. Identification and treatment of AC within a region should reduce the reservoir and thus transmission intensity in that area. Using computer simulation, the factors that influence the impact of this intervention, i.e. community screening campaigns (CSC) followed by artemether-lumefantrine (AL) treatment on disease transmission were explored. The model of Okell et al (2008) was modified with malaria vector seasonality added and components modified to represent screening and treatment of AC with AL. The age grouping, relative distribution of age in a region, and degree of heterogeneity in disease transmission were maintained. The impact of the number of CSC and their timing on malaria transmission throughout a period of 1 year was explored. A sensitivity analysis to determine factors with the greatest impact was done. The simulation showed the intervention reduces transmission in a region with marked seasonal transmission (6 months) of moderate intensity (EIR<100). Three CSCs scheduled in close succession (monthly intervals) at the start of the dry season had the greatest impact. Adding an extra CSC did not bring improvement. In areas with low transmission intensity (EIR<10) the reduction was sustained for years after a single intervention while gradually tapering off with return to initial setting. Repeated intervention at least every other year allowed to sustain the effect. The simulation results show that screening and treatment of asymptomatic carriers with AL in a region reduces malaria transmission significantly. Transmission intensity has the greatest impact on the magnitude and duration of malaria reduction. When combined with other strategies (LLINs, RDT, Prompt diagnosis & treatment, IRS), the effect of this intervention can persist for many years, and it may become a tool to accelerate the reduction transmission intensity to pre-elimination level. The modeling supports the evaluation of this approach in a prospective clinical trial in an area with marked seasonality.

874

PROTECTIVE EFFECTS OF WHO-RECOMMENDED LLINS AGAINST *ANOPHELES DARLINGI* IN THE FIELD

Elvira Zamora Perea[1], Daniel Del Cuadro Hidalgo[1], Hugo Rodríguez Ferrucci[2], Angel Rosas Aguirre[2], **Gregor J. Devine**[3]

[1]Laboratorio de Salud Publica, Iquitos, Peru, [2]Organismo Andino de Salud (ORAS), Lima, Peru, [3]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania

The Andean Malaria Program has adopted Long Lasting Insecticide Net (LLIN) distribution as an important strategy to prevent and control malaria in high risk areas of malaria transmission in the Amazon Region. The nets that have been distributed to date include Interceptor®, PermaNet 2.0®, and Olyset®. Respectively, these exploit the pyrethroids alpha-cypermethrin, deltamethrin and permethrin. All published studies on the field efficacy of these compounds when incorporated into LLINs refer only to African (An. gambiae s.l.) and Asian (An. culicifacies and An. fluviatilis) malaria vectors. Only a single comparative study between these compounds applied to bed nets has been completed (also in Africa). It is therefore essential to characterize the relative efficacy of these LLINs against An. darlingi, the most common and efficient malaria vector in the Amazon basin. This was done using a set of experimental huts sited close to the Amazon town of Iquitos, Peru. Using huts with open eaves and exit traps in the windows we examined 1) the lethal effects of the three LLINs on mosquitoes, 2) their impacts on mosquito exit and entry behavior, and 3) the protection that holed nets afforded their human occupants. We also examined the protection that nets gave to humans that were in the same house as the nets, but not under the nets.

Highly significant differences between the LLINs were noted, with some nets exerting far greater lethal effects, and some far greater repellent and irritant effects than others. An. darlingi is an early biting mosquito, which exhibits its peak in biting behavior before people have retired under their bed nets. These differences between the three LLINs therefore have profound implications for the patterns of protection that they give to humans against An darlingi.

875

INNOVATIVE TOOL TO EVALUATE MALARIA RISK: TOWARD THE DEVELOPMENT OF A BIOMARKER OF INFECTING BITE?

Sylvie Cornelie[1], Marie Rossignol[1], Edith Demettre[2], Martial Seveno[2], Fabrice Chandre[1], Vincent Corbel[3], Isabelle Morlais[4], Franck Remoue[3]

[1]Institut de Recherche pour le developpement, Montpellier, France, [2]plateforme protemique fonctionnelle -CNRS, Montpellier, France, [3]Institut de Recherche pour le developpement- CREC, Cotonou, Benin, [4]Institut de Recherche pour le developpement, OCEAC, Yaounde, Cameroon

Malaria causative agent, i.e. Plasmodium parasite, is transmitted to human during the blood meal of the Anopheles mosquito. During blood feeding, the vector injects parasite and saliva into the vertebrate host skin. This saliva contains bioactive components which induced an immune response in the vertebrate host. Our team has previously developed a serological biomarker to assess the human exposure to mosquito bite. This tool is based on the evaluation of human antibody response against mosquito salivary proteins. Here we investigate whether a salivary antigen could be specific of the infecting bite and constitute a biomarker of the risk of disease. To assess this question, we have compared the human antibody response against salivary extracts infected or not by P. *falciparum*. Experimental infections of An. gambiae by P. *falciparum* were carried out and salivary glands were dissected 14 days post-infection. The infective status of each salivary gland was confirmed by PCR. Then two-dimensional western-blots were realized with different pools of infected vs non infected sera. These pools were constituted with sera from Senegalese 1-2 y.o. children leaving in deeply exposed village to Anopheles and presenting or not a high parasitemia.

The results of 2D-blots showed that immunogenic proteins around 70kDa are detected in both infected and non infected vector. Mass spectrometry analyses identified these proteins as the 5'nucleotidase and Apyrase, proteins which inhibiting platelet aggregation. Furthermore one immunogenic protein from infected salivary glands extracts was detected only with sera from infected children by P. *falciparum*. Mass spectrometry analysis on this protein is underway. These results indicated that human immune system could discriminate between an infective bite and a non infective bite. This work opens the way to design epidemiological tools to evaluate the risk of malaria in area of (re) emergence, but also have strong implications for the vector control and monitoring.

876

STRATEGIES TOWARDS *PLASMODIUM FALCIPARUM* MALARIA ELIMINATION IN AFRICA USING CURRENTLY AVAILABLE TOOLS

Jamie T. Griffin[1], Deirdre Hollingsworth[1], Lucy C. Okell[1], Thomas S. Churcher[1], Michael White[1], Wes Hinsley[1], Teun Bousema[2], Chris J. Drakeley[2], Neil M. Ferguson[1], Maria-Gloria Basanez[1], Azra C. Ghani[1]

[1]Imperial College, London, United Kingdom, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

In the past decade malaria intervention programs have been scaled up across Africa. However, it remains unclear what levels of decrease in transmission are achievable using currently available tools. We developed an individual-based simulation model for *Plasmodium falciparum* transmission in an African context incorporating the 3 major vector species (*Anopheles gambiae* s.s., *An. arabiensis* and *An. funestus*) with parameters fitted to parasite prevalence data from 34 sites across Africa. We explored the impact on transmission of increased roll-out of of long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), mass screening and treatment (MSAT) and a future RTS'S/AS01 vaccine in six settings with varying entomological inoculation rate (EIR), vector species combinations and patterns of seasonality under a target of 80% coverage of interventions. In the low transmission setting (EIR approx. 3 infectious bites per person per year (ibppy)), LLINs alone can reduce malaria transmission to low levels if high usage levels are sustained. In two moderate transmission settings (EIR approx. 43, 81 ibppy) additional rounds of IRS with DDT coupled with MSAT could drive parasite prevalence below the 1% level. However, in the third (EIR=46) with *An. arabiensis* prevailing, these interventions are insufficient to reach this threshold. In both high transmission settings (EIR approx. 586, 675 ibppy) either unrealistic coverage levels (>90%) or novel tools and/or substantial social improvements will be required, although existing tools with realistic coverage levels greatly reduce prevalence. In conclusion, interventions using current tools can result in major reductions in *P. falciparum* transmission and associated disease burden in Africa. Reduction to below the 1% parasite prevalence level is possible in low to moderate transmission settings when vectors are primarily endophilic, provided a comprehensive and sustained intervention program is achieved. In high transmission settings and those in which vectors are mainly exophilic, additional new tools are likely to be required to achieve this level of control.

877

QUANTIFICATION OF EASTERN EQUINE ENCEPHALITIS VIRUS IN FIELD-COLLECTED MOSQUITOES TO EVALUATE THEIR ROLE AS BRIDGE VECTORS

Philip M. Armstrong, Theodore G. Andreadis

The Connecticut Agricultural Experiment Station, New Haven, CT, United States

Eastern equine encephalitis virus (EEEV) is maintained in an enzootic cycle involving *Culiseta melanura* mosquitoes and avian hosts, whereas other mosquito species that feed opportunistically on mammals have been incriminated as bridge vectors to humans and horses. To evaluate the capacity of these mosquitoes to transmit EEEV, we estimated the infection prevalence and virus titers in mosquitoes collected in Connecticut by cell culture, plaque titration, and quantitative RT-PCR. *Cs. melanura* yielded the greatest number of EEEV isolations (n=83) followed by *Ochlerotatus canadensis* (10) and *Aedes cinereus* (6). Relatively few (≤4) or no EEEV isolates were obtained from the remaining mosquito species collected. *Cs. melanura* contained significantly higher titers of virus (mean= 6.53 log10 PFU/mosquito pool) than all other mosquito species combined (mean=2.26 log10 PFU/mosquito pool). None of the remaining mosquito species had high enough titers to transmit virus. Our findings suggest that *Cs. melanura* is the primary if not sole vector of EEEV in this region, which may explain the paucity of human cases. More generally, this study emphasizes the importance of evaluating virus titers from field-collected mosquitoes to assess their vectorial capacity.

878

WESTERN EQUINE ENCEPHALITIS VIRUS PATHOGENESIS IN OUTBRED MICE

Eric C. Mossel[1], Aaron T. Phillips[1], Christopher H. Logue[2], Steven W. Dow[1], Ann M. Powers[3], Ken E. Olson[1]

[1]Colorado State University, Fort Collins, CO, United States, [2]Health Protection Agency, Porton Down, United Kingdom, [3]Centers for Disease Control and Prevention, Fort Collins, CO, United States

Western equine encephalitis virus (WEEV) is a naturally occurring recombinant virus derived from ancestral Sindbis and Eastern equine encephalitis viruses. Relatively little is known about virulence determinants of WEEV. We previously showed that infection by WEEV isolates McMillan (McM) and IMP-181 (IMP) results in high and low mortality, respectively, in outbred CD1 mice when virus is delivered by either subcutaneous or aerosol routes. McM and IMP infectious clones were used to generate chimeric constructs. By characterizing the pathogenic phenotypes of these chimeras, the pathogenic determinants were mapped to a region within the E2 glycoprotein from McM nt 8950 through McM nt 9658. This region of 709 nt contains 18 nt and 7 aa differences. Additional mapping is underway to further narrow the pathogenic determinants. We have also conducted studies with cationic lipid DNA complexes (CLDC), a potent immunomodulatory compound, in mice before and after WEEV-McMillan challenge. Subcutaneous CLDC administration up to 12 hours after challenge can protect mice from WEEV-induced disease and mortality. In virus-infected animals, large increases in production of IFNγ, TNF-α, MCP-1, IL-12, and IL-10 in the brain were observed by 72 hours after infection, consistent with neuroinvasion and viral replication in the CNS. Mice receiving CLDC treatment show early increased serum IFNγ, TNF-α, and IL-12, suggestive of a T_H1-biased activation of the innate immune system, but no increase in brain cytokine levels. These studies are lending insight into the mechanisms of WEEV pathogenesis and protection from WEEV encephalitis.

879

INFIRMATUS VIRUS: A NEWLY DESCRIBED *ORTHOBUNYAVIRUS* (CALIFORNIA SEROGROUP) ISOLATED IN FLORIDA

Christy L. Ottendorfer[1], Hassan K. Hassan[1], Kevin J. Hill[1], Gregory S. White[2], Lillian M. Stark[3], Thomas R. Unnasch[1]

[1]University of South Florida, Tampa, FL, United States, [2]University of Alabama at Birmingham, Birmingham, AL, United States, [3]Florida Department of Health, Bureau of Laboratories, Tampa, FL, United States

A novel virus, tentatively designated Infirmatus virus, was isolated as part of an ongoing study on the ecology of arbovirus transmission in Florida. The virus was isolated by Vero cell culture from a pool of *Aedes infirmatus* mosquitoes collected in 2008. The virus was not detected using previously reported flavi-, alpha- and bunya-broad RT-PCR methods. To identify the virus, sequence-independent single-primer amplification and a sequence-specific genome walking technique was used to generate the nearly complete sequence of the virus. BLASTn analysis of the sequence identified 86% nt homology to Trivittatus virus in the small (S) segment, 77% homology to Trivittatus virus in the medium (M) segment, and 75% homology to La Crosse virus in the large (L) segment. Infirmatus virus was also detected in 6 other mosquito pools (3 *Ae. infirmatus* pools, 2 *Cx. quinquefasciatus* pools, 1 *Cx. nigripalpus* pool) at the site. Host species identification on blood-fed mosquitoes found that *Ae. infirmatus* and *Cx. nigripalpus* frequently fed on eastern cottontail rabbits in this area, suggesting rabbits as its natural reservoir host, similar to California encephalitis virus. Earlier surveillance studies in the area

isolated a "Trivittatus-like virus" in the 1960s that was not classified or further characterized by molecular techniques. An isolate of this virus collected in 1965 was obtained for comparison; 98% nucleotide sequence homology (~900 bp) was found to the Infirmatus strain collected in 2008. Intracerebral inoculation of suckling mice with the novel virus resulted in neurovirulence and mortality three days post inoculation. This virus thus represents a novel California serogroup *bunyavirus* first isolated in the 1960s that may pose a risk to public health in Florida.

880

IGM PERSISTENCE IN PROBABLE CASES OF CALIFORNIA SEROGROUP INFECTION

Michael Drebot, Kai Makowski, Kristina Dimitrova, Harvey Artsob

National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada

Snowshoe hare (SSH) and Jamestown Canyon (JC) viruses are mosquito-borne zoonotic agents belonging to the California serogroup (CS) of viruses (Genus *Orthobunyavirus*). These viruses have a wide geographical range throughout North America and are associated with febrile and neurological disease. Serological procedures to identify probable cases of CS-associated illness usually consist of IgM detection in patient sera. Persistent IgM can cause diagnostic issues with case identification, therefore, a study was performed to evaluate lingering SSH and JC specific IgM in exposed individuals. Fifteen patients / individuals positive by SSH and JC IgM ELISA screening and neutralization assays were tested for CS virus IgM antibody at yearly intervals. In addition, 90 sera collected from patients during the winter months in Canada were screened for CS IgM to provide additional evidence for lingering IgM antibody during times when mosquitoes were not present. Evidence for California serogroup virus IgM persistence was identified among patients screened for arbovirus antibody over several years. Fifteen individuals had significant JC or SSH IgM titres that persisted for over a year with several exhibiting positive IgM levels for 2-5 years. Of 90 sera collected from randomly picked febrile patients during the months of January to March approximately 9% (8/90) were positive for California serogroup virus specific IgM. The high number of IgM positive sera collected during the late spring and winter months is consistent with JC and SSH exposures occurring the previous mosquito season, a time frame of 6 months or longer. Our studies indicate that JC and SSH IgM titres may be maintained for at least a year, a finding previously observed with West Nile virus infections. Persistent IgM may pose a diagnostic dilemma when identification of California serogroup infections is based only on presence of IgM in patient sera. Confirmation of current JC and SSH- associated illness should be demonstrated by diagnostic rises in antibody titre or the presence of virus in clinical samples.

881

EXPANDED FRAMEWORK OF HANTAVIRUS EVOLUTION FROM NEWLY IDENTIFIED MYOSORICINE SHREW HOSTS IN TANZANIA

Hae Ji Kang[1], William Stanley[2], Jake Esselstyn[3], Shannon N. Bennett[1], Joseph A. Cook[4], Richard Yanagihara[1]

[1]University of Hawaii, Honolulu, HI, United States, [2]Field Museum of Natural History, Chicago, IL, United States, [3]University of Kansas, Lawrence, KS, United States, [4]University of New Mexico, Albuquerque, NM, United States

Sub-Saharan Africa has long been considered the birthplace of numerous vector-borne and zoonotic infectious diseases affecting humans and domestic animals. For example, numerous viruses, including prototype viruses that define families and genera (such as Bunyamwera, Lassa and Ebola), were first discovered in Africa many decades ago. By contrast, hantaviruses have only recently been detected, in the African wood mouse and Therese's shrew from Guinea. Recent observations that multiple species of shrews (Order Soricomorpha, Family Soricidae) harbor genetically distinct hantaviruses in widely separated geographic regions throughout Eurasia and North America suggest the existence of additional shrew-borne hantaviruses in Sub-Saharan Africa, where numerous unique shrew lineages have evolved and diversified. To investigate this issue, archival tissues from the geata mouse shrew (*Myosorex geata*) and Kilimanjaro mouse shrew (*M. zinki*), two myosoricine shrew species restricted to Tanzania, were analyzed for hantavirus RNA by RT-PCR. Pairwise alignment and comparison of nearly full-length S- and L-genomic sequences of Uluguru virus (ULUV) and Kilimanjaro virus (KMJV) indicated moderately low nucleotide and amino acid sequence similarities with representative rodent- and soricid-borne hantaviruses. Phylogenetic analyses, using the maximum-likelihood and Bayesian methods under the best-fit GTR+I+Γ model of evolution, showed that ULUV and KMJV shared a common ancestry and were most closely related to Thottapalayam and Imjin viruses, two hantaviruses harbored by crocidurine shrew species in Asia, in keeping with the evolutionary relationship between crocidurine and myosoricine shrews. However, the newfound hantaviruses were distantly related to Tanganya virus in *Crocidura theresae* from Guinea. Discovery of genetically divergent hantaviruses in myosoricine shrews in eastern Africa further expands the host range and distribution of soricid-borne hantaviruses across four continents, lending additional support to the emerging paradigm-altering concept that ancestral soricomorphs, rather than rodents, may have served as the original mammalian hosts of primordial hantaviruses. Studies, now underway, of other African shrew genera, such as *Sylvisorex*, may provide greater insights into the evolutionary history of hantaviruses.

882

FACTORS ASSOCIATED WITH PERSON TO PERSON NIPAH VIRUS INFECTION

M. J. Hossain

ICDDR,B, Dhaka, Bangladesh

Since 2001, we have identified 9 Nipah virus (NiV) outbreaks in Bangladesh with a high case fatality (106/146; 73%). Family members or friends care for patients in the home or hospital. Since more than half of all cases followed close contact Nipah infected patients we attempted to identify factors responsible for NiV transmission to close contacts from 2007. Close contact was defined as sharing a room, veranda, or vehicle with a Nipah case for at least 15 minutes. We defined secondary infection as one developed evidence of NiV infection 5-20 days after close contact with a Nipah patient. All contacts except five deaths were serologically tested for NiV IgM antibody. Confirmed NiV infection was defined as a positive NiV IgM antibody test or features of encephalitis without laboratory confirmation. We identified 38 Nipah cases and 612 contacts; mean16 contacts per case. Only three (8%) cases transmitted NiV. The overall proportion of secondary cases was 2.5% (15/612) of which 25% (4/15) had mild febrile illness, and none were asymptomatic. The mean age (36 years vs. 33 years) and sex (53% male vs. 47% male) of secondary cases and contacts were similar. Compared to seronegative contacts, secondary cases were more likely to have spent time in a vehicle with a case (OR 2.9; 95% CI 0.9-9.5), had close contact with a case within the first 5 days of illness (OR undefined; 95% CI 1.0-undefined), fed a case with their hands (OR 3.2; 95% CI 1.0-10.4), helped patient to use the toilet (OR 4.7; 95% CI 1.3-15.3), cleaned vomit from the patient's body (OR 5.3; 95% CI 0.9-21.0) and received a cough in the face (OR 4.8; 95% CI 1.1-17.0). In conclusion, person-to-person transmission of NiV was uncommon among persons who cared for Nipah patients, but hand contamination with body fluids, receive cough in the face and close contact within five days of onset of illness increased risk. Hand washing with soap and water and avoiding to receive cough in the face while caring for a Nipah patient could interrupt transmission of NiV to caregivers.

883

HOSPITAL-BASED SURVEILLANCE FOR INFLUENZA VIRUSES IN GHANA, WEST AFRICA

William K. Ampofo[1], Karl C. Kronmann[2], Michael Adjabeng[3], Kofi Bonney[1], Ivy A. Asante[1], N. Talla Nzussouo[4], Richard Davies[5], Frederick Addo-Yobo[1], Prince Parbie[1], Harry Opata[6], John McCauley[7], Guillermo Pimentel[8], Robert V. Barthel,[8], Buhari A. Oyofo[8], Lawson Ahadzie[3], Joseph Amankwa[3]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]United States Naval Medical Research Unit 3, Accra, Ghana, [3]Ghana Health Service, Public Health Division, Accra, Ghana, [4]Centers for Disease Control and Prevention/United States Medical Research Unit #3, Accra, Ghana, [5]Centers for Disease Control and Prevention, Atlanta, GA, United States, [6]World Health Organisation Country Office - Ghana, Accra, Ghana, [7]Medical Resarch Council, National Institute for Medical Research, London, United Kingdom, [8]United States Naval Medical Research Unit 3, Cairo, Egypt

Ghana began sentinel investigation of influenza-like-illness (ILI) in selected health institutions in 2007. This is in line with global systems to identify circulating/novel influenza virus strains, inform annual vaccine composition and provide early warning for an influenza pandemic. Initial sites were located in 3 regions where Avian Influenza outbreaks in poultry had occurred. For the past 32 months, health facilities across Ghana have investigated patients presenting with respiratory disease signified by fever >38ºC and/or cough, sore throat, headache, body-aches and coryza. At the national influenza center, Influenza (flu) virus presence was determined by virus isolation in cell culture and real time reverse transcriptase polymerase chain reaction. Antigenic, phylogenetic and anti-viral susceptibility analyses were also performed. Five thousand, eight hundred and ninety-two cases of ILI have produced 1284 flu viruses (22% of samples). The 6-15 years age group was most affected (38%). Initially, in 2007, flu A H3N2 subtype was predominant but this was replaced in 2008 by flu A H1N1 with flu B Yamagata strains also in circulation. Flu A H3N2 then dominated in 2009 alongside with flu B Victoria strains. By early 2010, pandemic flu A H1N1 2009 (pH1N1) became the prevailing flu virus with A H3N2 and B Victoria strains also present. Fever, cough, headache and coryza were the most frequent clinical presentations. Molecular markers of resistance to Oseltamivir in flu A H1N1 isolates was first observed in 2008 and has persisted, but has not been found in other subtypes. The pH1N1 isolates have anti-viral resistance to Adamantanes with genomic comparability of 99% with A/California/7/2009 (pH1N1) strain. Four cases of flu AH3N2 and pH1N1 co-infections have been recorded. Amongst hospital admissions for severe illness, seasonal flu viruses have now been replaced by pH1N1. In conclusion, scrutiny for flu virus is now part of Ghana's public health system with full regional coverage by mid-2010. Soon, the profile of flu virus activity over the tropical climate will be discerned that would be applicable to West Africa as well. Due to the data obtained, the contribution of influenza virus to respiratory disease burden in Ghana is now better understood. As a result, control and response strategies for pandemic influenza have been established.

884

FEMALE UROGENITAL SCHISTOSOMIASIS IN TANZANIA'S LAKE ZONE REGION: A HIGHLY-SPORADIC DISTRIBUTION AMONG WOMEN IN EIGHT RURAL VILLAGES

Jennifer A. Downs[1], Charles Mguta[2], Godfrey M. Kaatano[3], Katrina B. Mitchell[1], Harusha Simplice[4], Safari Kinung'hi[3], Samuel E. Kalluvya[2], John M. Changalucha[3], Warren D. Johnson, Jr.[1], Daniel F. Fitzgerald[1]

[1]Weill-Cornell Medical College, New York, NY, United States, [2]Weill-Bugando Medical Centre, Mwanza, United Republic of Tanzania, [3]National Institute for Medical Research, Mwanza, United Republic of Tanzania, [4]Sengerema District Hospital, Sengerema, United Republic of Tanzania

Female urogenital schistosomiasis (FUS) is a parasitic infection caused by *Schistosoma haematobium* that leads to genital tract inflammation and ulceration and may predispose to incident HIV infection. FUS affects an estimated 45 million girls and young women in sub-Saharan Africa. The prevalence among women of reproductive age has been reported to be as high as 30-40% in areas where the parasite is endemic, including regions of northern Tanzania. We conducted a community-based prevalence study among women aged 18-50 living in northwest Tanzania's Lake Zone region, where *Schistosoma haematobium* infection had been diagnosed in 30-90% of schoolchildren in 2006. Women received screening for urogenital schistosomiasis, HIV, sexually-transmitted infections, intestinal schistosomiasis, and cervical cancer in collaboration with local cancer screening programs. In 472 women living in eight villages, the prevalence of FUS varied by village and ranged from 0 to 14%. Living in a village in which at least 3% of adult women had FUS and age less than 25 years old were highly-significant risk factors for FUS in this population ($p<0.0001$ for each risk factor). The overall prevalence of HIV in these women was 5.9% (28 of 472), but the prevalence of HIV in women with FUS was more than 50% greater (3 of 33, 9.1%). In conclusion, FUS is sporadically distributed among women in northwest Tanzania, with some villages having rates of *Schistosoma haematobium* infection as high as 14% in women of reproductive age, while other nearby villages have none. Women younger than 25 years old were almost four times more likely to have FUS than older women. As these young women are also at increased for HIV and FUS has been associated with HIV, this may have implications for schistosomiasis and HIV control programs in the region.

885

ASSESSMENT OF QUALITY OF LIFE AS A TOOL FOR MEASURING MORBIDITY DUE TO SCHISTOSOMIASIS AND THE IMPACT OF TREATMENT

Kimberly Y. Won[1], Bernard Abudho[2], Susan P. Montgomery[1], Erin D. Kennedy[1], Bobbie Person[1], Pauline N. Mwinzi[2], Elizabeth Ochola[2], Karen T. Foo[1], Allen W. Hightower[1], Diana M. Karanja[2], W. Evan Secor[1]

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

Mass treatment programs targeting schistosomiasis in a number of African countries are designed to reduce morbidity associated with the infection. In order to better monitor and evaluate treatment programs, tools for assessing morbidity are needed. Because schistosomiasis can persist for years as a chronic infectious disease, one approach is to utilize questionnaires that capture measures of quality of life. To evaluate whether the WHO quality of life assessment (WHOQOL-BREF) is a useful measure of the health impact of *Schistosoma mansoni* infections, we enrolled nonpregnant adult members of the community of Usoma, Kenya who had no recollection of previous treatment with praziquantel. Based on WHO recommendations and previous studies showing high prevalence of schistosomiasis in children, the entire community was eligible for mass drug distribution. Prior to treatment, the WHOQOL-BREF was administered to consenting participants. These individuals were

evaluated for schistosomiasis by both stool examination and presence of urine circulating cathodic antigen (CCA). Additionally, participants were tested for infection with soil transmitted helminths, malaria, and HIV. Two days after taking praziquantel, individuals were asked about tolerance of treatment and tested again for CCA. Preliminary results suggest no association between schistosome infection status (positive or negative) or intensity of infection and quality of life at baseline. However, following praziquantel treatment, persons with higher intensity infections demonstrated more pronounced side effects than individuals with light or no infections ($p < 0.0001$). Urine CCA levels were reduced within 2 days of treatment ($p < 0.0001$) and those with light infections were more likely to become negative. Ongoing work will reassess WHOQOL-BREF scores at 6 months after treatment to help determine how useful this tool will be to evaluate and compare the impact of mass drug administration programs.

886

PREVALENCE AND INTENSITY OF *SCHISTOSOMA* SPP TWO YEARS AFTER A PRAZIQUANTEL TREATMENT AMONG SCHOOL-AGE CHILDREN FROM A RURAL VILLAGE IN MALI

Ngoy P. Mutombo[1], Mary-Louise McLaws[1], Wing Y. Man[1], Artemis Koukounari[2], Moussa Sacko[3], Robert Dembélé[4], Joanne P. Webster[2]

[1]University of New South Wales, School of Public Health and Community Medicine, Faculty of Medicine, Sydney, Australia, [2]Schistosomiasis Control Initiative, Department of Infectious Disease Epidemiology, Faculty of Medicine, Imperial College London, London, United Kingdom, [3]Institut National de Recherche en Santé Publique, Bamako, Mali, [4]Programme National de Lutte Contre la Schistosomiase, Ministère de la Santé, Bamako, Mali

Schistosomiasis remains a major neglected and important health problem in developing countries. In regions where praziquantel (PQZ) has been used over prolonged periods for schistosomiasis morbidity control one central question builds around the optimal interval between PQZ treatment rounds to achieve significant long term decrease in worm loads. Our aim was to study the impact of a single dose of PQZ treatment on the prevalence and infection intensity of *Schistosoma mansoni* and *S. haematobium* in a rice irrigated village in Mali. Two cross-sectional parasitological surveys among children (6-14 years old) were carried out in 2005 and 2007 within a single village in Mali. Stool and urine samples were examined for *S. mansoni* and *S. haematobium* eggs, respectively. Difference in prevalence and infection intensity between the two years was tested using age and sex adjusted logistic and negative binominal regression models, respectively. At 2 years post-treatment, the overall prevalence of *S. mansoni* and *S. haematobium* infection fell from 93% to 88% [OR 0.55, CI95 0.26-1.10] and 74.5% to 28.0% [OR 0.12, CI95 0.07-0.20], respectively. Geometric means of *S. mansoni* and *S. haematobium* infections decreased significantly from 179 to 83 eggs/gram of faeces [egg count ratio (ECR) 0.58; CI95 0.42-0.78] and 12.3 to 1.8 eggs/10 ml urine [ECR 0.074, CI95 0.044-0.127]. The proportion of children with heavy infections decreased significantly from 42% to 26% for *S. mansoni* and 26% to 0.9% for S. haematobium. The validity of these results needs to be put into the context of the epidemiological setting, drawing attention to the issue of scale and specific control measures. In conclusion, praziquantel appeared to have a long term effect on *S. haematobium* but not on *S. mansoni* thought this might also suggest species-specific differences in praziquantel treatment. Current control efforts do not attain sufficient reduction of schistosomiasis infection in this particular setting which points us to the need for additional control measures specific to the 'Office du Niger' irrigation scheme.

887

IMPACT OF TWO ROUNDS OF PRAZIQUANTEL TREATMENT ON *SCHISTOSOMIASIS HAEMATOBIUM* IN SELECTED SENTINEL COMMUNITIES OF DELTA STATE, NIGERIA

Emmanuel Emukah[1], **Julie R. Gutman**[2], Emmanuel S. Miri[3], Donald Hopkins[4], Moses Katabarwa[4], John Eguagie[3], Paul Yinkore[5], Ndudi Okocha[5], Victoria Jibunor[5], Frank O. Richards[4]

[1]The Carter Center, Nigeria, Owerri, Nigeria, [2]Emory University, Atlanta, GA, United States, [3]The Carter Center, Nigeria, Jos, Nigeria, [4]The Carter Center, Atlanta, GA, United States, [5]Ministry of Health, Asaba, Nigeria

Urinary schistosomiasis is a parasitic disease caused by the trematode *Schistosoma haematobium* (SH). Nigeria is one of the most endemic countries for SH in Africa. Following an initial prevalence survey in 2003, annual mass drug administration (MDA) for SH was initiated in Delta State in 2004. Following 1993 WHO guidelines, single dose praziquantel (PZQ) was provided to all school-aged children in 57 communities where reagent strip (dipstick) testing of urine show hematuria prevalence of ≥20% in a sample of 30 school aged children (aged 6-14 yrs), and to the entire village in 7 communities where the prevalence in children was ≥50%. Children had a physical examination and were asked about passing blood in urine ('red urine'). A visual diagnosis of bloody urine was recorded by nurses (NVD) before results of dipstick testing was known. Cross-sectional surveys of school children were conducted in 8 sentinel villages (3 mass, 5 school-aged) at baseline (n=240) and after two annual doses (n=402) to determine the impact of PZQ. Following 2 rounds of PZQ MDA hematuria measured by dipstick decreased by 88.5% and only 1 sentinel village (SV) still qualified for MDA (≥20% prevalence). Mid upper arm circumference (MUAC) increased by 6.8% ($p<0.001$). Although specific, history of hematuria and NVD had a low sensitivity compared to dipstick results (31% and 44%, respectively). Prior to treatment, history and NVD identified only 1 (31%) and 3 (44%), respectively, of the 8 SVs as needing treatment. Neither history nor NVD identified the remaining SV still in need of treatment after two MDA rounds. PZQ mass treatment was associated with a decrease in the prevalence of hematuria and an increase in MUAC. Questioning children or using NVD failed to identify the majority of the communities in need of treatment. In this part of Nigeria, history of hematuria or gross hematuria are not sufficiently sensitive for either initial mapping or impact assessment.

888

DYNAMICS OF TRANSMISSION AND REINFECTION PATTERNS OF SCHISTOSOMIASIS AFTER TREATMENT

Louis-Albert Tchuem Tchuenté[1], Oumar Talla Diaw[2], Amadou Garba[3], Bonnie Webster[4], Russell Stothard[4], David Rollinson[4], Thomas Kristensen[5]

[1]University of Yaoundé I, Yaoundé, Cameroon, [2]Institut Sénégalais de Recherches Agricoles, Dakar, Senegal, [3]Schistosomiasis Control Initiative, Niamey, Niger, [4]The Natural History Museum, London, United Kingdom, [5]DBL Life Sciences, University of Copenhagen, Frederiksberg, Denmark

The high burden of schistosomiasis in Africa is due to *Schistosoma haematobium*, which causes urinary schistosomiasis, and *S. mansoni*, which causes intestinal schistosomiasis. Evidence has accumulated of the dynamics of schistosomiasis epidemiology and increasing co-endemicity of these two species. In order to assess the dynamics of transmission, the efficacy of treatment and the re-infection patterns in mixed infection foci, a multicentre study was conducted in several countries including Cameroon, Senegal and Niger, where suitable villages with *S. haematobium* and *S. mansoni* co-infections were selected. The studies were conducted following a designed standard protocol, including parasitological baseline survey, treatments and follow up surveys at several time intervals up to 12 months after treatment. The results showed an overall good efficacy of praziquantel against *S. haematobium* and *S. mansoni*. However, there were significant differences in transmission dynamics, cure rates and re-infection patterns. The efficacy of praziquantel

against *S. mansoni* was lower in Senegal and Niger compared to Cameroon. In the mixed infection foci in Senegal, the re-infection rates were higher for *S. mansoni*, contrary to Cameroon where *S. haematobium* exhibited higher re-infection patterns. These studies provided an important insight into the understanding of praziquantel efficacy and post-treatment re-infection dynamics of schistosome in co-infections with *S. mansoni* and *S. haematobium*. The findings and a better understanding of schistosome interactions are important for recommendations to optimize control strategies in mixed infection foci.

889

GEOSTATISTICAL MODEL-BASED ESTIMATES OF SCHISTOSOMIASIS RISK IN WEST AFRICA

Nadine Riedel[1], Eveline Hürlimann[1], Thomas K. Kristensen[2], Jürg Utzinger[1], Penelope Vounatsou[1]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Danish Bilharzias Laboratory, Department of Veterinary Disease Biology, University of Copenhagen, Copenhagen, Denmark

Schistosomiasis is a water-based disease caused by trematodes of the genus Schistosoma. It is believed that the disease affects some 200 million people worldwide with more than 95% of the infections concentrated in Africa. However, the current figures are largely based on population re-adjusted estimates originally published by Utroska and colleagues in 1989. These estimates might be outdated due to, for example, water-resource development and management, improved sanitary facilities and large-scale preventive chemotherapy. For planning, coordination and evaluation of control activities, it is essential to have reliable schistosomiasis risk maps and burden estimates. We analyzed survey data compiled on a newly established open-access global neglected tropical disease (GNTD) database (www.globalntddatabase.org) to (i) create smooth empirical risk maps for Schistosoma mansoni and S. haematobium for children aged below 20 years in West Africa, including Cameroon, and (ii) to calculate country prevalence estimates. We used Bayesian geostatistical models based on environmental and climatic predictors to take into account potential spatial clustering due to common spatially structured exposures. Our estimates suggest that a total of nearly 70 million West African children are currently infected with either S. mansoni or S. haematobium. The country prevalence estimates range between 0.7% (Gambia) and 35.5% (Liberia) for S. mansoni and between 17.7% (Gambia) and 50.8% (Sierra Leone) for S. haematobium. We observed that the combined schistosomiasis risk for both species is two-fold lower in Gambia than previously reported, while the existing estimates for Liberia seem to be seriously underreported (24.0% compared to our estimates of 61.7%). However, our predictions might overestimate overall country prevalence, since modelling was based on children aged below 20 years who are known to carry the highest infection risk.

To our knowledge, these are the first empirical estimates of S. mansoni and S. haematobium risk at high spatial resolution throughout West Africa.

890

OPTIONS FOR THE TREATMENT AND CONTROL OF SCHISTOSOMIASIS *HAEMATOBIA* AND *MANSONI*: EVIDENCE FROM TWO SYSTEMATIC REVIEWS AND META-ANALYSES

Anthony Danso-Appiah[1], **Piero L. Olliaro**[2], Paul Garner[3], Jürg Utzinger[4]

[1]Internal Health Group, Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]World Health Organization (WHO), Geneva, Switzerland, [3]International Health Division, Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [4]Unibas, Basel, Switzerland

Treatment and control of schistosomiasis relies on praziquantel with virtually no alternatives. Uncertainties remain on praziquantel doses. Systematic reviews and meta-analysis can help inform both policy and research. A systematic search for randomized controlled trials of drugs used alone or combined for treating *Schistosoma haematobium* and *S. mansoni*. We used risk ratio (RR) or weighted mean difference (WMD) to analyze combined dichotomous or continuous outcomes, including confidence intervals. Overall, 24 trials (6,315 participants) met the inclusion criteria for *S. haematobium* and 49 trials (9,608 participants) for *S. mansoni*. Praziquantel, metrifonate (against *S. haematobium*) and oxamniquine (against *S. mansoni*) were better than placebo in producing parasitological clearance and egg reduction. Praziquantel shows no dose-effect for *S. haematobium* between 20 and 40 mg/kg, while there is a dose-effect on *S. mansoni* (40 mg/kg > 20-30 mg/kg, no increment with 60 mg/kg). For *S. haematobium*, metrifonate is effective, but requires multiple dosing (10 mg/kg fortnightly x 3); no study compared directly the standard doses of praziquantel and metrifonate. With oxamniquine, there is a dose-effect for parasitological efficacy against *S.mansoni* (40 mg/kg > 10-30 mg/kg; no increment with 50-60 mg/kg). Oxamniquine (20-60 mg/kg) was not different to praziquantel (40 mg/kg). No dose-effect was demonstrable on clinical improvement with oxamniquine or praziquantel. Data on artemisinins for both *S. haematobium* and *S. mansoni* are inconclusive. In conclusion, praziquantel is effective and well-tolerated at the WHO-recommended dose of 40 mg/kg for both *S. haematobium* and *S. mansoni* in all endemic areas. There is no advantage with higher doses. Alternatives are metrifonate for *S. haematobium* and oxamniquine for *S. mansoni* but both have limitations and neither is available today for use in programmes. There is a pressing need for studies of combination therapy (e.g. praziquantel plus metrifonate, oxamniquine and artemisinins).

891

INCIDENCE AND ETIOLOGY OF ACUTE DIARRHEA IN A FRENCH MILITARY COHORT IN CHAD

Vincent Pommier de Santi[1], Elisabeth Nicand[2], Gisèle Lagathu[2], Carlos Grimaldos[3], Jean-Paul Boutin[1], **Catherine Marimoutou**[1]

[1]DESP, Institut de Médecine Tropicale du Service de Santé des Armées, Marseille, France, [2]Hôpital d'instruction des armées Val de Grâce, Service de biologie clinique, Paris, France, [3]Direction générale de la Gendarmerie, Paris, France

The objective of this study was to assess the incidence, etiology and behaviors associated with acute diarrhea among French Forces deployed to N'Djamena, Chad Sub-Saharan Africa. Prospective study based on physician consultation for diarrhea during a 5-month French forces mandate. Diarrhea was defined as >=3 stools per 24h or >=2 stools per 8 h. For each diarrheic episode an anonymous clinical-administered questionnaire and stools sample were collected. The global incidence rate was calculated using the mean number of soldiers based in N'Djamena during the study period (N=1024) as denominator. As the overall number of military personnel staying in N'Djamena slightly varied during the study period, diarrhoea incidence rates were also estimated for the 11 two-week-periods of stay. A case-crossover analysis estimated behaviors associated to diarrhea. A total of 240 acute diarrheas were notified by military physicians, resulting to an overall incidence rate of 49 diarrheas per 1000 person-months and a 23% risk to develop diarrhea. The incidence rate raised from 8.8/1000 person-two-weeks at the beginning of stay to 54.4/1000 person-two-weeks after one month and decreased after two months to stabilize between the end of November 2007 to early January 2008. Pathogens were identified in 40% stool samples, enteric viruses were predominant (28.6%, 14.8% for noroviruses). Three behaviors were significantly associated to acute diarrhea in the case-crossover multivariate analysis: diarrhoea in the close circle in the previous days increased the risk (OR:3.8 [2.0-7.0]); always eating at Mess (OR: 0.2 [0.1-0.5]) or staying in temporary encampment (OR:0.3 [0.1-0.8]) were protective. In conclusion, identification of viruses lead to high risk of man to man transmission. Independently of the risk of traveler's diarrhea due to poor sanitation environment confirmed by the study, our results underline the importance of relevant hygienic measures and primary care during military deployment.

892

POOR TOLERANCE OF NIFURTIMOX IN TREATMENT OF CHAGAS DISEASE IN UNITED STATES-BASED POPULATION

Aiman M. Smer, **Mahmoud I. Traina**, Salvador Hernandez, Jason Bradfield, Sheba K. Meymandi

Olive View-University of California at 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 million persons are infected. Chronic CD results in symptomatic cardiac and/or gastrointestinal disease in 10-30% of infected persons with 20,000 deaths annually. Approximately 17 million persons born in the endemic countries currently reside in the U.S. and roughly 300,000 of these immigrants are thought to have chronic CD. There are only two drugs available for the treatment of CD: nifurtimox and benznidazole. It has been over 40 years since the development of both drugs, yet neither is approved by the FDA. Currently, The CDC has obtained special approval for the use of nifurtimox. Though numerous clinical studies have evaluated efficacy and tolerance for nifurtimox in acute CD, little data exists on the tolerance of nifurtimox in older patients in the chronic or indeterminate stage of CD. Twenty nine consecutive Latin American immigrant patients with CD were treated with nifurtimox at the Center of Excellence for CD at Olive View-UCLA Medical Center for a planned treatment course of 90 days. The majority of patients were symptomatic with either chest pain or palpitations and 48.3% of patients had evidence of early cardiac involvement on electrocardiogram. None of the patients had clinical congestive heart failure. The median age of patients was 45 with ages ranging between 15 and 58. The most observed side effects of nifurtimox were: anorexia (79.3%), psychiatric manifestations including depression, anxiety, insomnia and memory loss (75.9%), nausea & vomiting (69.0%), headache (65.5%), myalgia & arthralgias (48.3%), abdominal pain (34.5%), fatigue (24.1%), parasthesias & neuropathy (20.7%), and rash (6.9%). Eleven (37.9%) patients required dose adjustment because of side effects. Nine patients (31%) stopped treatment temporarily, then resumed at a lower dose. Three of these patients (10.3%) were unable to tolerate the lower dose and stopped treatment prematurely. Most patients who required dose adjustment cited the psychiatric manifestations as the primary reason. The poor side effect profile of nifurtimox is the primary reason behind treatment intolerance and significantly hinder treatment efforts. Given the significant limitations of the current drugs, new drugs with better efficacy and lower side effect profiles need to be developed for CD.

893

QUALITY SYSTEMS IMPROVEMENT FOR COLLEGE OF AMERICAN PATHOLOGISTS ACCREDITATION OF A CLINICAL LABORATORY TO SUPPORT BIOMEDICAL RESEARCH IN BAMAKO, MALI

Joseph P. Shott[1], **Merepen A. Guindo**[2], Renion Saye[2], Diakite M. Lamine[2], Sintry Sanogo[2], Moussa B. Dembele[2], Keita Sekouba[2], Issaka Sagara[2], Richard Sakai[2], Gary Mays[3], Mary Nagel[4], Patricia A. Rizzo-Price[4], Louis H. Miller[5], Ruth D. Ellis[6], Ogobara Doumbo[2], Dapa Diallo[2]

[1]Clinical Monitoring Research Program, SAIC-Frederick, Inc., Bethesda, MD, United States, [2]Faculty of Medicine, Pharmacy and Odonto-Stomatology, University of Bamako, Bamako, Mali, [3]Office of the Director, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [4]Global Health Partnerships, Clinical and Laboratory Standards Institute, Wayne, PA, United States, [5]Laboratory of Malaria and Vector Research, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [6]Laboratory of Malaria Immunology and Vaccinology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

High-quality clinical laboratory capacity is oftentimes lacking in resource-poor settings and areas of high disease endemicity, impacting the ability to support biomedical research where it is needed most. As the regulatory climate changes globally, human research studies will require higher quality laboratory support for protecting study volunteers in assessing biological parameters in natural history studies and in assessing safety and immunogenicity which guide the clinical development of products. The University of Bamako in Mali and its NIAID partners have undertaken a comprehensive Quality Management Systems (QMS) improvement plan to scale-up productivity, technical ability and quality by utilizing Clinical and Laboratory Standards Institute (CLSI) internationally accepted consensus standards and guidelines. This QMS improvement and accreditation is part of the NIAID program for promoting best practices in clinical research in developing countries. The laboratory passed inspection by the College of American Pathologists (CAP) in April 2010. We report on the QMS implementation process, pitfalls in capacity building, staff training initiatives, and CAP-accreditation timeline as it may apply to other laboratories in resource-poor settings that support clinical research.

894

MILD AND SEVERE CLINICAL FORMS OF URBAN LEPTOSPIROSIS: ACTIVE OUTPATIENT-BASED SURVEILLANCE IN A SLUM COMMUNITY IN BRAZIL

Juan I. Calcagno[1], Helena C. Lima[1], André H. Goncalves[1], Federico Costa[1], Daniele Takahashi[1], Elsio A. Wunder[1], Jailton Silva[1], Mitermayer G. Reis[1], Albert I. Ko[2], Guilherme G. Ribeiro[1]

[1]Centro de Pesquisa Goncalo Moniz, Fundacao Oswaldo Cruz, Salvador, Brazil, [2]Department of Epidemiology and Public Health, Yale School of Medicine, New Heaven, CT, United States

Outbreaks of leptospirosis occur each year in slum communities in Brazil. The burden due to these outbreaks is underestimated since routine surveillance relies on identifying hospitalized cases. We performed active outpatient-based surveillance to measure the leptospirosis incidence in a slum community in Salvador, Brazil and determine the proportion of cases which develop severe disease forms. We identified all patients with acute fever and >5 years of age at an urgent care facility which serves a community of 62,952 inhabitants. The study team recruited a sample of patients five days a week to obtain information on clinical outcome and paired serum samples. The microscopic agglutination test was performed to confirm cases of leptospirosis. We recruited a sample of 1,119 patients among 4,509 identified with acute fever during a nine-month period

(04/09-12/09). Of these, 15 (1.4%) were confirmed cases of leptospirosis. The annual incidence of leptospirosis was 143 (95%CI, 120-166) cases per 100,000 population. Cases were mostly males (67%), had a mean age of 27 years (SD± 16.8 years) and were not suspected as having leptospirosis during their outpatient evaluation. The majority (11) of cases had a self-limiting illness while 4 developed Weil's disease requiring hospitalization. Risk factors for leptospirosis among subjects with acute fever were residence <10 meters from open sewage (OR 4.5; 95%CI 1.5-13.7) and sighting of rats (OR 7.0; 2.0-25-0), contact with trash (OR 5.7; 2.0-16.0), sewage (OR 5.9; 2.1-16.1), and mud (OR 3.4; 1.2-9.4) in the household environment. In conclusion, our study found that severe disease accounted for a small proportion (23%) of leptospirosis cases, indicating that the burden of urban leptospirosis is greater than previously believed. Outpatient physicians were unable to diagnose leptospirosis during the initial phase of illness. However, we identified environmental risk exposures which can be evaluated during outpatient evaluations and used to identify cases who would benefit from antimicrobial therapy.

895

CHARACTERISTICS OF MALNOURISHED CHILDREN WITH DIARRHEA IN A RURAL AREA IN EGYPT

Manal Mostafa[1], Adel Mansour[1], Hanan El Mohammady[1], Peter Sebeny[1], Sylvia Young[1], Nasr El Sayed[2]

[1]United States Naval Medical Research Unit 3, Cairo, Egypt, [2]Ministry of Health, Cairo, Egypt

Diarrhea and its association with malnutrition are major causes of morbidity and mortality in children in developing countries. Our aim was to compare the epidemiologic and clinical characteristics associated with better-nourished and malnourished children seeking diarrhea medical care. A hospital-based surveillance study was conducted during 2000-2007 in rural Egypt enrolling children less than 5 years of age seeking medical care for diarrhea. Data was collected, including height and weight to categorize a malnourished child as wasted (weight for age z-scores ≤ -2 SD) and/or stunted (height for age z-scores ≤ -2 SD). Better-nourished (not wasted and/or stunted) children served as a comparison group. Stool samples were collected for routine microbiological diagnostics. Of 3813 children enrolled, 610 (16%) were malnourished (6% wasted, 6% stunted, and 4% both wasted and stunted). For malnourished children, the mean age was 16 months and 59% were male, compared to 13 months and 54%, respectively, for better-nourished children. Compared to better-nourished children, stunted and both [wasted and stunted] children were less likely to be breastfed [(OR=0.6, p=0.003), (OR=0.6, p-value=0.02) respectively]; they were also more likely to be dehydrated [(OR=2.4, p=<0.0001), (OR=2.1, p=<0.0001) respectively]. Malnourished children were more likely to be hospitalized due to diarrhea than better-nourished children (OR=2.4, 2.4 and 3.5) for wasted, stunted and both [wasted and stunted] compared to better nourished children, respectively (p≤0.0001). *Cryptosporidium spp.* was the only pathogen more commonly found among wasted and stunted children (10%, 9.5%) compared to better-nourished children (6%), p=0.006 and 0.01. In conclusion, a significant percentage of children in rural Egypt seeking diarrhea medical care are malnourished and experience greater severity of illness than better-nourished children with diarrhea. Steps to improve sanitation and nutrition (including breastfeeding) may help to decrease diarrhea-associated morbidity in children.

896

RELATEDNESS OF *VIBRIO CHOLERAE* O1/O139 FROM PATIENTS AND THEIR HOUSEHOLD CONTACTS, DETERMINED BY MULTILOCUS VARIABLE NUMBER TANDEM REPEAT ANALYSIS (MLVA)

Emily A. Kendall[1], Fahima Chowdhury[2], Yasmin Begum[2], Ashraful I. Kahn[2], Shan Li[3], James H. Thierir[3], Jason Bailey[3], Carol O. Tacket[3], Regina C. LaRocque[4], Jason B. Harris[4], Edward T. Ryan[4], Firdausi Qadri[2], Stephen B. Calderwood[4], **O.C. Stine**[3]

[1]Vanderbilt University, Nashville, TN, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]University of Maryland, Baltimore, MD, United States, [4]Massachusetts General Hospital, Boston, MA, United States

The genetic relatedness of *Vibrio cholerae* O1/O139 isolates obtained from 100 patients and 146 of their household contacts in Dhaka, Bangladesh, between 2002 and 2005 was assessed by multilocus variable number tandem repeat analysis (MLVA). Isolate genotypes were analyzed at five loci containing tandem repeats. Across the population as well as within households, isolates with identical genotypes were clustered in time. Isolates from individuals within the same household were more likely to have similar or identical genotypes than isolates from different households, but even within a household, isolates from different individuals often had different genotypes. Isolates with genotypes related to the index case appeared in household contacts on average ~3 days after the household index case, while isolates with unrelated genotypes appeared in contacts ~6 days later. Limited data revealed that multiple isolates from the same individual may have identical, similar, or unrelated genotypes as well. Our results demonstrate that genetically-related *V. cholerae* cluster in local outbreaks, but also suggest that multiple distinct strains of *V. cholerae* O1 may circulate simultaneously within a household.

897

LUCIO'S PHENOMENON IN LEPROMATOUS LEPROSY: A VASCULITIS "MIMIC" IN TWO IMMIGRANTS FROM MEXICO

Glenn E. Mathisen, Riffat Muzzafer

Olive View-University of California at Los Angeles Medical Center, Sylmar, CA, United States

First described by Lucio in 1852, Lucio's phenomenon is a necrotizing skin reaction associated with non-nodular diffuse lepromatous leprosy (Latapi's leprosy). Although rare in the United States, we have seen two cases of Lucio's mimicking cutaneous vasculitis in patients from endemic regions of Mexico (Jalisco; Sinaloa). Case #1: A 36 year old male presented with a one month history of painful, erythematous lesions on the lower extremities; within two weeks, similar lesions appeared on the hands, arms and subsequently ulcerated. The patient also had a history of eyebrow loss x 1 year and evidence of lepromatous leprosy (+FITE stain) on skin biopsy with full-thickness epidermal/dermal necrosis. The patient responded to a multidrug regimen including rifampin/dapsone/clofazamine. Case #2: A 49 year old male with cirrhosis/ESLD presented with peripheral neuropathy and bilateral necrotic lower extremity ulcers. The patient also had coarse facial features, partial loss of eyebrows/eyebrows, hepatosplenomegaly and thickening of peripheral nerves. Biopsy of skin lesions demonstrated mononuclear cell infiltrate with numerous AFB on Fite stain. The patient was started on multi-drug therapy for leprosy (rifampin/dapsone/clofazamine); however, he died two weeks later after developing aspiration pneumonitis and associated respiratory failure. Patients with Lucio's phenomenon present with painful erythematous macules that evolve into discrete purpuric or necrotic lesions suggesting cutaneous vasculitis. Diagnosis is often delayed since patients may have positive serologies (RPR; ANCA; anti-cardiolipin) suggesting other infectious or rheumatologic conditions (anti-phospholipid syndrome; cryoglobulinemia). Associated findings of lepromatous leprosy are commonly present but definitive diagnosis depends on pathological confirmation of lepromatous

leprosy (+ Fite stain) and characteristic findings of vasculitis and/or tissue necrosis on skin biopsy. Patients usually respond to multi-drug therapy for lepromatous leprosy; however, in severe disease, the outcome may be fatal despite appropriate therapy. The role of adjunctive therapy (corticosteroids; thalidomide) remains controversial but specific agents may be helpful in selected cases. Consider the possibility of lepromatous leprosy with associated Lucio's phenomenon in immigrants from endemic regions with cutaneous vasculitis.

898

THE EFFECT OF VISCERAL LEISHMANIASIS ON GENE EXPRESSION IN HUMAN MONONUCLEAR CELLS: GLOBAL PROFILING OF CELLS FROM PATIENTS DURING DISEASE AND AFTER SUCCESSFUL TREATMENT

Gloria R. Monteiro[1], Eliana T. Nascimento[1], João Firmino Neto[1], Núbia N. Pontes[1], Richard D. Pearson [2], Upasna Gaur[3], Mary E. Wilson[3], Daniella R. Salha[1], Selma M.B. Jeronimo[1]

[1]UFRN, Natal, Brazil, [2]University of Virginia, Charlottesville, VA, United States, [3]University of Iowa, Iowa City, IA, United States

Visceral leishmaniasis (VL) is a potentially fatal disease caused by Leishmania infantum chagasi (Lic) in South America. Lic infection can be symptomatic or lead to progressive disease. The factors that determine whether an individual will develop asymptomatic or symptomatic infection are not fully defined. Leishmania infection suppresses macrophage microbicidal responses and IFN-γ pathway signaling *in vitro*. In order to identify genes critical to the immune response to Lic *in vivo*, we evaluated gene expression in peripheral blood mononuclear cells (PBMCs) from Brazilians with acute VL and compared this to PBMCs three months after successful treatment. PBMCs were stimulated with soluble leishmania antigen for 4 hours before RNA extraction. RNA was hybridized to Affymetrix arrays. Comparisons showed 362 genes were differentially expressed during disease versus after resolution. Transcripts that were up-regulated after resolution of VL included CXCL5 (p=0.032, fold change 1.92), CXCL10 (p=0.051 fold change 2.63), CCL22 (p= 0.046 fold change 2.00), FAM177B (p=0.006), BCL11A (p=0.011), AIDA (p=0.0007), ADAM28 (p=0.022), THBS1 (p=0.040). Transcripts that were up-regulated during acute VL included APOC1 (lipid metabolism; p=0.055 fold change -1.68), ARG1 (arginine metabolism; p=0.05 fold change -2.89) and DHFR (nitrogenous base biosynthesis; p=0.01 fold change -2.15). The comparison of gene expression between disease and post-recovery from VL are consistent with suppression of chemokines as CXCL5 and CXCL10 and induction of lipid biosynthesis APOC1 and non-classical macrophage microbicidal response as Arginase 1 during acute VL. Data indicate previously unreported pathways are likely involved in pathogenesis of VL due to Lic.

899

SUBUNIT LEPTOSPIRAL IMMUNOGLOBULIN-LIKE (LIG) PROTEIN VACCINE PROTECTS AGAINST LETHAL CHALLENGE IN THE HAMSTER MODEL OF LEPTOSPIROSIS AND PROTECTION IS ANTIBODY MEDIATED

Paula Ristow[1], Flávia McBride[1], Elsio A. Wunder Jr.[1], Marco A. Medeiros[2], Gabriela S. Esteves[2], Claudio P. Figueira[1], Alan J. McBride[1], Gisele Rocha[1], Adenizar Chagas Jr.[1], Cleiton S. Santos[1], Akira Homma[2], Ricardo Galler[2], Mitermayer G. Reis[1], Albert I. Ko[3]

[1]Centro de Pesquisas Gonçalo Moniz, Salvador, Brazil, [2]Fundação Oswaldo Cruz, Rio de Janeiro, Brazil, [3]Weill Medical College of Cornell University, New York, NY, United States

Subunit vaccines are a potential intervention strategy to prevent leptospirosis, an important neglected disease in developing countries. Lig proteins are a putative virulence factor which has bacterial Ig-like repeat domains and is expressed on the surface of *Leptospira*. We previously reported that immunization of recombinant Lig protein fragments in Freund's adjuvant conferred protection against lethal challenge in the hamster model of leptospirosis. This work aimed at evaluating Lig immunoprotection using aluminum hydroxide as an adjuvant, which is acceptable for human use. The terminal portion of LigA, the recombinant peptide LigANI, corresponding to nucleotides 1873-3675 of *ligA* gene from *Leptospira interrogans* Copenhageni strain Fiocruz L1-130, was produced as a soluble peptide and adsorbed to aluminum hydroxide. Golden Syrian hamsters (n = 8 or 10) were immunized with two doses (20-80 µg) of purified LigANI in aluminum hydroxide adjuvant, by either subcutaneous or intramuscular routes, and challenged two weeks afterwards with a lethal dose (2.5× LD50%) of *L. interrogans* strain Fiocruz L1-130. Immunization with LigANI conferred 100% protection against mortality (five experiments, P≤0.0005) by either route used. By Real Time PCR, leptospiral charge was statistically different in LigANI-immunized hamsters when compared to adjuvant-immunized hamsters in the lungs, spleen and eyes, but not in the liver and kidneys. Immunofluorescence studies of pre-challenge sera found that immunized hamsters produced surface-binding antibodies. Specific hyperimmune serum anti-LigANI was raised in New Zealand White rabbits immunized with LigANI. Passive transfer of rabbit hyperimmune sera conferred protection against lethality of 50 to 85% in hamsters (two experiments, P≤0.02). Albeit LigANI-based vaccine protected hamsters against lethal infection and serum anti-LigANI partially protected hamsters against mortality, no sterilizing immunity was observed. Together these findings indicate that immunization with recombinant Lig proteins in aluminum hydroxide confers robust immunoprotection in the standard animal model of leptospirosis and that the mechanism of immunity is antibody-dependent. Lig proteins may therefore serve as a sub-unit vaccine candidate for human and animal leptospirosis. Improvement efforts should focus on sterilizing immunity and heterologous protection.

900

ETIOLOGY OF NONSPECIFIC ACUTE FEBRILE SYNDROME IN A DENGUE ENDEMIC URBAN AREA IN COLOMBIA

Luis A. Villar[1], Gustavo Valbuena[2], Marylin Hidalgo[3], Martha González[4], Gloria Rey[4], Ruth Martínez[1], Carolina Coronel[1], Diana Tiga[1], Fredi Díaz[1], Piedad Agudelo[5]

[1]Universidad Industrial de Santander, Bucaramanga, Colombia, [2]Center of Biodefense and Emerging Infectious Diseases, University of Texas Medical Branch, Galveston, TX, United States, [3]Pontificia Universidad Javeriana, Bogotá, Colombia, [4]Instituto Nacional de Salud, Bogotá, Colombia, [5]Instituto Colombiano de Medicina Trópical, Medellín, Colombia

Although it is assumed that the nonspecific acute febrile syndrome (SAFI) is cause by this arbovirus, the etiology of this syndrome is still unknown in Colombian cities where dengue is endemic. Methods: We conducted a prospective cohort study (2003-2008) in subjects ≥ 5 years with SAFI in Bucaramanga. The etiologies were defined as follows: Dengue Negative: Negative test for specific IgM (ELISA) in convalescent serum. Influenza: Seroconversion or fourfold increase in antibody titers anti Influenza A or B of an acute and convalescent serum or titers> 1:40 (HIA test) in the latter. Leptospira: titers ≥ 1:50 by microagglutination technique. Rickettsiae: fourfold increase in the titers from the acute to the convalescent serum (IFA test). Rubella and Measles: Seroconversion of specific IgM (ELISA). Unusual Virus: We used cell culture in order to rule out Flavivirus (Yellow Fever, Encephalitis, St. Louis), Arenaviruses (Tacaribe Group) Bunyavirus (Oropuche) and Alphavirus (Venezuelan Equine Encephalitis) Results: Between March 2003 and August 2008, 2063 subjects with SAFI were followed and 1124 (54%) from those subjects were negative for dengue. In the latter group 228 (20.2%) had influenza and 52 (4.6%) had Leptospira. 11 suspected cases of Rubella and 7 of Measles were negative on confirmatory testing. None was positive for Rickettsiae or other virus. The majority 454 (40.3%) of total had no defined etiology..The acute sera of 27 cases initially considered to be dengue-negative, were positive for dengue in the cell culture. In conclusion, in a dengue endemic city in Colombia, other agents different from this virus are prevalent as the etiology of SAFI.

901

EFFECTS OF VARIOUS METHODS OF INACTIVATION OF HEMORRHAGIC FEVER VIRUSES ON CLINICAL LABORATORY PARAMETERS MEASURED IN HUMAN BLOOD

Emanuel L. Wani[1], Jeff Shaffer[2], Robert F. Garry[1], Daniel G. Bausch[2]

[1]Tulane University Medical Center, New Orleans, LA, United States, [2]Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States

Viral hemorrhagic fever (VHF) is caused by infection with one of over 25 different lipid-enveloped viruses, including Ebola, Marburg, and Lassa. These viruses pose a threat not only to infected persons, but also to healthcare workers, laboratorians, and researchers in potential contact with blood and other bodily fluids. Although specialized Biosafety Level 4 laboratories have been developed for researchers to safely manipulate these viruses and diagnose VHF, few standard clinical laboratories have these means of protection, potentially putting workers in these laboratories at risk. However, various means of inactivation of lipid-enveloped viruses have been reported that may help protect workers in clinical laboratories, including solvent/detergent combinations (Triton X-100/TnBP/Tween 80), heat, gamma-irradiation, formalin, psoralens, and UV light. However, since the point of testing in clinical laboratories is to measure parameters important in guiding clinical management, it is important to understand the effect of the various inactivation techniques on each parameter in question. We performed a study to evaluate the effects of proven or assumed virus inactivation techniques on clinical laboratory parameters commonly measured in the blood and useful in the treatment of patients with VHF, including complete blood cell counts, electrolytes, and chemistries, including coagulation parameters. Each parameter is measured before and after the inactivation step on the Piccolo Xpress blood analyzer (Abaxis Co.), a point-of-care instrument, and the percent change noted. Triton X-100/TnBP/Tween 80 has minimal effect on tested clinical parameters. In contrast, there were significant changes after heat and formalin inactivation. Results from gamma irradiation, UV light, and psoralen/UV light are pending but will be discussed, as well as proposed guidelines for safe handling and testing of blood from patients with VHF in the clinical laboratory.

902

CLINICAL STUDY OF SAFETY AND APPARENT EFFICACY OF ANTIVIPMYN® AFRICA FOR THE TREATMENT OF SNAKEBITE IN KINDIA, A FOREST REGION OF GUINEA

Cellou M. Baldé[1], Achille Massougbodji[2], Mamadou Y. Boiro[1], Edouard Millimono[1], Mamadou C. Diallo[1], Roberto P. Stock[3], **Jean-Philippe Chippaux**[4]

[1]Institut Pasteur de Guinée, Kindia, Guinea, [2]Faculté des Sciences de la Santé, Cotonou, Benin, [3]Instituto de Biotecnologia, UNAM, Cuernavaca, Mexico, [4]Institut de Recherche pour le Développement, Paris, France

An open phase IV pragmatic clinical study was conducted to measure the safety and assess the apparent efficacy under field conditions of Antivipmyn® Africa (AA), an equine lyophilized $F(ab')_2$-based antivenom. The study was conducted at the Insitut Pasteur de Guinée (IPG) from August 2009 until February 2010. All people reporting snakebite, presenting clinical symptoms of envenomation (edema, necrosis or neurological signs) and who formally accepted to participate in the study were included. Antivenom was administered by slow direct intravenous push. In 6 months 228 snakebite victims arrived for consultation at the IPG. The mean delay from bite to consultation was greater than 24 hours. Of these, 150 (65.8%) were included, mostly young men; of these, 124 (82.7%) exhibited signs of viper envenomation (inflammation and/or bleeding and/or necrosis) and 26 (17.3%) exhibited manifest neurological signs compatible with Elapid envenomation (local-regional paresthesias, cranial nerve paralyses, dyspnea, severe problems of awareness.) All patients received treatment, a mean of 1.41 vials per patient (± 0.99), and more for those with signs of neurotoxicity ($P < 10^{-5}$). Four patients (2.7%), apparently bitten by Viperidae, had a necrosis of variable extension which healed without sequels. Four others, in all likelihood bitten by Elapidae, died with hours (range: 1-7) of arrival to the IPG in spite of antivenom administration. For 2 of them, the delay between bite and arrival might explain in part the absence of a therapeutic response; for the other 2, the evolution of neurotoxicity continued in spite of treatment. Ongoing analysis of blood samples collected during the trial should permit an identification of the offending species and inform on the evolution of blood venom levels. Ten patients exhibited mild adverse events (pruritus or eruption), 5 of which are likely to be attributable to treatment.

903

FALSE-POSITIVE RAPID PLASMA REAGIN TESTING IN PATIENTS WITH ACUTE *PLASMODIUM VIVAX* MALARIA

Ryan C. Maves, Andres G. Lescano, Nilda Gadea, Eric S. Halsey, Paul C. Graf

United States Naval Medical Research Center Detachment, Lima, Peru

Prior to the introduction of penicillin, a common therapy for patients with neurosyphilis was the induction of fever with deliberate *Plasmodium* infection. Investigators at that time described positive serologic tests for syphilis in malaria-infected control patients without evidence of syphilis infection. Currently, the diagnosis of syphilis consists of screening with non-treponemal tests such as the rapid plasma reagin (RPR), followed by confirmation with a specific test such as the *Treponema pallidum* hemagglutination assay (TPHA). In this study, we compare the rate of false-positive RPR tests in patients with vivax malaria compared with patients with other febrile illnesses in Peru. Patients ≥ 5 years of age were offered enrollment into an ongoing febrile surveillance protocol in Peru if they had a temperature of ≥ 38.0 degrees C for ≤ 7 days without distinct localizing symptoms, such as a purulent cough or meningismus. Malaria was diagnosed by microscopy and PCR. RPR and TPHA were performed on all acute serum specimens. Groups were compared by two-tailed Fisher's exact test. 73 patients with vivax malaria and 76 control patients with other febrile illnesses were identified. 54.9% of patients with malaria were male with a mean age of 31.5 years, compared with 40.8% and 28.9 years in patients without malaria. In patients with malaria, positive RPRs were detected in 8/73 (11.0%), of whom 2/8 (25%) had positive TPHA tests. RPR titers ranged from 1:1 to 1:16 among the false-positive tests. In patients without malaria, a positive RPR and TPHA were detected in 1/76 (1.3%) with no false-positives. Overall, false-positive RPRs were detected in 6/73 patients with malaria (8.2%) versus 0/76 patients with non-malarious fever (0%) (p=0.0124). The positive predictive value of the RPR in patients with malaria was 25% (95%CI: 4.4-64%). In conclusion, false-positive RPRs are common in patients with vivax malaria. The RPR is a widely-used screening test for syphilis in pregnant women, persons with HIV, and other groups at risk for malaria. As such, the RPR should be interpreted with caution in malarious settings.

904

RISK FACTORS FOR METABOLIC SYNDROME IN CHILDREN AND ADOLESCENTS FROM URBAN AND RURAL AREAS OF NORTHEASTERN VENEZUELA

Marcos De Donato[1], Hectorina Rodulfo[1], Dayana Melim[1], Mariela Trybiec[1], Elvia Michelli[2], Brunnell Gonzalez[3], Del Valle Guilarte[2], Isaurea Quijada[1]

[1]IIBCA, Universidad de Oriente, Cumana, Bolivarian Republic of Venezuela, [2]Departamento Bioanalisis, Universidad de Oriente, Cumana, Bolivarian Republic of Venezuela, [3]Departamento Enfermeria, Universidad de Oriente, Cumana, Bolivarian Republic of Venezuela

Obesity represents one of the major problems associated to public health for its association to increased risk to type 2 diabetes, cardiovascular disease and stroke. Recent studies have showed that obesity is a world

wide epidemic, with the age of onset for diabetes, metabolic syndrome and cardiovascular disease decreasing every year. In order to study the prevalence of children and adolescents with increased risk of obesity in urban areas compared to rural areas, we studied 1,764 individuals (3 to 17 years old) from the cities of Barcelona (n=972), Cumana (n=325), and four rural communities (n=467). We have calculated body mass index (BMI), fasting glucose, total and HDL cholesterol, triglycerides, uric acid, creatinine, and total serum proteins. The prevalence for the risk of obesity (BMI>85 percentile) was 30.2% in the cities and 9.9% in the rural areas, without statistical differences between sexes. There were differences among age groups of the prevalence for risk of obesity. High fasting glucose (BMI>100 mg/dL) was found in 2.2 and 4.9% of the individuals from urban and rural areas, respectively. High cholesterol (above the 90 percentile, according to age) was found in 9.0 and 13.5% of individuals, respectively, and high triglycerides (above the 90 percentile) were found in 8.3 and 5.7% of the subjects. We found a relationship between age and fasting glucose, creatinine and total serum protein in both sexes, and total cholesterol but only in males. Individuals with high risk for obesity showed higher values of triglycerides and total cholesterol. These results show that there is a high prevalence of overweight among children and adolescents in urban areas but not in rural areas, most likely related to physical activity and nutrition. The risk factors found in the studied individuals can contribute to the progression of diseases such as type 2 diabetes, cardiovascular disease and metabolic syndrome. Prevention programs have to initiate in very early stages, thus more studies in children and adolescents have to be carried out.

905

ACCEPTABILITY OF A PRE-REFERRAL LIFE SAVING DRUG ADMINISTRATION FOR THE PREVENTION OF SEVERE MALARIA AND UPPER RESPIRATORY INFECTION RELATED DEATHS IN CHILDREN LIVING IN RURAL AREAS

Audrey Vermeersch[1], Anaelle Libaud[1], **Pascal Millet**[1], Elizabeth Ashley[2], Amabelia Rodrigues[3], Melba Gomez[4], Piero Olliaro[4], Nicholas J. White[2]

[1]Centre Rene Labusquiere, Bordeaux, France, [2]University of Oxford, Nuffield Department of Medicine , Oxford, United Kingdom, [3]Bandim Project, Bissau, Guinea-Bissau, [4]World Health Organization, Geneva, Switzerland

Malaria and acute respiratory infections (ARI) worldwide account together for 25% of deaths among less than 5 years old children. Clinical manifestations of those diseases make differential diagnosis difficult in the absence of medical doctors and proper diagnosis. Co-infections between malaria and ARI are generally treated as a malaria case, and result in a number of treatment failures leading to late antibiotic administration, enhanced morbidity and death. In rural areas, difficulty in reaching health facilities is responsible for many deaths during transportation of a child to the nearest hospital. Therefore, implementation of a home based treatment directed against these two major infectious diseases is of great interest in reducing infant mortality. In this context, we are developing a pre-referral intra rectal antibiotic - antimalarial combination to initiate the treatment of aggravated febrile illness in children. Beyond the rational for the R&D related to the development of a proper drug formulation, one of the current objective is to evaluate whether a pre-referal drug combination administered before any clinical or biological diagnosis is done, will be acceptable by the health authorities, health professionals, and the community. A qualitative study was conducted from May to July 2009 in Guinea Bissau. Health officials, health professionals, and community members leaving in rural areas were interviewed. At the institutional level, participants were asked about the acceptability of the combination and how to integrate the drug within the health system. The overall intervention strategy was well accepted by all health representatives in Guinea Bissau, but should be based on proper trainings aiming to optimize the use of the drug at the community level and to ensure proper treatment after admission to a referred health structure. Such therapy should be proposed in agreement with other health programmes related to malaria control such as the TDR/WHO artersunate rectal project, and the introduction of rapid diagnostic tests for malaria at the community level. Severity of illness was evaluated differently depending on mothers at the community level, but fever associated to at least one symptom of severity was mostly associated to the need for an emergency intervention. However, drugs for the treatment of non-severe malaria should also be implemented to prevent misuse of the emergency treatment at the community level.

906

ACCEPTABILITY TO PARTICIPATE IN A DENGUE VACCINE TRIAL AMONG RESIDENTS IN PUERTO RICO

Carmen L. Pérez-Guerra[1], Rosa L. Rodríguez-Acosta[1], Eunice Soto-Gómez[1], Marisol Peña-Orellana[1], Luis M. Santiago[1], R. Rhode Cruz[1], Viani Ramírez[1], D. Fermín Argüello[1], Kay M. Tomashek[1], Gustavo Dayan[2]

[1]Centers for Disease Control and Prevention/CCID/NZVED, San Juan, PR, United States, [2]Sanofi Pasteur, Swiftwater, PA, United States

Dengue is endemic in Puerto Rico with the most recent outbreak occurring in 2007. Vector control has shown limited impact. Dengue vaccines may be effective for disease control. Knowledge of dengue prevention and the acceptability to participate in a Phase III dengue efficacy trial in Puerto Rico was assessed. Two structured questionnaires with open-ended questions were developed; 1) key informant interview questionnaire for university researchers, mayors, school principals/teachers, community leaders, and parents; 2) focus group questionnaire for children and parents. Sixty-four interviews with key informants and 45 focus groups with 96 parents and 111children were conducted in 12 municipalities with the highest dengue rates. Most participants knew about dengue prevention and perceived children as the most affected population; all felt at risk for dengue. Participants knew that vaccines prevent illness and indicated that a dengue vaccine would be "fabulous"; 99% of parents would not allow their child to take part in a clinical trial. Barriers to child participation included: lack of trust and information on vaccine development and trial procedures; fear of infection due to vaccination; side effects (SE) and sequelae; and lack of transportation. Lack of trust and fear was associated with news reports on Influenza A-H1N1vaccine SE. Motivators for participation were: altruism, information on vaccine development and trial procedures, gaining protection against dengue, and getting free medical care and stipends for transportation or participation. Researchers stated that community and captive populations were the best settings for a clinical trial. Face to face interventions may be the best strategy to motivate parents to allow their children to participate in a dengue vaccine trial. Therefore, the following information should be included: study purpose, procedures and duration; results of prior studies; vaccine development, risks of SE and sequelae; differences between Influenza A H1N1and dengue vaccines; benefits; safety regulations for subjects; and transportation.

907

UPDATE ON TETRAVALENT DENVAX: PREPARATION FOR PHASE I CLINICAL TRIALS IN THE US AND COLOMBIA

Jorge E. Osorio[1], Claire Y.H. Huang[2], Aurelia Haller[3], Jill Livengood[3], Ivan D. Velez[4], Sarah George[5], Cindy Thomson[6], Joseph Santangelo[6], Dan T. Stinchcomb[3]

[1]University of Wisconsin, Madison, WI, United States, [2]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [3]Inviragen, Fort Collins, CO, United States, [4]Universidad de Antioquia, Medellin, Colombia, [5]St. Louis University, St. Louis, MO, United States, [6]Inviragen, Singapore, Singapore

Inviragen's DENVax tetravalent dengue vaccine consists of a mixture of the live, attenuated DEN-2 PDK-53 virus and three chimeric recombinant viruses, each bearing the DEN-2 PDK-53 non-structural gene backbone while expressing DEN-1, DEN-3 or DEN-4 structural genes. The four live, attenuated dengue virus strains (DENVax 1, DENVax-2, DENVax-3 and

DENVax-4) express the antigenic prM and E proteins from each of the four dengue serotypes and share the common dengue 2 (strain PDK-53) genetic background. Inviragen is currently preparing for two phase I clinical trials of DENVax formulations. The first trial will be conducted in the US at St. Louis University. In the study, two formulations (high and low dose) will be tested and administered by the subcutaneous and intradermal routes. A similar study is being planned for Colombia in the town of Rionegro (Ant), a high altitude area with low incidence of dengue. Required documents for both trials have been submitted to the US (FDA) and Colombia (INVIMA) regulatory authorities.

908

INCREASED REPLICATION OF DENGUE-2 VIRUS SEROTYPE IN MIXED CO-INFECTION STUDIES

Carolina Quintero Gil[1], Marlen Martínez-Gutierrez[1], Francisco Díaz[2], Marta Ospina[3], Jorge Emilio Osorio[4]

[1]Programa de Estudio y Control de Enfermedades Tropicales (PECET), Universidad de Antioquia, Medellin, Colombia, [2]Grupo de Inmunovirología, Universidad de Antioquia, Medellin, Colombia, [3]Laboratorio Departamental de Salud de Antioquia, Universidad de Antioquia, Medellin, Colombia, [4]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, WI, United States

A double mosquito infection with two Dengue viruses (DENV) serotypes under field conditions has been documented recently. To our knowledge, no studies have been conducted characterizing experimental infection in mosquitoes exposed to two distinct DENV serotypes and the impacts of coinfection on mosquito biology and transmission dynamics remain unknown. We have conducted preliminary studies *in vitro* and *in vivo* of mosquito mixed co-infection using strains of DENV serotypes 2 and 3 isolated from the Medellin area. To test this, C6/36 HT cells were co-infected with 2x108 genome copies/ml of DENV-2 and -3 serotypes and the replication capacity was evaluated at 96 hours post-infection. Additionally, A. aegypti mosquitoes were artificially fed with a mixture of DENV -2 and -3 at 2x108 genome copies/ml of each serotype. Mosquitoes were also infected with single viruses; negative controls consisted of blood without virus. At different days post-feeding viral genome was quantified by RT-qPCR and viral antigen was detected by immunofluorescence. Our results *in vitro* show that there are significant differences in the replication capacity between the DENV-2 and -3 different serotypes, but not between strains of the same serotype isolated from the same geographic area 5-10 years apart. Similar results showing significant differences between serotypes, but not between DENV strains, were obtained from co-infection studies in A. aegypti mosquitoes. The amount of antigen and viral genome were significantly ($P<0.05$) greater to DENV-2. These results are of great importance considering the epidemiology of DENV infection, since it is known that the frequency of infection with DENV-2 was higher than others serotypes.

909

ESTIMATING THE MAGNITUDE AND DIRECTION OF ALTERED ARBOVIRUS TRANSMISSION DUE TO VIRAL PHENOTYPE

Rebecca C. Christofferson, Christopher N. Mores

Louisiana State University, Baton Rouge, LA, United States

Vectorial capacity (C) is used as a measure of the transmission potential of a vector borne pathogen within a susceptible population. Vector competence (b), a component of the vectorial capacity equation, is the ability of an arthropod to biologically transmit an infectious agent following exposure to that agent. Comparisons of arbovirus strain-specific vector competence estimates have been used to support observed or hypothesized differences in transmission capability. Typically, such comparisons are made at a single (optimal) time point during the extrinsic incubation period, the time in days it takes for the virus to replicate and disseminate to the salivary glands. Instead of evaluating vector competence at discrete time points, utilization of the rate of change gives a more accurate measurement of the transmission potential of an arbovirus within its vector. Accordingly, we investigated the rate of change in vector competence of dengue virus in *Ae. aegypti* mosquitoes and combined it with the survival function of the vector to produce a vectorial capacity curve. The areas under the resulting curves represent the cumulative transmission potentials of the arboviruses within a population of mosquitoes. We used the calculated area under the curve for 5 dengue strains and the corresponding variance estimates to test for differences in cumulative transmission potentials between strains of dengue based on our dynamic model. To further characterize differences between dengue strains, we devised a displacement index (DI) which we define as the capability of a newly introduced strain to overtake and displace the established, dominant circulating strain. The computation of a displacement index can be used to better understand the transmission dynamics in systems where multiple strains/serotypes/arboviruses circulate. We postulate that this displacement index will lead to a better measurement of the true differences in transmission potential of pathogens, as well as inform models of the serotype/strain switching phenomena such as that seen in dengue transmission systems.

910

USING HUMAN MOVEMENT DATA TO DERIVE DENGUE VIRUS TRANSMISSION NETWORKS

Gonzalo M. Vazquez-Prokopec[1], Steven T. Stoddard[2], Valerie Paz-Soldan[3], Amy C. Morrison[2], Brett M. Forshey[4], John P. Elder[5], Tadeusz J. Kochel[4], Thomas W. Scott[2], Uriel Kitron[1]

[1]Emory University, Atlanta, GA, United States, [2]University of California, Davis, CA, United States, [3]Tulane University, New Orleans, LA, United States, [4]United States Naval Medical Research Center Detachment, Lima, Peru, [5]San Diego State University, San Diego, CA, United States

Movement patterns and social structure play an important role in modulating human-vector contact rates, affecting transmission dynamics, and the spread and persistence of vector-borne pathogens. For dengue virus (DENV), limited dispersal range of its day-biting vector, *Aedes aegypti*, points to movement of viremic humans as a plausible explanation for the rapid spread of infection across urban environments. We used field data from spatially-explicit semi-structured interviews (SSI) and GPS data-loggers to derive contact networks of individual humans for DENV transmission in Iquitos, Peru. We obtained movement data for 300 participants and expressed their contact network as an undirected bipartite graph representing the locations participants had in common as a consequence of their routine movements. Different measures of network topology were estimated for the full contact network and "key sites" network containing only those locations where exposure to *Aedes aegypti* is most likely (houses and schools). Places where participant's spent the most time outside their home were other residential locations (71% of total time); markets and stores (18%); parks, cemeteries, and recreational areas (3%); and hospitals and health posts (2%). Average degree of a participant (number of locations visited) increased with age from an average (SD) of 2.8 (1.1) for 3-8 yr-olds to 7.1 (4.3) for 45-69 yr-olds. The derived key-sites network had a main component with 69% of all the participants, indicating a high degree of connectivity at residential locations. Current targeted vector control programs focus on neighboring homes within 100 m of a diagnosed dengue case's house. Our quantitative empiric contact networks indicate that residential exposure can occur beyond 100 m of a person's home and are consistent with the notion that movement of viremic people is a prime driver of rapid DENV propagation in urban environments.

911

INTERACTION BETWEEN PRECURSOR MEMBRANE AND ENVELOPE PROTEINS OF DENGUE VIRUS

Wen-Yang Tsai, **Wei-Kung Wang**

JABSOM, University of Hawaii at Manoa, Honolulu, HI, United States

The envelope (E) protein of dengue virus (DENV) is the major immunogen for vaccine development. The C-terminus of E protein contains two α -helices (EH1 and EH2) in the stem region and two transmembrane domains (ET1 and ET2) in the anchor region. After synthesis, E protein forms a heterodimer with the precursor membrane (PrM) protein; such interaction is critical for the assembly of virus particles in the ER and maturation along the secretary pathway. At the C-terminus of PrM protein, there is an α-helical domain (MH) and two transmembrane domains (MT1 and MT2). Previous studies of the tick-borne encephalitis virus reported the domains of E protein important for PrM-E interaction and production of virus-like particles (VLPs). In addition, PrM protein was reported as a chaperone for proper folding of E protein. However, the domains of PrM protein interacting with E protein remain largely unclear. A series of constructs containing C-terminal truncation of E protein in the DENV4 PrM/E, E or PrM expression vector were generated. After transfection to 293T cells, cell lysates and cultural supernatants were collected and subjected to Western blot analysis, radioimmunoprecipitation, ultracentrifugation and co-sedimentation assays. In the absence of E protein, the expression of PrM protein was reduced and completed abolished after truncation of MT1. In the presence of E protein, the expression of PrM protein was greatly reduced after truncation of ET1 and EH2, suggesting that they are important for expression of PrM protein.Radioimmuno-precipitation by using anti-E mAb revealed greatly reduced PrM protein after truncation of ET1 and EH2, suggesting they are required for PrM-E interaction. Pulse-chase experiment suggested that EH2 is important for the stability of PrM protein. The production VLPs was reduced for ET2 truncation mutant, suggesting that ET2 is involved in the efficient production of VLPs. In conclusion, our results suggest that PrM protein with C-terminal truncation is not stable. EH2 and ET1 domains are involved in PrM-E heterodimerization, and EH2 domain is important for maintaining the stability of PrM protein. Moreover, ET2 domain is involved in the production of VLPs. These information adds to our understanding of the biology of the major immunogen of dengue vaccine development.

912

CLIMATE AND MAJOR DENGUE EPIDEMICS IN THE AMERICAS

Natalia V. Blanco, **Ivo M. Foppa**

Tulane University, New Orleans, LA, United States

Dengue is a major public health threat, with at least an estimated one third of the world population at risk of infection. This study explored the relationship between several climate indicators and the likelihood of major dengue epidemics. The analysis was based on annual dengue incidence data from 46 countries from North, Central and South America, as well as the Caribbean, as reported by PAHO from 2000-2008. The climatic factors examined were annual average of daily mean, maximum and minimum temperature, annual average of monthly total precipitation and the El Niño Southern Oscillation (ENSO) phases. Multivariate logistic regression models were fitted to the proportion of major dengue epidemics. Annual incidence rates of 200 per 100,000 population or larger were considered as a major dengue epidemic. Precipitation alone explained 20.90% of the total variation in the occurrence of major dengue epidemic. The final multivariate model contained precipitation, the cold ENSO phase, mean annual temperature, minimum annual temperature as well as the interaction of mean and minimum temperature. This model explained 39.14% of the total variation. Our ecological analysis suggests that global indicators of precipitation and temperature predict the probability of large dengue epidemics. Understanding the climate's role in the probability of large dengue epidemics may aid in the prediction of such events.

913

DENGUECON: AN ECONOMIC TOOL FOR INFORMED VACCINE DECISION-MAKING

Rebekah Borse[1], **Mark E. Beatty**[2], Martin I. Meltzer[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]International Vaccine Institute, Seoul, Republic of Korea

Several dengue vaccines are in phase 2 clinical trials. Preparing for introducing with well-thought out introduction plan could accelerate introduction of an effective vaccine; however health economic data for dengue is often not available. The objective of this study was to design a model that introduces basic cost-effectiveness concepts, that is sufficiently simple so that practicing public health officials can readily use it and interpret input and output values and users can use the model without specialized software, programming skills, or advanced knowledge of statistics to identify data needs and stimulate country-specific economic studies for more precise evidence-based decision making. We designed interactive, Excel-based models that require four types of data: Disease burden, vaccine effectiveness, cost of treatment and cost of vaccination. The models were made self-contained, have low RAM requirements, are easily down loaded or emailed, and can be operated on any computer using the commly available Microsoft Office software. To limit the complexity of the model we assumed a healthcare perspective for calculating medical costs and cost-effectiveness ration. We developed two primary models: DenguEcon and DenguEconDALY. These models will allow for the analysis of Dengue Vaccine introduction for a single cohort over 10 years that examine the cost-per-case averted, cost-per-death averted or cost-per-disability-adjusted life year averted, respectively. In addition, we developed a third model, DenguEconCompare, which will allow for head-to-head comparison of two vaccine introduction strategies (e.g. addition to EPI vs. catch-up campaigns) examining up to 10 cohorts over a 10 year period. In conclusion, this analysis will allow the user to project the economic and health consequences of vaccination in a specific country setting. However, users should cautiously interpret their findings. Estimated cost-effectiveness ratios should serve as just one barometer for policy-makers when evaluating the appropriateness new vaccine introduction.

914

THE TEMPORAL AND SPATIAL ANALYSIS ON THE EFFECTIVENESS OF DENGUE INTERVENTION ACTIVITIES IN TAINAN, 2007

Ching-Yi Chuo[1], Tzai-Hung Wen[2], Susan C. Hu[3], Kun-Hsien Tsai[1], Chwan-Chuen King[1]

[1]Graduate Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan, [2]Department of Geography, National Taiwan University, Taipei, Taiwan, [3]Department of Public Health, College of Medicine National Cheng Kung University, Tainan, Taiwan

Dengue hemorrhagic fever has become one of the major causes of pediatric deaths, particularly in endemic/hyperendemic countries. In many tropical and subtropical regions, climate conditions facilitate the viral transmission through local mosquitoes. The specific aims of this study are to analyze epidemiological data and to evaluate the two most important disease control strategies - source reduction and insecticide spraying. We used 1403 total laboratory-confirmed dengue cases occurred in Tainan from the 1st week of July 2007 to the 2nd week of January 2008 to construct models illustrating both the temporal and spatial effects of dengue interventions on the epidemic. To consider mosquito's life cycle, accumulated 3-week total numbers of dengue cases in the smallest administrative unit - Li in Taiwan were compiled. Data of intervention measures, including information of spraying insecticides, and source reduction, obtained from the work logs of Tainan City government, were served as the two independent variables. Finally, we used Li-specific case differences as the outcome measures (subtracting case numbers in the prior three weeks from the current three weeks) and spatial lag models

with k-nearest neighbors set as 4 to analyze the effects of the two control measures. The results showed source reduction significantly decreased dengue cases ($p<0.05$), particularly at early stage of the epidemic. When the case numbers increased strikingly, neither insecticide nor source reduction efficiently controlled the epidemic. Only until all the manpower and resources were allocated properly with more integrated efforts during the peak period of the epidemic, both spraying insecticide and source reduction demonstrated significant effectiveness in reducing number of dengue cases. In conclusion, our data revealed that timely and thoroughly city-wide intervention by source reduction of mosquito breeding sites once dengue cases were identified early through surveillance system was the most effective strategy at the initial stage of dengue season and/or epidemic period when case numbers were low. Prevention and control of the mosquitoes has to be implemented at the right place, time and most importantly based on the data analysis of spatial epidemiology. We believe our experiences can shed more light on to set up standard operation procedures and evidence-based dengue prevention/control-related public health policies.

915

ADVANTAGES OF A FIELD SITE CONSORTIUM

Mark E. Beatty, Harold S. Margolis, William G. Letson

International Vaccine Institute, Seoul, Republic of Korea

Dengue is a major public health issue in developing countries. Several dengue vaccines are in currently in development. The Pediatric Dengue Vaccine Initiative (PDVI) provided funding and/or technical support to researchers to establish field sites in anticipation of future trials. Non-funded sites also expressed interest to form what has been called the PDVI Field Site Consortium. The goal of the Consortium was to open a forum for dengue researchers to allow sharing of experience and research, in preparation for the sites to be used in clinical vaccine trials. There are currently 9 field sites in the Consortium in 8 countries: Managua (Nicaragua, 2004-9), Medellin (Colombia, 2008-9), Patillas (Puerto Rico, 2005-7), Colombo (Sri Lanka, 2008-9), Kolkata (India, 2008-10), Kampong Cham (Cambodia, 2006-8), Kamphaeng Phet (Thailand, 2004-7) Ratchaburi (Thailand, 2006-8), Long Xuyen (Vietnam, 2004-8) and Cebu (Philippines, 2009-10). We review the development of the Consortium, the meetings proceedings, and PDVI work plans to assess the advantages of Consortium participation. The first Consortium meeting was March 2007 in Bangkok, Thailand, where terms of reference were developed and agreed upon. Since that time, the Consortium assisted field sites by [1] developing standardized case report forms [2] sharing programs and databases for data collection in the field (e.g. using hand held computers); [3] development and sharing of epidemiologic databases; [4] sharing of a sophisticated laboratory database; [5] implementation of a Good Clinical Practice program; [6] negotiating with industry for lower unit prices on dengue test kits; [7] provision laboratory training to and sharing of testing protocols; and [8] development of mult-country protocols for additional dengue research. In conclusion, the Consortium has accelerated the development of field sites ideal for phase 3 clinical trials. Sharing of methods and skills has led to accelerated infrastructure, staff development and over-all reduced costs of field site development.

916

ECONOMIC IMPACT OF DENGUE OUTBREAK ON TOURISM

Mark E. Beatty

International Vaccine Institute, Seoul, Republic of Korea

Dengue fever is endemic in most tropical and subtropical tourist locations around the world. Dengue infection has been documented in returning traveler. We compared the volume of hotel room reservations collected routinely and prospectively by the World Tourism Organization to national surveillance data for four countries: Thailand, Vietnam, Brazil, and Puerto Rico. We categorized data from National Surveillance as either outbreak or non outbreak years based on National Public Health alerts for dengue outbreaks. The average cost of a room was multiplied by the total volume of rooms reserved, to determine the total lost revenues.

917

GLOBAL RISK AND BURDEN OF TRAVEL-ASSOCIATED DENGUE

Mark E. Beatty

International Vaccine Institute, Seoul, Republic of Korea

Dengue fever is endemic in most tropical and subtropical tourist locations around the world. Dengue infection has been documented in returning traveler. We conducted a systematic review of literature to determine the risk of dengue travels. We then multiple the volume of hotel room reservations collected routinely and prospectively by the World Tourism Organization to national surveillance by that average risk to estimate the total number of dengue infections occurring in travelers.

918

SEROEPIDEMIOLOGICAL EVALUATION AFTER THE OUTBREAKS OF DENGUE IN SOUTHERN TAIWAN AREAS WITH AND WITHOUT FREQUENT PAST EPIDEMICS

Tiger Z.R. Li[1], Scott Y.J. Huang[2], Hsiu-Pin Lin[1], Shu-Fang Chuang[3], Chuin-Shee Shang[1], Tzai-Hung Wen[4], Chi-Yin Ting[5], Chuan-Liang Kao[3], J.H. Huang[6], Chwan-Chuen King[1]

[1]Institute of Epidemiology, National Taiwan University, Taipei City, Taiwan, [2]University of Texas Medical Branch, Galveston, TX, United States, [3]Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University, Taipei City, Taiwan, [4]Department of Geography, National Taiwan University, Taipei City, Taiwan, [5]Institute of Health Policy and Management, National Taiwan University, Taipei City, Taiwan, [6]Centers of Disease Control, R.O.C, Taipei City, Taiwan

Residents lived below N 23 latitude in Taiwan have suffered dengue epidemics in past years. From 2007 to 2008, Tainan experienced the largest outbreak in 60 years caused by dengue virus serotype 1 (DENV-1) with total 1,498 confirmed cases. Recently, Kaohsiung had also faced several epidemics of dengue/dengue hemorrhagic fever (DHF) caused by multiple serotypes of dengue viruses (DENV-1, -2, and -3), with total 675 confirmed DF cases and 9 DHF cases from May 2009 to May 2010. To understand the magnitude of dengue virus infection and the correlations between infection and cases, we conducted two community-based seroepidemiological studies at one year post-epidemic period in Tainan (Aug. and Sept., 2008), and at mid-epidemic period in Kaohsiung, (Nov., 2009). Moreover, two school-based cohort studies were implemented to evaluate possible asymptomatic infection and the seroprevalence and seroincidence of dengue infection in children. Laboratory tests included dengue-IgM, dengue-IgG, and dengue-specific NS1 antibody. The preliminary results showed that seroprevalence of dengue-IgG in the communities of Kaohsiung with high dengue incidence in 2009 reached 33%, which was similar to the seroprevalence evaluated in the communities of Tainan with high dengue incidence in 2007. Further spatial analysis showed that the magnitude of dengue infection was higher and broader than the officially identified 2007 cases. On the other hand, the seroincidence in the communities in Kaohsiung during the intermediate phase of the 2009 epidemic was quite high (16%, 14/87). Interestingly, three dengue-seroincident persons were located in the same family about two months after the 2009 outbreak, and most dengue-IgM positive cases distributed quite locally in HisaoKang District. Furthermore, the seroprevalence of dengue-IgG in Tainan's schoolchildren was 4%, significantly lower than in adults indicated that Tainan had not become a dengue-endemic area yet. Since Kaohsiung has multiple-serotypes of DENV versus occasional outbreaks by a single serotype of DENV in Tainan are quite different, future efforts in serotyping by dengue-specific NS1 antibody, prospective epidemiological analysis, and quality research of risk communication will provide better clues on the roles of asymptomatic

persons and isolated dengue viruses in the series of chains of transmission and on establishing a community-based effective health education program as well.

919

ADULT DENGUE HEMORRHAGIC FEVER CASES WITH SECONDARY INFECTION FACILITATED VIRAL TRANSMISSION IN KAOHSIUNG, TAIWAN

Tsai-Ying Yen[1], Day-Yu Chao[2], Shu-Fang Chuang[3], Zheng-Rong Tiger Li[1], Tzai-Hung Wen[4], Chuan-Liang Kao[3], Chwan-Chuen King[1]

[1]Institute of Epidemiology, National Taiwan University, Taipei City, Taiwan, [2]Graduate Institute of Veterinary Public Health, National Chung-Hsing University, Taichung, Taiwan, [3]Clinical Virology Laboratory, NTU Hospital, Taipei City, Taiwan, [4]Department of Geography, National Taiwan University, Taipei City, Taiwan

Dengue viruses (DENV, genus Flavivirus, family Flaviviridae), one of the most significant emerging threats to global public health, have increased in geographic range, prevalence, and disease severity in recent years. DENV is mosquito-borne human pathogen which causes diseases varying from asymptomatic infection, dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). The clinical manifestations have been examined from genetic, immunologic, and virological aspects. To further elucidate the disease progress, 63 serum samples (paired or triple samples) of the 24 DENV-2 laboratory-confirmed dengue patients at different stages after the onset of illness, including 5 primary DF cases, 1 primary DHF case, 7 secondary DF cases, and 11 secondary DHF cases were collected and analyzed. The preliminary findings show that the mean of viral load at defervescence stage for secondary DF cases was higher than primary DF cases, and such trends lasted until the convalescent stage. In addition, the mean of viral load for secondary DHF patients was significantly higher than secondary DF cases at convalescent stage ($p = 0.022$), although DHF cases reached the peaking viral load at a later time point than DF cases. These findings on the longer duration and higher levels of viral load in DHF cases than in DF cases imply that more efficient prevention and control measures are required once DHF cases occur. Future research will focus on integrating temporal and spatial information with viral load and cytokines/chemokines analysis for better understanding the interplays between viral pathogenesis and immunopathogenesis at different stages of disease progress.

920

SPATIAL EPIDEMIOLOGY OPENS A NEW DIRECTION FOR GLOBAL CONTROL OF DENGUE HEMORRHAGIC FEVER - TAIWAN'S EXPERIENCE

Chwan-Chuen King[1], Tzai-Hung Wen[2], James C.Y. Chuo[1], Z.Y. Tiger Li[1], Hsiu-Pin Lin[1], Tsai-Ying Yen[1], Shu-Fang Chuang[3], Betty Wu-Hsieh[4], Day-Yu Chao[5], Chuan-Liang Kao[3]

[1]Institute of Epidemiology, National Taiwan University, Taipei City, Taiwan, [2]Department of Geography, National Taiwan University, Taipei City, Taiwan, [3]Clinical Virology Laboratory, NTU Hospital, Taipei City, Taiwan, [4]Institute of Immunology, National Taiwan University, Taipei City, Taiwan, [5]Graduate Institute of Veterinary Public Health, National Chung-Hsing University, Taichung, Taiwan

Dengue epidemics involving the more severe dengue hemorrhagic fever (DHF) have been expanding and increasing worldwide. In Taiwan, where dengue is not endemic, we have had an unprecedented opportunity to develop spatial-temporal indices in Kaohsiung, southern Taiwan that may help identify risk patterns for predicting large-scale epidemics and the emergence of DHF. To understand the epidemiological pre-conditions for severe DHF epidemics, we initiated spatial epidemiological analysis using geographical information system (GIS) to analyze the relationship between dengue fever (DF) cases and DHF cases. Through past large-scale epidemics in southern Taiwan, we found the following important spatially epidemiological characteristics. First, the distribution of DHF cases were more focalized than DF cases. Second, DHF cases occurred more likely in those areas with more cluster dengue cases. Third, epidemiological linkage to identify sources of the infection will provide better clues than residential areas to be targeted for minimizing DHF cases. Fourth, spatial-temporal diffusion of dengue cases clearly showed the correlation between large clusters and temporal increases of dengue case numbers. Fifth, DHF cases were associated with a longer lasting waves and/or waves with more intense transmission, despite low annual incidence in these areas. Finally, dynamic transmission of dengue cases, environment factors and abundance of mosquitoes plus effectiveness of control measures can be clearly analyzed and evaluated through an integrated manner. Virologic/serologic surveillance monitoring high risk populations in high risk areas is necessary after the occurrence of DHF or a large-scale dengue epidemic. We believe that the world can prevent or minimize severe epidemics of DHF by improving surveillance, implementing more integrated community-based prevention and control programs, using GIS to closely monitor tempo-spatial trends of dengue clusters and developing dengue vaccine.

921

FIRST RECORDED OUTBREAK OF DENGUE 3 IN SENEGAL

Ousmane Faye[1], Mawlouth Diallo[1], Yamar Ba[1], Oumar Ba[2], Amadou Alpha Sall[1]

[1]Institut Pasteur Dakar, Dakar, Senegal, [2]Direction de la Prévention Médicale, Dakar, Senegal

In October 2009, a 44 years old patient living in Turin (Italy) for 20 years and who stayed in the region of Louga in Senegal from the 3rd of July to the 3rd of October 2009 was suspected then diagnosed with Dengue in the hospital of Lazzaro Spallanzani in Rome. During this period, dengue RNA were identified in the National Reference Centre for Arboviruses in Paris in a second patient living in Marseille, France who also visited families in the region of Thiès, Senegal. Faced with these two cases, the arboviruses and viral haemorrhagic fever unit of Pasteur institute in Dakar took hold of the health authorities in Senegal for increased surveillance. Thus, between October 2009 and January 2010, a total of 696 samples were received in the laboratory. The suspected cases lived mostly in Dakar but also in other regions of the country. All samples received were tested by two techniques (RT-PCR in real time using specific primers for dengue and by ELISA for detection of immunoglobulin M. Of the samples tested, dengue was identified by at least one of the techniques in 196 patients. Sequencing of the E, NS5 and NS5/3'UTR of the DENV-3 strains isolated in Dakar at the last trimester of 2009 in order to analyze the phylogenetic relationship among them. Our sequences were aligned with homologous DENV-3 retrieved from Genbank. All the trees showed that DENV-3 strains circulating in Senegal were more closely related to strains isolated in Cambodgia in Asia. One death was recorded among the positive cases. Entomological surveys were conducted in and around homes of patients. These investigations allowed the identification of the vector *Aedes aegypti* in almost all the visited site, the identification of the RNA of dengue virus by RT-PCR real time and the isolation of the virus in a cellular system in three batches of mosquitoes. These results indicate the first time dengue virus type 3 appears in Senegal following the outbreak of dengue 3 recorded in Ivory Coast.

922

REDUCTION IN THE USE OF UNNECESSARY INJECTIONS IN MALARIA TREATMENT AMONG TERTIARY HEALTH INSTITUTIONS IN NIGERIA

Hamisu Hassan[1], Anne Ikwang[1], J. D. Akilah[2]

[1]United States Agency for International Development | DELIVER PROJECT, Abuja, Nigeria, [2]National Malaria Control Program, Abuja, Nigeria

Following an intervention to reduce unnecessary injections at health care institutions in Nigeria, United States Agency for International Development's Making Medical Injections Safer (MMIS) project conducted

a follow-up study in five selected tertiary health institutions to collect information before and after the interventions. The goal was to reduce the unnecessary use of injectable medications. The main objective was to measure whether training and policy changes have had any effect on the way these medications are used. After the MMIS health care workers were trained for the study, they assessed prescription patterns, collecting baseline information from August 2004 to January 2005 and follow-up data from August 2007 to January 2008. A secondary analysis of the data from this study shed light on the treatment of malaria in tertiary facilities. Between the two periods of study, standard first-line treatment in Nigeria shifted from injectable to non-injectable medication. When baseline and follow-up study results were compared, a significant reduction was noted in the use of unnecessary injections to treat malaria cases, which reflects the change in treatment policy. However, it should be noted that, in the outpatient department, 7% of malaria cases sampled at the follow-up were prescribed injectable medication for treatment. This is one of the highest injection rates of all the sampled diagnoses that do not usually require an injection. Given that the new malaria treatment protocol in Nigeria calls for non-injectable treatment_unless there are clear signs of *falciparum* resistance or treatment failure with artemisinin-based combination therapy (ACTs)_malaria cases are probably still receiving more injectable medications than necessary. This study indicates that, in Nigeria, there is still a long way to go in the fight against malaria. A surprisingly large number of malaria cases in that country are still being treated at tertiary health care facilities instead of the primary health care level.

923

THREE-LEVEL APPROACH FOR ENSURING THE QUALITY OF MEDICINES IN RESOURCE-LIMITED COUNTRIES

Victor S. Pribluda

United States Pharmacopeia, Rockville, MD, United States

The availability and use of medicines for the treatment of diseases are basic components of any health care system. Poor quality medicines may result in impaired therapies and jeopardize patients' safety, posing a serious threat to consumers and wasting significant financial resources. Assessing a product's compliance with the appropriate quality standards requires performing quality control (QC) analysis by the Official Medicine Control Laboratory (OMCL). However, OMCLs in resource-limited countries may be understaffed and not have appropriate financial support, lacking the necessary infrastructure, equipment, and personnel to perform QC analysis according to product specifications. Geographical barriers to accessing OMCLs, which tend to be located in large cities, impose an additional constraint. Because of the above limitations, a three-level approach for QC is proposed that could help resource-limited countries improve quality control within their regulatory framework. This approach encompasses the following: Level 1 analyses that include visual inspection of the package and label and physical inspection of the product; Level 2 analyses that utilize easy-to-use, simple, rapid, and cost-effective basic analytical methodology that can be implemented in the field to assess medicines quality; and Level 3 analyses that require the assessment of all critical quality attributes of a medicine via complete validated or compendial methodologies performed at the OMCL. The level to employ for a product at a given stage in the supply chain is based on risk-benefit analysis. By strategically implementing this approach throughout the supply chain, from procurement to patients' use, health authorities may increase the frequency and number of medicines tested within their limited financial and human resources, resulting in more effective control of the national pharmaceutical market and the quality of medicines.

924

MARKET ACCESS HURDLES FOR INNOVATIVE DISEASE CONTROL TOOLS

Helen V. Pates Jamet, Tessa B. Knox, Navneet Garg

Vestergaard Frandsen, Lausanne, Switzerland

Recent innovations in vector control tools include combination long lasting insecticidal nets (LNs), and insecticide incorporated Durable Lining which is installed on the walls of houses as a long lasting alternative to Indoor Residual Spraying (IRS). There is no specific category for either of these tools, which creates confusion amongst decision and policy makers regarding their utility. There are currently no appropriate testing guidelines for independent evaluation of these novel tools. As such, clear recommendations on efficacy - which are required by most vector control programs and donors as an external 'stamp of approval' - cannot be given, thereby delaying the deployment of these tools. Another example of a novel health product is an instant microbiological water purifier designed to reduce or eliminate the need for repeat interventions for effective use. Similar market access hurdles have been encountered with this tool due to the absence of a separate category, and hence most donors cannot even request this tool because policy is linked with products that require repeat intervention (e.g., chlorine). Despite continuous calls from the international community to expand the existing disease control toolbox, there remains a need to develop internationally accepted and clearly defined categories in order to maintain the motivation for innovation. Analysis of market access hurdles for innovative disease control tools needs to be addressed on both an international and local level. This is exemplified by the development of the LN category within the WHO Pesticide Evaluation Scheme (WHOPES) that established a platform for increased competition thereby enabling the significant and rapid scale-up in net coverage necessary to achieve the Millennium Development Goals.

925

DOGS AND THE SOCIAL EPIDEMIOLOGY OF VISCERAL LEISHMANIASIS: KNOWLEDGE, PERCEPTION AND PUBLIC HEALTH POLICY

Kevin J. Esch[1], Paulo Arruda[1], Annette O'Connor[1], Mary E. Wilson[2], Selma M. Jeronimo[3], Christine A. Petersen[1]

[1]Iowa State University, Ames, IA, United States, [2]University of Iowa, Iowa City, IA, United States, [3]Universidade Federale do Rio Grande do Norte, Natal, Brazil

Zoonotic visceral leishmaniasis (VL), caused by *Leishmania chagasi infantum,* was responsible for over 51,000 new cases of human VL between 1980 and 2003 in Brazil. Infected dogs are a major risk factor for human infection. Culling of seropositive dogs has been utilized in endemic areas in northeastern Brazil as a result. Despite these efforts, the seroprevalence of *Leishmania* infection in the region remains stable, suggesting this method of control is not effective. We developed a survey which was administered in interview format to adults in 270 randomly selected households in peri-urban areas of Natal, Brazil; an area endemic for VL with a canine seroprevalence rate greater than 25%. Sampling was based on Global Positioning System (GPS) random sampling selection. The purpose of this cross-sectional survey was to enhance the current understanding of environmental, social, and cultural factors which predispose to VL. This study quantitatively described demographics related to the risk of canine and human infection in this location and identified behavioral, cultural, and disease perceptions associated with a higher risk for *L. chagasi* exposure. We also assessed the perception of dogs as a risk for disease and the role of knowledge and perception in the social epidemiology of visceral leishmaniasis in Brazil. Lastly, the study evaluated the role of canines in household life and its effect on how the current culling program is perceived. This study identified multiple gaps in the knowledge of the study population regarding VL, and the current system of addressing canine disease in high risk populations. This information is being used to develop educational materials to address these areas of

need to prevent further spread of disease. Understanding the beliefs, priorities, and capabilities of the population at highest risk for VL will help develop disease prevention policies which will be more effective in this area of endemicity.

926

IMPACT OF ESTUARINE WETLAND DEGRADATION ON ZOONOTIC PATHOGEN TRANSMISSION

Karen Shapiro[1], **Patricia A. Conrad**[1], Woutrina A. Miller[1], Jonna A. Mazet[1], Wes W. Wallander[2], John Largier[3]

[1]One Health Center, University of California, Davis, Davis, CA, United States, [2]Land, Air and Water Resources, University of California, Davis, Davis, CA, United States, [3]Bodega Marine Laboratory, University of California, Davis, Davis, CA, United States

The loss of estuarine wetlands is a global phenomenon; 67% of wetland habitats along estuaries and coastal seas have been lost worldide. Rising sea levels resulting from global climate change are expected to lead to increased inundation and subsequent loss of estuarine wetlands in locations where the marshland cannot retreat inland due to urbanization or agricultural practices. Increased intensity of rainfall events predicted to occur as a result of climate change in some African countries could also contribute to greater nonpoint source fecal pathogen pollution into waterways and estuaries. The goal of our research was to evaluate the effect of coastal wetland degradation on contamination of estuarine and coastal waters with terrestrially derived zoonotic pathogens. *Toxoplasma gondii*, whose oocyst stages are shed in feline feces and infective to a diverse range of warm-blooded animals, including marine mammals and humans, was our model system. Experiments were conducted with surrogate microspheres and a specially designed flume that was deployed in vegetated and mudflat (non-vegetated) estuarine wetland habitats. The flume-in-field study design allowed for replication of experiments with specific hydrological parameters, while conducting the study within a natural estuarine environment using in-situ vegetation, substrate, and water. Compared to vegetated sites, significantly more surrogates were recovered from unvegetated mudflat habitats that represent degraded wetlands. Specifically, in Elkhorn Slough where a large proportion of otters are infected with *T. gondii*, erosion of 36% of vegetated wetlands to mudflats was calculated to increase the flux of oocysts by greater than two orders of magnitude. Total degradation of wetlands may result in increased *Toxoplasma* oocyst transport of six orders of magnitude or more. Our results provide novel insights into the consequences of changes in wetland habitats on the ecology of zoonotic infectious disease organisms in coastal marine ecosystems.

927

ADDRESSING HEALTH WORKFORCE CRISIS IN RURAL HEALTH FACILITIES THROUGH INTEGRATED INFECTIOUS DISEASE CAPACITY BUILDING OF MID- AND LOWER-LEVEL HEALTH PRACTITIONERS IN UGANDA

Milly Namaalwa

Infectious Disease Institute/IDCAP, Kampala, Uganda

The Integrated Infectious Disease Capacity Building Evaluation Program (IDCAP) is providing On-Site Support (OSS) to 36 rural health facilities spread out in all regions of the country. IDCAP conducted a training needs assessment (TNA) in the rural facilities of Uganda and noted that the already constrained workforce mainly comprising of mid level health Practitioners (Clinical officers and Registered Nurses) is challenged with inadequate and insufficient skills. They feel incompetent in offering care and management of infectious diseases among others. The package for OSS consists of Multi Disciplinary Training (MDT) of all the health workers involved in the patient care pathway (Medical officers to nursing assistants), Continuous Quality Improvement training and one on one coaching and mentoring of key staff who are involved in making daily clinical decisions on site. In the first IDCAP OSS field work conducted in April 2010 in 18 study sites, on average 8 clinical staff were mapped out for one on coaching and mentoring. Of these, 2 were Medical Clinical Officers, 2 Nursing Officers and 4 enrolled Nurses per site. On average, 30 health staff participated in the MDT training per site. This number appears to be big because most of the staff involved in patients' care are of lower cadre which often times can be attracted and retained in these rural health facilities i.e Enrolled Nurses, Nursing assistants, Health Educators and Inspectors, Records officers and counselors. The scope of work for non-professional personnel is tailored operationally to enable them perform less technical tasks such as counseling, adherence monitoring, home visiting, patient registration and maintaining flow client. All these aspects are addressed in the MDT sessions. In conclusion, participation of health facility staff in on-site activities i.e. mentoring, coaching, multi-disciplinary and Continuous Quality Improvement training could be one of the strategies towards improving individual and site performance given the staff shortages in rural health settings in delivering health services.

928

THE EFFECT OF IDCAP IMPLEMENTATION ON IMPROVEMENT OF HEALTH MANAGEMENT INFORMATION SYSTEMS IN RURAL UGANDA

Martin Mbonye

Infectious Disease Institute/IDCAP, Kampala, Uganda

Integrated Infectious Diseases Capacity Building Evaluation (IDCAP) is providing classroom and on-site training and continuous quality improvement of mid-level practitioners in 36 rural health centers in Uganda. IDCAP seeks to measure incremental impact and cost-effectiveness of integrated infectious diseases management. In order to measure the impact of site-level performance, IDCAP has had to strengthen the Ministry of Health (MOH) Management Information System (HMIS). This paper discusses the HMIS improvements that have been achieved due to IDCAP efforts. A Data surveillance system was set up to collect data on management of infectious diseases in Outpatients, Maternity, TB, HIV, Antenatal and Postnatal clinics. For Outpatients, the MOH Medical Form (MF5) was modified to capture in a coded format patients' demographics, history, examinations, diagnosis, treatment, referral and dispensed drugs. Health centers received a computer, printer, power supply and/or backup systems and modems for electronic reporting. Records personnel were trained in electronic data entry, analysis, reporting, quality assurance, facilitation and receive technical support. The rate of data completeness has improved to over 95%. Over 90% of clinicians using the new MF5 have found it timesaving and easy-to-use. Data losses and reporting timeliness have improved. Over 300,000 records have been collected during the four months of baseline. Using a computer, data entry, analysis and reporting is timely reducing from over seven days to less than two days. In conclusion, efforts to collect quality data to measure IDCAP site performance and impact has had a profound effect on improvement and strengthening of the HMIS in the rural health centers implementing IDCAP. The new MF5 and data surveillance system have improved quality of collected data, timely electronic entry, data analysis and reporting. It is also strengthening treatment and drug supply systems.

929

CARETAKERS KNOWLEDGE, ATTITUDES AND PRACTICES ABOUT ANTIBIOTIC USE IN CHILDREN IN A SETTING WHERE ANTIBIOTICS ARE AVAILABLE WITHOUT MEDICAL PRESCRIPTION

Lucie Ecker[1], Theresa J. Ochoa[2], Joaquin Ruiz[3], Martha Vargas[4], Luis J. Del Valle[5]

[1]Instituto de Investigación Nutricional, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Fundación Clinic para la investigación Biomédica, Barcelona, Spain, [4]CRESIB, Hospital Clínico/IDIBAPS, Barcelona, Spain, [5]Universitat Politécnica de Catalunya, Castelldefels, Spain

The misuse of antibiotics is associated with the emergence of resistant pathogens. It has been assumed that the availability of antibiotics without prescription has lead to higher self-medication rates. We surveyed caretakers in settings where antibiotics are available without prescription in order to determine their knowledge, attitudes and practices about antibiotic use. In a house-to-house survey performed in three peri-urban districts of Lima, 1201 caregivers were asked in a semi-structured questionnaire regarding antibiotic use in children less than five years of age. An educational leaflet about antibiotics and resistance concepts was explained after the interview. Only 3% of the caregivers identified correctly all antibiotics. Amoxicillin and cotrimoxazole were the best known drugs. Anti-inflammatory drugs like ibuprofen and naproxen were missed indentified as antibiotics in 45%. Only 11% of the caregivers knew the concept of antibiotic resistance. 83% of the children had used antibiotics before one year of age, 40% before 6 months of age. In 86% of the cases antibiotics were prescribed by a physician. Given the hypothetical case of common cold caretakers would seek medical advice in 76% of cases and 14% would use the drug of last prescription; 51 believed necessary to use antibiotics in this case. In the hypothetical case of non-dysenteric acute diarrhea, 87% would ask for medical advice and 4% would use the drug of last prescription; 65% of caregivers believed that an antibiotic was necessary in this case. In general, 84% of caregivers respected medical decision even if an antibiotic was not prescribed. In conclusion, knowledge about antibiotics and resistance is poor in this setting. However caregivers usually ask for medical advice when their children get ill and respect the medical recommendations. Despite this, infants are often and early exposed to antibiotics. Interventions to improve use of antibiotics should emphasize on physicians recommendations.

930

CULTURAL EFFECTS IN EPIDEMIOLOGY: FACTORS AFFECTING POLYPARASITISM RESEARCH IN COASTAL KENYA

Ruth Magtanong[1], Esther Atsabina Wanjala[2]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]DVBD/Case Western Reserve University/Emory Polyparasitism Project, Msambweni, Kenya

Epidemiological studies are impacted by low participation and high attrition rates. One frequently cited explanation is the lack of participant comprehension of the study's aims and objectives. In an evaluation of informed consent practices for an epidemiology study in Coastal Kenya, our findings provide support to this explanation; participants scored well on two standard measures of comprehension. However, semi-structured interviews revealed that these scores may not be a direct reflection of participant comprehension. Participant and fieldworker interviews revealed key issues impacting participation and comprehension. Specifically, these issues address participant perceptions of research processes and how these perceptions affect participant behavior. Factoring these issues in epidemiological studies may lead to greater participation and less attrition among research participants.

931

A SYSTEMATIC REVIEW OF THE SAFETY OF LICENSED LIVE ATTENUATED VACCINES

Nicole Thomas[1], Judith E. Epstein[2], Stephen L. Hoffman[3]

[1]National Capitol Consortium Pediatric Infectious Diseases Fellowship, Bethesda, MD, United States, [2]United States Military Malaria Vaccine Program, Naval Medical Research Center, Silver Spring, MD, United States, [3]Sanaria Incorporated, Rockville, MD, United States

The concept of vaccination with live organisms has been around for centuries, and was initially inspired by the practice of variolation in China, so named because infectious matter was taken from dried scabs or pustules from people with a milder form of the disease (Latin varius=speckled) and used deliberately to infect healthy recipients. Live attenuated vaccines today are derived mainly from cultures of living microorganisms that have been modified such that their virulent properties are diminished or eliminated, or which use closely related but less dangerous organisms to produce a broad immune response. Modern advances have facilitated the development of subunit vaccines, which rely on technology including the utilization of recombinant proteins. Nevertheless, immunization via live attenuated vaccines continues to be a highly effective means of generating protective immunity. Because our group is conducting a Phase 1 trial of a live metabolically active, non-replicating, radiation atttenuated *Plasmodium falciparum* sporozoite vaccine we reviewed the world's literature on the safety of live vaccines. There are currently US licensed vaccines against 25 pathogens with live attenuated vaccines available for 16 of these pathogens. The risks associated with receipt of a live vaccine include 1) reversion of virulence 2) risk of disease due to immunocompromised state 3) risk of transmission to others via shedding of the vaccine strain and 4) risk of autoimmune response to the vaccine. For each live vaccine, we review the method of attenuation, the associated safety issues, and the populations at highest risk for complications after exposure to the vaccine.

932

EVALUATING THE EFFECT OF CULTURAL COMPETENCE TRAINING ON PHYSICIAN-PATIENT INTERACTION AND OUTCOMES

Jayda Batchelder, Latha Rajan, Bonnie Nastasi

Tulane University, New Orleans, LA, United States

In the context of global health and migration, the need for cultural competence in health care is urgent. There have been some such initiatives in the sexual health field. However, the claims regarding outcomes have not been substantiated by formal research and evaluation. The current study hypothesized that the education and training of doctors in culturally appropriate practice has a positive effect on assessment, diagnoses, and treatment of their patients with sexual health concerns. Using data from a recent 5 year project (RISHTA) conducted in 3 low income communities in India, this study examined the effect of cultural competence training on the effectiveness of 44 trained (intervention) and control doctors. Doctors were trained in a holistic approach to treatment of men's sexual health concerns called Narrative Prevention Counseling (NPC). NPC incorporated biological, psychological, relational, and social-cultural factors. Coding methodology was developed for analysis of qualitative data. Coding was done independently by two researchers to control for inter-rater variability. The physicians who had received formal NPC scored significantly higher on total cultural competence as well as separately in assessment, diagnosis, and treatment regardless of case type. The independent samples t-test for Total Cultural Competence was significant ($t(42)=6.143$, $p<.001$, 95% CI:12.06-23.71). Separate t-tests for subcomponent scores revealed significantly increased scores for the individual subcomponents Construction 7.51 ($p<.001$, 95% CI:5.55-9.48), Deconstruction 4.91 ($p<.001$, 95% CI:2.71-7.11), and Reconstruction 5.56 ($p<.001$, 95% CI:3.03-8.08). Exploratory analysis suggested that practitioners tended to have higher cultural competence when treating non-STI cases in

comparison to STI cases. This study shows that a culturally competent curriculum has positive effects on medical practice. Further studies that explore the effects of such training on individual fields of healthcare as well as individual types of medicine are recommended.

933

BUILDING COMMUNITY PARTNERSHIPS FOR RESEARCH IN LIMPOPO PROVINCE, SOUTH AFRICA

Vhonani Netshandama[1], Jane R. Boissevain[2], Amidou Samie[1], Edgar Musie[1], Richard Guerrant[2], **Rebecca A. Dillingham**[2], Pascal Bessong[1]

[1]University of Venda, Thohoyandou, South Africa, [2]University of Virginia, Charlottesville, VA, United States

Rural populations are under-represented in biomedical research, particularly in sub-Saharan Africa. The Mal-ED project is a multi-country effort to evaluate the interactions of malnutrition and enteric disease in eight communities. Two of the sites for the project are in Africa, and one site, in rural South Africa, had not previously participated in international biomedical research. We describe the development of relationships with the rural communities which have agreed to participate in this important research. The University of Venda is a comprehensive university located in Thohoyandou, South Africa. Its mission includes a commitment to engagement with the surrounding rural communities. The University of Virginia's Center for Global Health has partnered with the University of Venda since 2003 to develop joint educational experiences for students and to pursue collaborative research. Since 2008, the partners have developed a close relationship with two communities through connections bolstered by projects led by teams of students from the two universities. These projects have been completed with significant input from community members and, in many cases, in response to their concerns. The projects have included the construction and revision of a slow sand filter to aid in purification of surface water for home use; a series of PhotoVoice projects to elicit the communities' perceived strengths and challenges relative to the provision of water and to health; geographic information system (GIS) mapping of the communities' water supplies and sanitation facilities; and a census. Participants in the PhotoVoice project were queried about their impressions. Many noted how "empowered" they felt by participation in the research process. One noted, "I felt so proud when I talked to the University people... when I saw that they were interested in our photos." In addition to the ties fostered by the student projects, community meetings of the research team with the traditional and civic leadership were essential and defined the communities' expectations which included: efforts to hire staff from the community, the formation of a community advisory board, and the development of a health education program. Informed community consent is not institutionalized in the way that individual consent is. Documenting strategies to promote and achieve community consent, particularly in rural areas, must be a priority.

934

CHALLENGES IN THE DEVELOPMENT, REVIEW AND APPROVAL OF MULTI-CENTER STUDIES OF MALARIA CONTROL AND ELIMINATION STRATEGIES

Ousmane A. Koita[1], Seydou O. Doumbia[1], Daouda Ndiaye[2], Davis Nwakanma[3], Amadou Diallo[1], Kalifa Bojang[3], David J. Conway[3], Sarah K. Volkman[4], Danny A. Milner, Jr.[4], Mard A.t. Muskavitch[5], Joseph Keating[6], Dyann F. Wirth[4], Donald J. Krogstad[6]

[1]University of Bamako, Bamako, Mali, [2]University Cheikh Anta Diop, Dakar, Senegal, [3]Medical Research Council Laboratories, Fajara, Gambia, [4]Harvard School of Public Health, Boston, MA, United States, [5]Boston College, Chestnut Hill, MA, United States, [6]Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States

Studies of tropical diseases at single sites face challenges similar to the studies of other diseases. However, the challenges of study development, review and approval are substantially greater for multi-center studies of malaria control and elimination strategies. The first challenge will be to develop consensus protocols because the results of these studies will be interpretable only if the protocols used are the same at each site (so differences in the results reflect differences among sites, not differences in study protocols). The second challenge is that the review and approval of protocols by multiple Institutional Review Boards (each of which is an independent entity with its own FWA Number) must be sufficiently independent to guarantee the integrity of the process, and sufficiently expeditious to ensure that the time for the initial review of individual protocols is ≤ 2-3 weeks, not months to years. Preliminary discussions with IRBs suggest the possibility of a two-stage strategy. The first stage would be simultaneous submission of the initial protocol to endemic area IRBs which would be responsible for recruitment strategies, supportive care and confidentiality because of their closeness to these culturally-related issues. The second stage (after the addition of those revisions to the protocol) would be the simultaneous submission of the revised protocol to all IRBs to examine the entire range of issues being studied. This approach, if successful has the potential to yield consensus protocols across substantial numbers of IRBs and to do so within a reasonable time frame.

935

INTERNET ACCESS AND BAND WIDTH AS FACTORS THAT LIMIT THE IMPROVEMENT OF MALARIA CONTROL AND PUBLIC HEALTH IN WEST AFRICA

Donald J. Krogstad[1], Ousmane A. Koita[2], Ayouba Diarra[2], Seydou O. Doumbia[2], Daouda Ndiaye[3], Christopher Whalen[4], Clarissa Valim[5]

[1]Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]University of Bamako, Bamako, Mali, [3]University Cheikh Anta Diop, Dakar, Senegal, [4]Research Data, Bethesda, MD, United States, [5]Harvard School of Public Health, Boston, MA, United States

In contrast to East and South Africa, which have benefited from an array of Internet cables in the Indian Ocean, Internet access and band width are less available and more expensive in West Africa. For example, a digital subscriber line (DSL) with a band width of 250 kb is typically $300-500 per month with a $500-1000 installation charge and a 2-3 month wait for installation. As a result, the Internet resources readily available elsewhere (on-line clinical trial and study software, molecular data bases, sample size and other statistical programs) are either less available or unavailable - e.g., on-line clinical study software cannot be used because it requires consistent Internet access and band widths ≥ 250 kb. The current situation in West Africa has resulted from a number of factors, which include: government monopolies, limited competition among other Internet service providers and years of delay in the provision of long-promised high-speed (fiber-optic) cable by international aid agencies. Limited Internet access

and insufficient band width are two of the most important and least recognized obstacles that will need to be addressed in order to improve malaria control and public health in West Africa.

936

EVIDENCE FOR NEGATIVE SELECTION ACTING ON THE GENE ENCODING THE MEROZOITE SURFACE PROTEIN-9 (MSP-9) IN *PLASMODIUM* SPP

Stella M. Chenet, M. Andreina Pacheco, Ananias A. Escalante

Center for Evolutionary Medicine and Informatics, Arizona State University, Tempe, AZ, United States

Merozoite Surface Protein 9 (MSP-9) is a vaccine candidate found in all malarial parasites species. In order to investigate the extent and maintenance of the genetic diversity found in MSP-9, we analyzed a total of 52 sequences of the following species: *Plasmodium falciparum, P. vivax, P. cynomolgi, P. knowlesi, P. coatney, P. fieldi* and *P. simiovale* and evaluated the signature of natural selection by estimating the difference between synonymous (dS) and non synonymous (dN) substitutions. The Z- test was used to determine the significance of such differences and, the null hypothesis was that the polymorphism was strictly neutral (dN=dS) at the 5% level. We found that in all orthologs of MSP-9 there was an excess of dS over dN, which suggest that this gene is under negative or purifying selection. We further explore how selection affected different regions of MSP-9 for *P. falciparum, P. vivax, P. knowlesi* and *P. cynomolgi*. We found that the C-terminal repetitive region of *P. cynomolgi* and *P. vivax* and the N-terminal region of *P. knowlesi* and *P. cynomolgi* were under negative selection. In contrast, evidence for positive selection was found in the N-terminal region of *P. falciparum* which may suggest that such polymorphism was important for the parasite in terms of avoiding recognition by the host immune response. In conclusion, we found evidence of negative selection acting on the MSP-9 protein in several *Plasmodium* species, this result may be indicative of the importance of this protein in the invasion of the red blood cell. However, the N-terminal region in *P. falciparum* shows a different pattern consistent with positive selection. Overall the effect of natural selection on the N-terminal region of the gene is different in *P. vivax* and *P. falciparum*. This observation implies that the genetic polymorphism observed in these human parasites may have a different interaction with the host immune response. Thus, the findings in one species cannot be directly translated into others.

937

PREGNANCY-ASSOCIATED MALARIA IN RELATION TO FREE FETAL HEMOGLOBIN IN MATERNAL BLOOD AND SUSCEPTIBILITY TO DEVELOP PREECLAMPSIA

Caroline Pehrson[1], Christentze Schmiegelow[1], Mayke Oesterholt[2], Daniel Minja[3], Pamela Magistrado[1], Stephanie Boström[4], Martha Lemnge[3], John Lusingu[3], Thor Theander[1], Stefan R. Hansson[5]

[1]Centre for Medical Parasitology at Department of International Health, Immunology and Microbiology, University of Copenhagen and Department of Infectious Diseases, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark, [2]Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, [3]National Institute for Medical Research, Tanga Medical Research Centre, Tanga, United Republic of Tanzania, [4]Department of Immunology, Wenner-Gren Institute, Stockholm University, Stockholm, Sweden, [5]Department of Obstetrics and Gynecology, Lund University, Lund, Sweden

Pregnancy-associated malaria (PAM) and preeclampsia (PE) are major causes of maternal and perinatal mortality in developing countries. PAM results in sequestration of parasites in the placenta, causing placental inflammation and impaired placental function. Placental malaria (PM) has been associated with an increased risk of pregnancy-induced hypertension (PIH), but the mechanisms linking PAM and PE are not known. PE is believed to progress in two stages. In stage 1 placental hypoxia causes oxidative stress and local inflammation. Stage 2 is a systemic syndrome characterized by endothelial dysfunction, multiorgan damage, and clinical symptoms. PE is associated with placental over-expression of hemoglobin (Hb) and increased concentrations of free fetal Hb (HbF), adult Hb (HbA), and oxidation markers in maternal plasma from early gestation. Free HbF may have a role in the etiology of PE by induction of oxidative stress in the placenta and leakage over the fetomaternal barrier to induce a maternal systemic oxidative stress. The objective of this study was to investigate the association of PAM with free HbF in maternal plasma and development of PE in a cohort of pregnant women in an area endemic of malaria. In a longitudinal prospective study in Korogwe, northeast Tanzania, 1000 pregnant women are followed throughout their pregnancy with clinical and parasitological examination and collection of blood samples at 4 antenatal visits, emergency visits, and at delivery. Plasma from women with positive rapid diagnostic test and women developing PIH, PE, or eclampsia will be analyzed for free HbF and compared to a healthy control group. Follow-up is expected to be complete in September 2009. Preliminary results will be presented. The interrelation between PAM, levels of free HbF in maternal plasma, and development of hypertensive disorders in pregnancy will be investigated. Measurements at multiple time-points will be used to investigate the longitudinal changes in plasma HbF throughout the pregnancy in relation to subsequent development of PIH, PE and eclampsia.

938

COPPER FLUORESCEIN COMPOUND CONFIRMS THE PRESENCE OF NITRIC OXIDE IN *PLASMODIUM FALCIPARUM* TROPHOZOITES

Graciela Ostera[1], Fuyuki Tokumasu[2]

[1]Georgetown University, Washington, DC, United States, [2]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

The presence of nitric oxide (NO)-derived reactive nitrogen species (RNS) in the food vacuole (FV) of *Plasmodium falciparum* parasites suggests that this free radical might play a role in the biochemical processes that take place in this organelle. Previously, we showed the presence of NO-derived RNS in the FV of 3D7 *P. falciparum* trophozoites using the fluorescence NO indicator DAR-4M AM. This fluorophore provided topographical information on the presence of NO-derived radicals, which was typically in close proximity with hemozoin deposits in trophozoites as well as in other blood stages of the malaria parasite. Although the function of NO in this organelle is not known, recent work suggests that NO can modulate heme speciation in isolated FVs exposed to NO generated *in situ.* There is, however, some uncertainty about the nature of the nitrated species present in the FV *in vivo*. Using anti-nitrotyrosine antibodies we observed nitrosation activity specifically localized in the FV envelope of trophozoite and gametocyte stage parasites, suggesting that nitrated radicals with strong oxidative potential, such as peroxynitrite (ONOO-), are present in this environment. The fluorophore we used previously, DAR-4M AM, does not react with NO directly but with N2O3, an oxidation product of NO in aqueous media. Consequenlty, we wanted to verify the actual presence of the NO radical in FVs. In this study we used a Cu(II)-complexed fluorescein compound, a new generation NO indicator that specifically reacts with NO over other RNS (N2O3, NO2, NO2-) present in biological systems. The Cu(II)-fluorescein compound has Ex/Em: 503-530 nm respectively, it is not toxic and safe to use in live cell imaging. *P. falciparum* 3D7 trophozoites treated with the Cu(II)-fluorescein compound displayed fluorescent signals in the food vacuole region of trophozoite stage parasites, lacking any noticeable fluorescence from the erythrocyte cytoplasm. These results confirm that NO radical is present in trophozoite food vacuoles and strongly suggests its involvement in FV biochemistry.

939

THE NEUROLOGICAL IMPACT OF *IN UTERO* EXPOSURE TO EXPERIMENTAL PLACENTAL MALARIA

Chloe E. McDonald, Karlee L. Silver, Keith Ho, Howard T. Mount, John G. Sled, Kevin C. Kain

University of Toronto, Toronto, ON, Canada

Placental malaria (PM) is associated with poor fetal outcomes including pre-term delivery, intrauterine growth restriction (IUGR) and low birth weight (LBW). LBW is correlated with abnormal cognitive and neurological development. Severe malaria infection in children has been associated with long-term cognitive impairments, including deficits in language, pragmatics and non-verbal functioning. Taken together, these studies suggest that PM may also impact neurological and cognitive development in the fetus. We hypothesize that offspring of mothers with PM will show behavioural impairment in tests of learning and memory, as well as abnormal neuroanatomical development, compared with control offspring from uninfected mothers. We will also examine a possible role of complement in PM-induced neurological impairments. Excessive activation of complement has previously been associated with adverse clinical outcomes in severe malaria and placental malaria. We hypothesize that mice lacking C5a-C5a receptor signaling will be protected from the neurological impairments associated with *in utero* exposure to PM. Using a mouse model of PM that replicates the pregnancy outcomes and placental pathology of human malaria, we examined the impact of PM on the neurological development of offspring. BALB/c wild type and C5aR-/- dams were infected at gestational day 13 with the rodent malaria parasite, *Plasmodium berghei* ANKA. Control animals were offspring brought to term by uninfected BALB/c and C5aR-/- dams. All offspring were reared by uninfected, healthy surrogate dams. Control and PM offspring were tested in a battery of behavioural tests including the open field test as a control to assess normal behaviour and the novel object recognition test to assess learning and memory. Following cognitive testing, neurological changes were examined using neural imaging data collected from magnetic resonance imaging. Volumetric analysis of imaging data was used to compare neurological development between offspring of PM infected mothers as well as control animals.

940

IDENTIFICATION OF PROTEINS ON THE SURFACE OF RETICULOCYTES INFECTED WITH THE RODENT MALARIA PARASITE *PLASMODIUM YOELII*

Elamaran Meibalan, Mary A. Comunale, Amy Cernetich Ott, Anand Mehta, James M. Burns, Jr.

Drexel University College of Medicine, Philadelphia, PA, United States

Malaria parasites replicate inside host red blood cells and export parasite proteins out of the parasitophorous vacuole to the erythrocyte plasma membrane. Previous studies have shown that multigene families of *Plasmodium* species encode antigenically variable proteins on the membrane of infected RBCs. However, non-variant parasite-encoded erythrocyte membrane proteins have also been suggested to be involved in import/export pathways, adherence to vascular endothelium and localization to reticulocyte-rich tissues. Such surface exposed parasite proteins represent potential vaccine targets as antibodies generated against them could block essential functions, enhance phagocytosis and/or promote complement mediated lysis of infected RBCs. The long-term goal of this project is to identify and characterize the subset of non-variant parasite-encoded proteins expressed on the surface of infected erythrocytes. Our initial approach was to identify parasite-encoded proteins associated with the infected RBC membrane that possessed an extra-cellular domain. *P. yoelii* 17X infected RBC membranes were isolated by surface biotinylation, neutravidin pull down and analyzed by immunoblotting. In this analysis, we identified a subset of putative surface exposed parasite-encoded membrane proteins including unusually high molecular weight proteins (>300 kDa). We also showed that membrane proteins around 30-35 kDa bind to recombinant mouse CD36, an endothelial cell receptor known to be critical in adherence of infected RBCs to vascular endothelium. In order to identify CD36-binding parasite proteins, we performed two dimensional electrophoresis of infected membrane proteins and mass spectrometry (LC-MS/MS) analysis which revealed four candidate *P. yoelii* proteins, such as PY06644(Enolase), PY00631(putative adhesin), PY01841(14-3-3 protein) and PY07648. These candidate antigens are currently being studied for their role in binding to mouse CD36 and in tissue specific sequestration of parasitized RBCs.

941

EXAMINING THE FINE SCALE STRUCTURE OF THE CROSSOVER LANDSCAPE IN *PLASMODIUM FALCIPARUM* BY HIGH-THROUGHPUT ALLELE SCANNING

Upeka Samarakoon, Allison Regier, Asako Tan, Scott J. Emrich, Michael T. Ferdig

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

Malaria is one of the most persistent and devastating parasitic diseases in humans. Understanding the mechanisms of genetic variation is fundamental to understanding the origin and spread of drug resistance and the influence of selection pressures on parasite virulence. Recombination generates new variants and regulates linkage disequilibrium (LD). Crossovers (CO) break up linkage, while non-crossover gene conversions (GC) weaken LD between nearby loci. Existing marker systems, e.g. microsatellites, lack the resolution necessary to capture the subtle, localized allele changes that occur as a result of COs and GCs which may have important phenotypic relevance. The detection of COs and precise measurement of their breakpoints and distribution is challenging - ideally relying on the observation of all four products derived from a single meiosis. This is not possible in malaria parasites; therefore an alternative is to directly scan the genomes of independently recombinant progeny at fine-scale resolution. We describe a method for SNP allele detection using massively parallel shotgun sequencing of two *Plasmodium falciparum* progeny clones resulting from a cross between a multi drug sensitive (HB3) and multi drug resistant line (Dd2), using the 454/Roche FLX sequencing platform. Of 24,599 high quality SNP markers identified, approximately 8000 allelic positions were used to differentiate between the progeny clones. A sliding window approach was used for the prediction of recombination break points (BPs). We reclassify eight previously misidentified COs as GCs, as well as discover previously unknown COs and GCs. Of 63 putative GCs, 36% span genes from polymorphic gene families associated with pathogenesis, cell adherence and rosetting. This global, high resolution genome view begins to clarify the types and locations of genetic exchange in the context of local sequence. This is a necessary step to reveal mechanisms, hotspots and gene functions that comprise the repertoire of genetic variation contributing to drug resistance, gene diversification and genome organization.

942

RHOPH COMPLEX FROM MOUSE MALARIA PARASITE INTERACTS WITH ERYTHROCYTE CALMYRIN

Satoru Takeo, Toyokazu Miura, Hitoshi Otsuki, Tatsuya Sawasaki, Yaeta Endo, Motomi Torii, Takafumi Tsuboi

Ehime University, Matsuyama, Japan

It is essential for blood parasitism of plasmodial merozoites to recognize hosts' erythrocytes and to establish parasitophorous vacuoles. The proteins from three apical microorganelles, i.e. micronemes, dense granules, and rhoptries, should be involved in this critical process. Here we focused on the high molecular weight rhoptry protein (RhopH) complex and searched the recombinant mouse erythrocyte proteome for interaction partners of *Plasmodium yoelii* (Py) RhopH. The PyRhopH extracts was prepared by freeze-thawing schizont-rich pellet and labeling with monoclonal antibody (mAb) #32 specific to RhopH3 component of the complex. We then

referred to the study of human erythrocyte proteome (Blood 2006: v108: p791) to select mouse orthologs. A total of 441 biotinylated recombinant mouse proteins were prepared by wheat germ cell-free synthesis to conduct the PyRhopH interaction partner(s) analysis. Among positives the strongest signal was detected with calcium binding protein calmyrin (also called CIB-1). The specificity of this interaction was confirmed by dose-dependence test, mAb species selection, and divalent cation-chelator (EDTA) inhibition. Finally, the detection of calmyrin in mouse erythrocytes suggested that the *in vitro* results above reflect *in vivo* interaction between PyRhopH complex and mouse calmyrin.

943

FREQUENT SEVERE THROMBOCYTOPENIA IN CASES OF *PLASMODIUM VIVAX* MALARIA FROM THE PERUVIAN AMAZON

Salomon Durand[1], Cesar Cabezas[2], Andres G. Lescano[1], Paul C. Graf[1]

[1]Naval Medical Research Center Detachment, Lima, Peru, [2]Instituto Nacional de Salud, Lima, Peru

Thrombocytopenia has been described as a complication related to *Plasmodium falciparum* malaria and less frequently to vivax malaria, but recent reports in South America have described a frequent association between thrombocytopenia and vivax malaria. To determine the prevalence of low platelet counts in the Peruvian Amazon we studied 224 vivax malaria cases enrolled in a study on the efficacy of antimalarial drugs and 179 afebrile, thick smear-negative subjects from the same area. A platelet count per µl of blood was performed in each participant using a Giemsa-stained thin blood smear obtained by finger stick. Follow-up platelet counts were made in a small, convenience sample of malaria cases who were receiving the prescribed antimalarial treatment, chloroquine plus primaquine. Counts of <100,000 platelets/µl were defined as low. There was no statistical difference in the average age of malaria positive and negative groups (26.0±16.3 vs. 25.2±16.7, p=0.637), but malaria cases were more frequently male (122/224=54% vs 77/179=43%, p=0.022). The geometric mean of platelet counts was 74,919/µl (23,000 - 198,000) and 138,071/µl (66,000 - 293,000) in the malaria and non-malaria groups, respectively. Low platelet counts were clearly more frequent in malaria cases than in the group without malaria (176/224=79% versus 17/179=9.5%, p<0.001), and 12.2% (28) of malaria cases had a severely low platelet counts (<50,000/µl) versus zero in malaria-negative subjects (p<0.001). Among malaria cases, higher parasitic density (parasites per µl of blood) correlated significantly with lower platelet counts (Spearman Rho=-0.342, p<0.001). After commencement of malaria treatment, platelet recovery was rapid and occurred in all 10 studied cases by 48 or 72 hours. In conclusion, thrombocytopenia is very frequent in cases of *P. vivax* malaria in Peru, but it resolves within a few days after antimalarial treatment.

944

FY^A AND FY^B DUFFY ANTIGEN POLYMORPHISM AFFECTS BINDING OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN (PVDBP) AND BLOCKING BY SPECIFIC ANTIBODIES AND CHEMOKINES

Lior J. Greenberg[1], Xianli Jia[1], Brian T. Grimberg[1], John H. Adams[2], Peter A. Zimmerman[1], Christopher L. King[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]University of South Florida, Tampa, FL, United States

The Duffy antigen has two known polymorphisms, Fya and Fyb, which differ by only one amino acid in the N-terminal region. This region, which is known for its critical role in *Plasmodium vivax* invasion of human red blood cells (RBC) by use of the malarial parasite protein *P.vivax* Duffy binding protein (PvDBP), is also important for chemokine binding, notably for interleukin-8 (IL-8) and RANTES. IL-8 has been shown to inhibit invasion of *P.knowlesi*, which also uses the Duffy antigen to invade human RBC. Previous results suggest that PvDBP shows poorer binding to RBC expressing Fya compared to Fyb. We tested the hypothesis that blocking antibodies directed to PvDBP and the chemokine IL-8 and RANTES preferentially block binding of PvDBP to erythrocytes expressing either the Fya or Fyb polymorphism. Binding of Fya and Fyb genotyped human erythrocytes to PvDBP was measured by flow cytometry. All variants of PvDBP (AH, O, P, C and SAL1) consistently showed an average of 40-50% lower binding to Fya erythrocytes. Blocking the Duffy antigen with polyclonal rabbit antibodies or pooled human antibodies with binding inhibitory activity showed 2-4 fold greater inhibition of PvDBP binding to Fya than Fyb erythrocytes. While both Fya and Fyb erythrocytes had similar PvDBP binding and inhibition of RANTES, Fya binding to PvDBP was significantly inhibited by IL-8 (at concentrations similar to levels identified in patients with acute malaria) to a greater extent than Fyb erythrocytes. In conclusion, these results show that in addition to the inherent decreased binding abilities of the Fya Duffy antigen polymorphism, binding inhibitory antibodies as well as chemokines may be more effective in blocking parasite invasion of RBC expressing Fya versus Fyb. These studies suggest that testing of a vaccine targeting PvDBP will be more effective in populations predominantly expressing the Fya allele.

945

DOES ENHANCED DETECTION AND ANALYSIS OF MALARIA INFECTIONS IN UMBILICAL CORD BLOOD SAMPLES EXPLAIN LOW BIRTH WEIGHT AND FETAL ANEMIA IN NEWBORNS OF THE KASSENA-NANKANA DISTRICT OF NORTHEASTERN GHANA?

David J. Fryauff[1], Andrea J. McCoy[1], Frank Atuguba[2], Mary I. Amati[2], Abraham R. Oduro[2], Thomas Anyorigiya[2], Victor Asoala[2], Timothy Awine[2], Kwadwo A. Koram[3], Abraham Hodgson[2], Samantha Richards[1], Francis Nkrumah[3]

[1]Naval Medical Research Center, Silver Spring, MD, United States, [2]Navrongo Health Research Centre, Navrongo, Ghana, [3]Noguchi Memorial Institute of Health Research, Accra, Ghana

Even with bednet use during pregnancy by >50% of women, and Fansidar-based intermittent preventive treatment (IPTp) that reached 82% of pregnant women, fetal anemia (FA, Hb <12.5g/dL) characterized 21.5% of live births in the rural Kassena-Nankana District of northeastern Ghana and 18% of these newborns were underweight (LBW, <2500g). Malaria microscopy identified *Plasmodium falciparum* infections in only 47 of 2258 (2.1%) umbilical cord bloods (2.1%) but these few positives revealed borderline statistical associations with first born status (P = 0.12), LBW (P = 0.09), and FA (P = 0.12). We hypothesized that a more sensitive screening of umbilical cord bloods could validate these associations and explain the high levels of LBW and FA observed. We further conjectured that higher rates of fetal malaria infection, and mutations in malaria genes associated with Fansidar resistance might reveal additional associations that could be acted upon to improve health and survival. This retrospective study aimed to determine the rate of infectivity and molecular characterization of *P. falciparum* in cord/heel blood taken from >2200 live births enrolled in an IRB-approved cohort study during March 2006-March 2007. Detection of *P. falciparum* in filter paper blood blots was based on nested PCR targeting the 18S ribosomal RNA. Genetic diversity within the *P. falciparum* PCR-positive samples was determined by analysis of *msp1*, *msp2* and *glurp* (glutamine-rich protein). Based on single nucleotide polymorphisms (snp), we determined the frequency of *dhps* and *dhfr* point mutations associated with Fansidar resistance in the parasitemias. Results are discussed in light of birth characteristics, seasonality, malaria protection, and demography.

946

MIXED-SPECIES INFECTIONS AND ALTERNATION OF INFECTIONS OF *PLASMODIUM VIVAX* (PV) AND *P. FALCIPARUM* (PF) IN LOW TRANSMISSION AREAS: HOW DO CYTOKINES MODULATE INFECTION DYNAMICS?

Rosalynn L. Ord[1], Jean Hernandez[2], OraLee H. Branch[1]

[1]New York University, New York, NY, United States, [2]Universidad Nacional de la Amazonia Peruana, Iquitos, Peru

Low transmission settings provide the opportunity to study single infections, the dynamics of mixed infections and the alternation of species detected over a period of just weeks ('cryptic infections' likely mixed but only one species detected any given time). Longitudinal detection in Zungarococha, Iquitos, from 2003-2009 show that individuals with mixed infections are presenting at clinics more frequently than those with single infections (83.3% and 73.5%, respectively; p=0.15) and individuals with mixed infections report a recent febrile episode more frequently than those with PV or PF infections (73.0% and 65.4%, respectively; p=0.33). When PCR diagnosis is considered, 33.9% of mixed infections are first observed at the clinic versus 8.9% of single infections (p<0.001). This suggests that individuals with mixed infections seek treatment much earlier compared to individuals with single infections. To further elucidate the host response during these parasite interactions, the levels of ten cytokines from single, mixed and alternating infections are being determined. Preliminary data show that all microscopy-positive individuals, regardless of infection type, show an increase in IL6, IFNa, IFNg, TNF, and CRP, compared to uninfected individuals. The main difference observed between single and mixed infections is that single show a trend towards a reduction in the level of IL4 and no difference in the level of IL8, compared to controls. However, the trend in mixed infections is no difference in IL4 but a reduction in IL8. Single-species and mixed-infections tend to also have higher IL12p40 but positive infections (both PV and PF) from alternating infections show a reduction in this specific cytokine. This might be evidence that alternating species allows parasites to modulate their environment with the greatest effect that protects the parasites and the host when multiple species are co-circulating. Determining human cytokine responses are essential to understanding host-parasite interactions and the mechanisms that underlie disease severity in these regions of co-infection.

947

DISTRIBUTION OF DEVELOPING GAMETOCYTE STAGES IN THE BLOOD OF MALARIA-INFECTED PATIENTS

Regina Joice[1], Jacqui Montgomery[2], Belinda Morahan[3], Karl B. Seydel[4], Danny A. Milner[5], Kim C. Williamson[3], Terrie E. Taylor[4], Curtis Huttenhower[1], Matthias Marti[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi, [3]Loyola University, Chicago, IL, United States, [4]Michigan State University, East Lansing, MI, United States, [5]The Brigham and Women's Hospital, Boston, MA, United States

In the context of malaria eradication, the transmission stage (gametocyte) is of high importance, as it is the only red blood cell stage that leads to the propagation of the disease. It is well noted that mature Stage V gametocytes, but not developing gametocyte stages (I-IV), are observed in the circulating blood. It has been hypothesized that developing stages are sequestered in tissues, but we assume that prior to sequestration, the sexually committed ring stage is also present in the circulating blood. Based on published microarray data, we developed a constrained regression model to predict the distribution of three populations in a given sample: early gametocytes (rings), developing gametocytes (I-IV), and mature gametocytes (Stage V). The application of the model to published patient blood sample microarray data predicts that a subset of these samples have early and/or late gametocytes. It also predicts a depletion of developing gametocytes in all samples, as we would expect, given our hypothesis that these stages sequester. We have used this model to define a small set of stage specific markers for qRT-PCR that accurately predict the distribution of these three gametocyte subpopulations in a sample. The assay was validated using *in vitro* gametocyte development time courses, and was subsequently applied to a large number of peripheral blood samples from malaria-infected patients in Blantyre, Malawi. For a subset of these samples in which an enrichment of a gametocyte marker was observed, IFA analysis was performed for confirmation of the results. The present work sheds light on the dynamics of gametocyte disappearance and reappearance in the peripheral blood over the approximately seven to ten day sexual development. The tools presented here will facilitate prediction of patient transmission potential over the course of drug treatment, and with different combination therapies. Furthermore, the application of this methodology to tissue samples could be used to investigate the sequestration of developing gametocytes.

948

MECHANISM OF BINDING OF *PLASMODIUM FALCIPARUM* TO VASCULAR ENDOTHELIUM IN CEREBRAL MALARIA

Vijaya L. Soma[1], Abhai Tripathi[2], Joel Janes[3], Monique Stins[2], David Sullivan[2], Joseph D. Smith[4]

[1]School of Medicine, University of Washington, Seattle, WA, United States, [2]Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD, United States, [3]Department of Global Health, University of Washington, Seattle, WA, United States, [4]Seattle Biomedical Research Institute, Seattle, WA, United States

There are an estimated 200,000 cases of cerebral malaria annually in Africa alone; mortality remains high at 20%, even with treatment. Hypoxia and tissue injury occur when large amounts of *Plasmodium falciparum*-infected erythrocytes (IE) adhere to vascular endothelium in the brain and prevent normal blood flow. Adherence of IEs is mediated by *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), a large family of clonally variant adhesion proteins encoded by *var* genes. Approximately 60 *var* gene copies are present in every parasite genome. It is unclear which PfEMP1 proteins and host receptors mediate binding to human brain microvasculature endothelial cells (HBMEC), though some studies suggest that intercellular adhesion molecule-1 (ICAM-1) is a possible host receptor. To study this binding, we took long-term parasite cultures that express a mixture of different *var* genes and a parasite line that was previously enriched for ICAM-1 binding and repeatedly panned these IE on HBMEC. We conducted initial and post-panning quantitative binding assays using common endothelial cell receptors to assess for changes in binding characteristics and applied real time-PCR to catalog *var* genes that are newly-induced in the panned lines. Initial binding assays revealed that IE that bound more highly to recombinant ICAM-1 than to other common human ligands also showed two to three times greater binding to HBMEC. Panning of non-ICAM-1 binding parasite lines on HBMEC led to upregulation of an ICAM-1 binding phenotype. We found that the genes *var6, var13, and var19* were consistently upregulated in HBMEC-panned IE. Our results suggest that a limited subset of *var* genes that encode ICAM-1 binding and potentially other phenotypes were consistently upregulated in *Plasmodium falciparum*-infected erythrocytes panned on primary human brain microvasculature endothelial cells.

949

KNOWLEDGE AND COMPLIANCE OF HEALTH WORKERS TO MALARIA RAPID DIAGNOSTIC TEST GUIDELINES IN RUKUNGIRI DISTRICT, WESTERN UGANDA, 2010

Ronald N. Tusiime, Fred Wabwire-Mangen, Robinah Najjemba

Makerere University College of Health Sciences, School of Public Health, Kampala, Uganda

Rapid diagnostic tests (RDTs) for diagnosis of malaria were introduced in November 2008 and rolled out to all health centre (HC) of level II in Rukungiri district.. Before their introduction, health workers used

presumptive clinical means for diagnosing and treating malaria. RDTs are cost effective and reduce errors in treatment if the prescribers comply with the test results. This study assessed the knowledge and compliance of health workers to RDT guidelines for diagnosis and management of malaria in Rukungiri district. The study used both cross sectional and retrospective designs. Twenty three HCs from the entire district were selected by simple random sampling. A total of 460 cases at the selected HCs that had an RDT test for malaria were selected by simple random sampling from patient registers. Data abstracted included client particulars, RDT results and drugs prescribed. Key informant interviews were conducted with health workers. Data was analysed using SPSS version 11.0. All health workers who manage patients had adequate knowledge of RDT use. Of all 460 patients seen at health facilities, two-thirds (69%) were managed in compliance with the national RDT guidelines. 79% of the patients tested with RDTs had fever, cough, flu and headache. Patients who were more likely to receive and anti-malarial were: those that presented with fever (OR: 56.1, 95% CI: 28.5 - 110); had a positive RDT result (OR: 401, 95% CI: 142.9 - 1000); The mean compliance score for all health facilities was 10.19 (SD 1.3), median score was 10 (Range 8.0, 15). In conclusion, knowledge of health workers of the RDT guidelines was high; however, compliance was still sub-optimal. District health authorities should actively monitor the health workers' implementation of the RDT policy guidelines and respond to unmet needs.

950

USING RAPID DIAGNOSTIC TESTS (RDTS) AS SOURCE OF MALARIA PARASITE DNA FOR MOLECULAR ANALYSES

Deus Ishengoma[1], Sudi Lwitiho[1], Rashid Madebe[1], Ola Persson[2], Nyagonde Nyagonde[1], Lasse Vestergaard[2], Ib Bygbjerg[2], Martha Lemnge[1], Michael Alifrangis[2]

[1]National Institute for Medical Research, Tanga, United Republic of Tanzania, [2]University of Copenhagen, Copenhagen, Denmark

Malaria prevalence has declined drastically in most parts of Tanzania possibly due to efficient treatment regimens (using artemisinin combination therapies), use of insecticide treated bed nets and climatic changes. Although surveillance of malaria to detect the occurrence of hidden parasite reservoirs and parasite resistance to antimalarial drugs using sensitive molecular tools is important, it increasingly becoming difficult to obtain malaria positive samples from studies, such as drug efficacy trials since they cannot be performed to the same extent as before. This study was conducted to establish if sufficient DNA could be successfully extracted from rapid diagnosistic tests (RDTs) and used for various molecular analyses. Serial dilutions were made (in triplicates) from a hyper-parasitaemic sample (131,260 parasite/µl) with whole blood donated by uninfected donor and blotted on RDTs (ParaHIT®f, Span Diagnostics - India) according to manufacturers' instructions. DNA was extracted using chelex method from the three sets of RTDs (either immediately or after storage for one month at room temperature with/without silica preservatives). The extracted DNA was amplified using a nested PCR for *Plasmodium* species detection. Used RDTs (n=29) obtained from ongoing projects in Korogwe and Muheza districts were analysed to confirm initial results. Furthermore, since false negative RDTs possibly pose a problem in malaria endemic areas in transition, the detection of parasite infections among negative RDTs will be examined during a malaria survey in Muheza district planned in May 2010. DNA was successfully extracted and amplified from the three sets of RDTs. For all sets of dilutions, the minimum detection limit of malaria parasites by PCR was down to 1 parasite/µl. DNA was also extracted and successfully amplified from all 29 positive RDTs received from ongoing studies in Muheza and Korogwe districts. Results of false negative RDTs in the malariometrict survey to be conducted in May 2010 will be obtained and discussed. This study has shown that DNA can be successfully extracted from RDTs and used for detection of malaria parasites by PCR. Since the Ministry of Health and Social Welfare is planning to introduce RDTs in all health facilities, availability of used RDTs will provide an alternative source of DNA for genetic studies such as surveillance of parasite resistance to antimalarial drugs.

951

QUALITY ASSURANCE OF MALARIA RAPID DIAGNOSTIC TESTS (RDT) AND ITS IMPLICATION FOR CLINICAL MANAGEMENT OF MALARIA

Vachagan Harutyunyan

Malaria Consortium, Kampala, Uganda

In 2010 WHO changed its guidelines for the treatment of malaria to recommend parasitological confirmation in all patients suspected of malaria before treatment is started. However perceptions of reliability of malaria diagnostic tests among the frontline health care workers in resource limited settings present considerable obstacles for the effective rollout of parasitologically confirmed malaria treatment. We assessed the implications of simple measures for monitoring accuracy of malaria RDT-s in the field settings on malaria treatment practices at the outpatient clinics in Kibondo district, Tanzania. We determined the sensitivity and specificity of the malaria RDT in patients diagnosed with suspected malaria at the outpatient clinics, by comparing the results of the RDT at the clinics with microscopy done by an independent blinded laboratory technologist. Results of this investigation were shared with the clinical staff and confirmation of all patients with suspected malaria by RDT was recommended. Clinical practice was monitored by comparing the proportion of laboratory confirmed versus presumptive malaria diagnoses during the period from June to December 2007. Malaria slides and RDT's from 513 patients diagnosed in September and October 2007 in the clinics were compared. Specificity and sensitivity of RDTs used were 92.2% and 93.7% respectively. Over-diagnosis of malaria at the clinics was common and 68.8% (95% CI: 61.8% - 75.8%) of children under five years old diagnosed with malaria had a negative malaria slide. After sharing these results with the clinicians, malaria diagnoses based on laboratory confirmation in the clinics rose within two months from 38% of total malaria diagnoses to 64%. Implementing field based malaria RDT quality control measures has important implications for the clinical performance of the frontline health care providers in resource limited settings. Clinicians in rural African settings need to be convinced by disseminating and discussing evidence about RDT's from their own and similar settings in order to improve diagnostic and prescription habits.

952

DEVELOPMENT OF FIELD USABLE RDT KITS FOR SIMULTANEOUS DIAGNOSIS OF MALARIA AND PREGNANCY

Seung Hee Han, Bo Choi, **Hyeonsuk Kim Kim**, Young Ho Choi

Access Bio Inc., Monmouth Junction, NJ, United States

Malaria is a serious parasitic disease which now occurs in more than 90 countries worldwide. In Africa, 30 million women living in malaria-endemic areas become pregnant each year. For these women, malaria is a threat both to themselves and to their babies, with up to 200,000 newborn deaths each year as a result of malaria in pregnancy. Pregnant women are more likely infected to malaria as pregnancy reduces a woman's immunity to malaria, making her more susceptible to malaria infection and increasing the risk of illness, severe anemia and death. Maternal malarial infection has serious consequences for the unborn child-it is highly to cause miscarriage, stillbirth, premature delivery and low birth weight, increasing risk of infant death. The simultaneous detection of malaria and pregnancy will help the management of complications, appropriate treatment of medication and may decrease the risk of prenatal mortality. Recently, we have developed three different types of rapid, one step qualitative assay for detecting malaria and pregnancy simultaneously. The test procedure is very simple. Just add 5 µl of whole blood to the sample well in the device and followed by adding 2 drops of assay buffer in the assay buffer well. The assay is rapid (<20 min), easy to

operate, inexpensive, portable, and have no special storage requirements and it is suitable for field situation with limited healthcare facilities and limited trained medical care staffs. *CareStart*™ Malaria /Pregnancy (pLDH/HCG) combo Test detects malaria *Plasmodium* species and pregnancy. The *CareStart*™ Malaria /Pregnancy (HRP2/HCG) combo Test detects the infection of malaria *P. falciparum* species and pregnancy. The *CareStart*™ Malaria /Pregnancy (pLDH/HRP2/HCG) combo Test distinguishes malaria *P. falciparum* species from other *Plasmodium (P. vivax, P. malaria and P. ovule)* species. These RDT kits detect less than 30 parasites/µl and as early as 1week of pregnancy.

953

SHARING GOOD PRACTICES! MCTA MICROSCOPY EXTERNAL QUALITY ASSESSMENT PROGRAM FOR CLINICAL TRIALS IN AFRICA

Bernhards R. Ogutu[1], James Sande[2], Pamela A. Onyor[2], Rita Biden[3], Erick J. Wagar[2], Douglas S. Walsh[2], Fred N. Binka[3], Peter B. Obare[2]

[1]INDEPTH Network/Malaria Diagnostics and Control Center of Excellence, Kenya Medical Research Institute/United States Army Medical Research Unit-Kenya, Accra, Ghana, [2]Malaria Diagnostics and Control Center of Excellence, Kenya Medical Research Institute/United States Army Medical Research Unit-Kenya, Kisumu, Kenya, [3]INDEPTH Network, Accra, Ghana

Microscopy remains the "gold standard" in clinical trials evaluating candidate drugs, biologics, and devices developed to prevent, treat, or diagnose malaria. Failure to perform malaria microscopy competently compromises the quality of clinical trials. False-positive diagnosis of malaria has a negative effect on the outcome of prophylactic clinical trials because the protective efficacy of the candidate product will be underestimated. Several methods of perform malaria microscopy exist but the benefits of harmonized diagnostic methods are great in multi-centre clinical trials. To standardize microscopy techniques across sites, the Malaria Clinical Trial Alliance (MCTA) supported the Kenya Medical Research Institute/U.S. Army Medical Research Unit-Kenya Malaria Diagnostics and Control Center of Excellence (MDCoE) to train microscopists and establish an external quality assessment (EQA) program in sub-Saharan Africa. The outcome was to improve competence and create sustained proficiency in preparation and staining of standardized quality slides, parasite detection and quantitation and species identification. Quarterly the sites sent 5 slides to MDCoE prepared in their laboratories with the results obtained by their microscopists. In turn MDCoE sent 5 standardized, validated slides to the sites which were read by site microscopists and results sent back. The slides provided by MDCoE consisted of negative slide, mixed species slide, and Pf parasites of differing densities. Concordance was high for parasite detection and *P. falciparum* species identification but lower for uncommon species (Pm, Po). Concordance on parasite density estimation was variable between sites and across different densities. However, all sites showed improved performance over time. In conclusion, the EQA program has demonstrated sustained proficiency by the microspcopists at trials sites and valuable lessons learned which can inform the design and implementation of future EQA programs.

954

NOVEL MOLECULAR DIAGNOSTIC TARGETS FOR THE DETECTION OF *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX*

Allison Demas[1], Naomi W. Lucchi[2], Jenna Oberstaller[3], Jeremy DeBarry[3], Ganesh Srinivasamoorthy[3], Deborah Sumari[4], Abdunoor M. Kabanywanyi[4], Leopoldo Villegas[5], Ananias A. Escalante[6], S. Patrick Kachur[1], John W. Barnwell[1], David S. Peterson[3], Jessica C. Kissinger[3], Venkatachalam Udhayakumar[1]

[1]Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Atlanta Research and Education Foundation, Atlanta, GA, United States, [3]University of Georgia, Athens, GA, United States, [4]Ifakara Health Institute, Dar-es-Salaam, United Republic of Tanzania, [5]Asociación Civil Impacto Social, Tumeremo, Bolivarian Republic of Venezuela, [6]Arizona State University, Tempe, AZ, United States

Accurate and rapid diagnosis of malaria is crucial for saving lives. Molecular diagnostic tools are important for detecting sub-clinical infections and differentiating between Plasmodium species. In spite of available genomic information for Plasmodium *falciparum* and P. vivax, the majority of PCR-based methods rely on the 18S RNA gene. While this gene has been a good target for diagnostic assays, it has certain limitations in multiplex assay platforms to detect mixed infections. Here we describe new DNA targets for the species specific detection of P. *falciparum* and P. vivax. Genomic sequences were obtained from PlasmoDB for P. *falciparum* and P. vivax and scanned with the de novo repeat finding tool RPTScout to identify potential repetitive diagnostic sequences. Candidates were screened via sequence similarity searches for vector contamination, human genome sequence, species-specificity, size and copy number, and aligned to assess conservation suitable for amplification. Repeat candidate R364 was identified in the P. *falciparum* genome and R47 was identified in the P. vivax genome. We evaluated these targets for use in PCR assays and compared them to the Snounou method, an 18S RNA gene-based nested PCR. Primers designed to candidate R364 specifically identified P. *falciparum*, and primers to candidate R47 detected only P. vivax. Both assays showed similar limits of detection to the Snounou method with a single, as opposed to nested, PCR reaction. Using known quantities of laboratory-cultured parasites, we were able to detect DNA in concentrations as low as 1parasite/µl. The method was further validated using microscopically-determined P. vivax samples from Venezuela (n=96) and P. *falciparum* samples from Tanzania (n=91). In comparison to Snounou nested PCR, preliminary data show P. vivax candidate R47 had 95.8% sensitivity and 100% specificity, and P. *falciparum* candidate R364 had 97.8% sensitivity and 100% specificity.

The novel PCR method presented here is a valuable alternative molecular diagnostic method for specifically detecting P. *falciparum* and P. vivax.

955

POINT-OF-CARE DIAGNOSIS OF MALARIA BY FLUORESCENCE LOOP MEDIATED ISOTHERMAL AMPLIFICATION

Naomi W. Lucchi[1], Allison Demas[2], Jothikumar Narayanan[3], Abdunoor M. Kabanywanyi[4], Deborah Sumari[4], Patrick S. Kachur[2], John W. Barnwell[2], Venkatachalam Udhayakumar[2]

[1]Atlanta Research and Education Foundation, Atlanta, GA, United States, [2]Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Parasitic Diseases Branch, Centers for Disease Control and Prevention, Chamblee, GA, United States, [4]Ifakara Health Institute, Dar-es-Salaam, United Republic of Tanzania

Molecular diagnostic methods can complement existing tools to improve the diagnosis of malaria. However, they require good laboratory infrastructure thereby restricting their use to reference laboratories and research studies. Therefore, adopting molecular tools for routine clinical use in malaria endemic countries will require simpler molecular platforms. The recently developed loop-mediated isothermal amplification (LAMP)

method is relatively simple and field-amicable. In this study, we attempted to improve this method for malaria diagnosis by combining the use of a fluorescence signal (SYBR Green) as the readout and the use of a portable isothermal amplification platform with real-time fluorescence reading capability. We refer to this as the RealAmp system. Published genus-specific primers were used to test the utility of this system. Amplification was carried out at 63oC for 90 minutes with the portable reader set to collect fluorescence signals at 1 minute intervals. DNA derived from different species of malaria was used for the initial characterization. Clinical samples of *Plasmodium falciparum* were used to determine the sensitivity and specificity of the RealAmp system compared to a nested PCR method. In addition, directly boiled parasite preparations were compared with a conventional DNA isolation method. The RealAmp system was found to be simple and allowed real-time detection of DNA amplification. The time to amplification was generally less than 60 minutes. All four human malaria parasites were detected. This method detected *P. falciparum* in clinical samples with 98.9% sensitivity and 100% specificity compared to a standard nested PCR method. In addition, this method consistently detected *P. falciparum* from directly boiled blood samples (up to 40p/mL). This RealAmp system is a simple field usable tool when compared to traditional PCR methods for the molecular diagnosis of malaria and potentially other infectious agents. This tool can be used in health care settings and field laboratories and may be valuable for field use in malaria elimination programs.

956

IMMUNOSENSOR DESIGN FOR DIAGNOSIS OF MALARIA USING THE RHOP-3 RHOPTRY PROTEIN OF *PLASMODIUM SP*

Tobili Y. Sam-Yellowe, Susan Moreno-Molek, Lakshmi S. Kaza, Pavan Maheswaram, Shravan Singh Thakur, Tongmin Wang, Alan Riga

Cleveland State University, Cleveland, OH, United States

Plasmodium falciparum causes the most lethal form of malaria resulting in approximately 500 million clinical cases annually and 1-2 million deaths in children under the age of 5 years. Currently, diagnosis of malaria depends on microscopy and is augmented by serological tests and rapid diagnostic tests. Etiological confirmation of the correct *Plasmodium* species remains challenging and molecular diagnostic tests such as the polymerase chain reaction (PCR), is not available in many malaria endemic areas. Reliable, specific and sensitive point-of care (POC) diagnostic methods that are of low cost are needed to facilitate rapid single step diagnosis of malaria. Diagnostic tests with prognostic value will increase treatment efficiency and reduce the development of drug resistant parasites in malaria endemic areas. The structure of the Rhop-3 rhoptry protein of *Plasmodium* sp. was investigated using the thermal analytical techniques of dielectric analysis (DEA), and differential scanning calorimetry (DSC) for potential immunosensor development for malaria diagnosis. The physical-chemical properties of the protein were analyzed. Antisera from patients in malaria endemic areas are highly reactive with recombinant Rhop-3 in an enzyme-linked immunosorbent assay (ELISA). The Rhop-3 protein is also secreted by the parasite and present in plasma during infection. The electrical conductivity (ps/cm) of Rhop-3 in aqueous solutions revealed bulk conductivity properties and was more reliable than surface sensor conductivity values by DEA. A change in Rhop-3 conductivity was observed in the first 5 minutes and again at 20 min in an overlay of ionic conductivity versus time, indicating a distinct electrical profile for Rhop-3. The electrical response of Rhop-3 varied by frequency at different sampling temperatures in tan delta versus frequency overlays. Calorimetry revealed unbound water crystallizing and melting at appropriate temperatures. An additional endotherm and corresponding exotherm at 14 J/g is probably related to the interaction of the protein and a "type" water. The latter may reflect specific amino acid water interactions which can be used to monitor the Rhop-3 protein in the process of immunosensor design.

957

AUTOMATED REAGENT-LESS DIFFERENTIATION OF *PLASMODIUM FALCIPARUM* FROM *P. VIVAX* IN HUMAN THIN FILM BLOOD SMEARS WITH FTIR MICROSPECTROSCOPY

Kenneth A. Puzey, Bryan Holmes, Adam Hunt, **McKale E. Santin**

Quantaspec, Inc., Burlington, VT, United States

In malaria cases species of infection affects course of treatment. Differentiation of *P. falciparum* from *P. vivax* by RDTs requires multiple antibodies, which increases test costs. Furthermore, RDTs are subject to reader error. Speciation by visual microscopy is dependent on the skill and availability of an expert microscopist. The objective of this study was to evaluate the utility of FTIR microspectroscopy for automatic reagent-less differentiation of *P. falciparum* from *P. vivax* infected human red blood cells. Geimsa-stained thin film blood smear slides were analyzed in this study. For *P. falciparum* positive controls, 240 slides with ring stage *P. falciparum* were prepared from culture. For *P. vivax* positive controls, 80 clinical *P. vivax* slides were collected and verified by expert microscopy (EM) and Polymerase Chain Reaction (PCR). For negative controls, 40 slides with *Salmonella*-infected blood (prepared from culture) and 40 uninfected human blood slides were prepared. Infrared spectra were measured from a small area of each slide (~12 micronsx12microns) usually containing only one red blood cell. Algorithms were written to differentiate red blood cells infected with *P. falciparum*, red blood cells infected with *P. vivax*, red blood cells infected with *Salmonella* and uninfected red blood cells based on their infrared spectra. Algorithms were tested by cross-validation. For *P. falciparum* sensitivity was 98.4 to 100% and specificity was 97.7% to 100% (95% CI). For *P. vivax* the sensitivity was 95.4% to 100% and the specificity was 98.8% to 100% (95% CI). These results suggest that FTIR spectroscopy may be useful for automated reagent-less differentiation of malaria infection. In high throughput settings spectroscopy testing may be lower cost because it does not require consumables.

958

AUTOMATED REAGENT-LESS DIFFERENTIATION OF *PLASMODIUM FALCIPARUM* FROM *P. VIVAX* IN HUMAN THIN FILM BLOOD SMEARS WITH FTIR MICROSPECTROSCOPY

Kenneth A. Puzey, Bryan Holmes, Adam Hunt, **McKale E. Santin**

Quantaspec, Inc., Burlington, VT, United States

In some regions of the world malaria parasite drug resistance is present in 50% of cases. Unfortunately, tests to determine drug resistance are not clinically available forcing health ministries and doctors to make difficult choices. An economical clinical test for drug resistance would enable doctors to administer less expensive chloroquine to susceptible cases, lowering health costs and slowing the spread of resistance to newer drugs. The objective of this study was a preliminary evaluation of the utility of FTIR microspectroscopy for differentiating red blood cells infected with drug resistant strains and drug susceptible strains of *Plasmodium falciparum.* 120 Geimsa-stained thin film blood smear slides were prepared with drug-susceptible ring stage *P. falciparum* from culture (40 slides strain 3D7, 40 slides strain 1776, 40 slides D6), and 120 Geimsa-stained thin film blood smear slides were prepare with drug-resistant ring stage *P. falciparum* from culture (40 slides strain HB3, 40 slides strain Dd2, 40 slides strain 7G8). Negative controls included 40 Geimsa-stained thin film blood smear slides of uninfected human blood as well as human blood infected with *Salmonella* from culture (40 slides). Additional *P. falciparum* negative controls included 80 clinical Geimsa-stained *P. vivax* slides collected and verified by expert microscopy (EM) and Polymerase Chain Reaction (PCR). Infrared spectra were measured from a small area of each slide (~12 microns x12microns) typically containing only one red blood cell. Algorithms were written to differentiate red blood cells infected with *P. falciparum*, red blood cells infected with *P. vivax*, red blood cells infected with *Salmonella* and uninfected red blood cells based on their infrared spectrum. Algorithms were tested by cross-validation. For drug

susceptible strains, sensitivity was 97% to 100% and specificity was 98.7% to 100% (95% CI). For drug resistant strains sensitivity was 97% to 100% and specificity was 98.7% to 100% (95% CI). These results suggest that FTIR spectroscopy may be useful for automated reagentless differentiation of drug resistant and drug susceptible strains of *P. falciparum* in thin film blood smears. This capability could enable more cost effective case management and reduce the spread of drug resistance to newer drugs.

959

THE EPIDEMIOLOGY OF MALARIA IN HOUSTON, 2000 - 2009

Paola Carrasco, Wayne X. Shandera

Baylor College of Medicine, Houston, TX, United States

The objective of this study was to study the epidemiology of malarial cases in Houston. All malaria reports in Houston, 1/03 and 12/09, were reviewed for demographics, site of acquisition, species, prophylaxis taken, and treatment. 115 cases from Harris County over 7 years (range 6-35/y) showed a sex ratio (M:F) of 1.96 (n=83) and mean age on presentation of 32.8. Speciation in 93 (81%) cases included 65 with *Plasmodium falciparum* (70%), 20 with *P. vivax* (22%) and 9 with *P. malariae* (10%), (1 concomitant F-M). The sex ratio for *P. falciparum* cases was 1.36. The sex ratio for *P. vivax* cases was 4.33 (n = 16, P = 0.24, Fisher's exact). The number of *P. vivax* cases decreased for the study interval (P = 0.08, test for trend). Age of onset did not differ by species. 72 of 85 with data acquired the infection in Africa. 55% (26/47 with data) of *P. falciparum* cases were reported by Nigerians. Isolates from all Central American cases speciated as *P. vivax* and 7 were from Africa. Prophylaxis was not was taken by 27/53 (51%) and was taken incompletely by 11 (21%). Prophylaxis was used exclusively by Africans (26/45, Africans vs 0/8, non-Africans, P = 0.003, 1-tailed Fisher's exact). Africans more often reported a history of travel (52/70 vs 8/17, P = 0.03, chi square) while Central American cases developed after immigration. Treatment data were available for 72 (63%), with popularly used regimens being doxycyline (23), followed by mefloquine (15) and chloroquine (12), all given most often with primaquine. In conclusion, malaria is reportedly frequently by community hospitals in Houston. Most *P. falciparum* cases are Africans who present after returning from travel to homelands. Less common *P. vivax* cases occur among non-Western sub-Saharan Africans and also Central Americans who immigrate. Attention should be directed toward completing prophylactic regimens among travelers and providing standardized therapeutic regimens (artemesinin combination therapy) currently not available in the United States.

960

POPULATION PHARMACOKINETICS AND PHARMACODYNAMICS OF CHLOROQUINE IN MALAWIAN CHILDREN WITH UNCOMPLICATED *FALCIPARUM* MALARIA

Fraction K. Dzinjalamala, M. Laufer, P.C. Thesing, O. Nyirenda, J.L. Reid, D. Hughes, K.B. Barnes, J.L. Wiesner, S.A. Ward, T.E. Taylor, C.V. Plowe

Blantyre Malaria Project, Blantyre, Malawi

Antimalarial pharmacokinetic variability can influence the therapeutic outcome in individuals treated for malaria. The aim of this study was to characterize the population pharmacokinetics and pharmacodynamics of chloroquine in Malawian children who received a standard dose of chloroquine alone or in combination azithromycin, atovaquone-proguanil or artesunate, for uncomplicated *falciparum* malaria. Concentration-time measurements obtained from 400 children who received the standard oral doses of chloroquine were pooled to create a dataset containing concentration data points spanning multiple dosing occasions. Pharmacokinetic parameters of chloroquine were estimated by nonlinear mixed effects modelling. We will report the mean population estimate of apparent clearance (CL/F), volume of distribution (V/F) and the variability in these parameters. An assessment of the relationship between the posterior individual estimates of pharmacokinetic parameters, *in vitro* pharmacodynamic parameters and important covariates will also be reported.

961

ACCURACY OF GESTATIONAL DATING IN AN OBSERVATIONAL PREGNANCY MALARIA COHORT IN MALAWI: AN ULTRASOUND DEMONSTRATION PROJECT

Blair Wylie[1], Patricia Mawindo[2], Osward Nyirenda[2], Linda Kalilani-Phiri[3], Mwayi Madanitsa[3], Redson Kuyenda[2], Albert Malenga[2], Gladys Membe[3], Bonus Makanani[3], Philip Thesing[4], Miriam Laufer[5]

[1]Division of Maternal-Fetal Medicine, Massachusetts General Hospital, Boston, MA, United States, [2]The Blantyre Malaria Project, University of Malawi College of Medicine, Blantyre, Malawi, [3]University of Malawi College of Medicine, Blantyre, Malawi, [4]The Blantyre Malaria Project and the Center for Vaccine Development, School of Medicine, University of Maryland, Blantyre, Malawi, [5]Center for Vaccine Development, School of Medicine, University of Maryland, Baltimore, MD, United States

Malaria during pregnancy is associated with an increased risk for low birth weight (<2500 g). Distinguishing infants that are premature (< 37 wks) from those that are growth-restricted (<10%) requires accurate assessment of gestational age (GA). Where ultrasound (U/S) is routinely accessible, antenatal sonographic confirmation of GA is more accurate than menstrual dating alone. Our goal was to pilot the feasibility and utility of adding U/S to an observational pregnancy malaria cohort. Research staff (1 MD, 3 clinician midlevels, 1 RN) from The Blantyre Malaria Project underwent an intensive 1-week U/S training in 07/09 led by a visiting perinatologist. Didactic lectures and observed hands-on instruction focused on acquiring images of the fetal biparietal diameter, abdominal circumference, and femur length using a portable SonoSite S180 U/S machine. Following 3 months of additional practice, fetal biometric images were obtained from subjects enrolled in an ongoing malaria cohort. After electronic image review by the perinatologist, a best U/S estimate of GA was determined using standard biometric tables, and compared with menstrual dates. Of 100 pregnancies imaged, 15 women had unknown last menstrual periods; U/S therefore established GA. Of the remaining 85, U/S re-dated the pregnancy in 23 (27%) secondary to a discrepancy between menstrual and U/S dates (discrepant if > 7 days difference when imaged < 20 wks; > 14 days if 21-28 wks; > 21 days if > 28 wks). U/S demonstrated the GA to be less than anticipated in 10 and more than anticipated in 13. Images were obtainable 93.6% of the time (89.1% in the 1st 50 scans; 98% in the 2nd 50). Comparison of U/S with postnatal assessment of GA (Ballard) awaits the deliveries. In conclusion, U/S should be strongly considered in prospective malaria studies with obstetric endpoints. Reliance on menstrual dating may lead to misclassification of infants as premature (earlier due date by U/S) or as growth-restricted (later due date by U/S).

962

EXPLORING THE ANTIMALARIAL EFFECT OF ANTIRETROVIRAL PROTEASE INHIBITORS IN A COHORT OF HIV-INFECTED WOMEN RESIDING IN MALARIA-ENDEMIC AREAS OF SUB-SAHARAN AFRICA

James S. McCarthy[1], Alice S. Butterworth[1], Kim A. Porter[2], Joe Eron[2], Grant Dorsey[3], Shahin Lockman[4], Sibtain Rahim[5], Fred Sawe[6], Doug Shaffer[6], Abraham Siika[7], Mina Hosseinipour[8], Elizabeth Stringer[9], Judith Currier[10], Tsungai Chipato[11], Robert Salata[12], Tina S. Skinner-Adams[1], Steven R. Meshnick[2]

[1]Queensland Institute for Medical Research, Brisbane, Australia, [2]University of North Carolina, Chapel Hill, NC, United States, [3]University of California, San Francisco, CA, United States, [4]Harvard School of Public Health, Boston, MA, United States, [5]Abbott Laboratories, New York, NY, United States, [6]Walter Reed Project, Kericho, Kenya, [7]Moi University, Eldoret, Kenya, [8]University of North Carolina Project, Lilongwe, Malawi, [9]Centre for Infectious Disease Research, Lusaka, Zambia, [10]University of California, Los Angeles, CA, United States, [11]University of Zimbabwe, Harare, Zimbabwe, [12]Case Western Reserve University, Cleveland, OH, United States

The distribution of malaria and HIV overlap in many regions of the world. Available evidence indicates that co-infection results in increased severity of malaria and increased viral replication, potentially accelerating the course of immunosuppression, and increasing HIV transmission. Some antiretroviral protease inhibitors (PI) have been demonstrated to show a moderate antimalarial effect. To explore the clinical relevance of this we examined the incidence of malaria in a cohort of HIV-infected women as a sub-study of ACTG 5208, a study investigating the impact of previous Nevirapine exposure for PMTCT on treatment outcomes. The substudy included subjects in 6 malaria-endemic study sites across sub-saharan Africa who received alternate antiretroviral regimens, one containing the PIs lopinavir/ritonavir, the other a NNNRTI, nevirapine. Data and serum samples were collected at scheduled visits over 48 weeks. Incidence of malaria was determined by clinical diagnosis, parasitologic diagnosis by blood slide, laboratory assay for the presence of *Plasmodium falciparum* HRPII in plasma by ELISA, or LDH by Rapid Diagnostic Test (RDT), and by increase in antibody titer to the recombinant *P. falciparum* proteins AMA1 and MSP1-19. 2,971 serum samples were collected from 447 HIV infected patients. 80 subjects had a clinical or parasitologic diagnosis of malaria, among whom sera were available for 66. Interim laboratory analysis of 2,509 samples indicated a very low incidence of laboratory-confirmed malaria, with a total 7 samples positive for HRPII, in 5 individuals, 2 of whom had a concordant clinical or parasitologic diagnosis. Sera from a further 2 individuals tested positive for non-*falciparum* malaria in RDTs. Among 26 subjects with available sera and a clinical, parasitologic or HRPII antigenemia-based diagnosis, a rise in malaria-specific antibody titer was uncommon (1, 1 and 2 respectively). The lower than expected incidence of malaria in this population impeded exploration of the potential protection against malaria conferred by an antiretroviral regimen containing PIs with an antimalarial effect.

963

POPULATION, BEHAVIORAL AND ENVIRONMENTAL DRIVERS OF MALARIA PARASITEMIA IN THE DEMOCRATIC REPUBLIC OF CONGO

Jane Messina[1], Michael Emch[1], Steve M. Taylor[2], Kashamuka Mwandagalirwa[3], Jeremie Muwonga[4], Atua Matindii[5], Antoinette K. Tshefu[3], Steven R. Meshnick[2]

[1]University of North Carolina Department of Geography, Chapel Hill, NC, United States, [2]University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC, United States, [3]Ecole de Sante Publique, University of Kinshasa, Kinshasa, The Democratic Republic of the Congo, [4]Laboratoire National de Reference SIDA et IST, Kinshasa, The Democratic Republic of the Congo, [5]Programme National de Lutte contre le Paludisme, Kinshasa, The Democratic Republic of the Congo

The prevalence of malaria in the Democratic Republic of Congo (DRC) is among the highest in the world, but there are limited data on individual and ecological risk factors for parasitemia. Real-time PCR assays were employed to detect *Plasmodium falciparum*, ovale and malariae parasites in dried blood spots collected from adult respondents to the 2007 Demographic and Health Survey, a representative sampling of the population. Using these data and the extensive questionnaire results from each of the approximately 8000 respondents, spatial statistical analyses and multilevel modeling were employed to estimate parasite prevalence and define individual and ecological drivers of infection. Of 7,778 respondents included in our models, 2,208 (28.4%) were parasitemic, with prevalence ranges from 0 to 82% between geographically-defined survey clusters across the DRC. *P. falciparum* infections were the most prevalent species, either as monoinfection (91%) or co-infecting with *P. malariae* (6%) or *P. ovale* (<1%). Using ArcGIS, parasite prevalence for all 300 GIS-linked survey clusters were input to create a comprehensive interpolated map of malaria prevalence in the DRC. Younger males were at higher risk for infection ($p < .0001$), while wealthier and more educated people who own bed nets were at lower risk ($p < .05$). Two measures of conflict were negatively associated with malaria risk ($p < .05$), suggesting that provision of antimalarial drugs by humanitarian groups in these areas may be contributing to lower prevalence. Overall, this study demonstrates the need for surveillance systems for infectious diseases that use a representative population-based sampling scheme.

964

PLASMODIUM VIVAX MEROZOITE SURFACE PROTEIN-3 GENETIC DIVERSITY SUGGESTS ALLELE SPECIFIC IMMUNITY IN LOW-TRANSMISSION COMMUNITY COHORT

Ulziijargal Gurjav, Patrick Sutton, Jean N. Hernandez, OraLee Branch

New York University, New York, NY, United States

Plasmodium vivax merozoite surface protein (MSP) 3 is a molecular epidemiologic marker for discerning highly polymorphic genome composite of the malaria parasite. In the present study, we sought to determine the overall *P. vivax* genetic diversity and to detect if the genotypes present in individual's first infection versus second infection of two infections occurring within 1.5 year were different. We included 65 individuals sampled in active case detection where there were two *P. vivax* microscopy infections detected from each individual, giving a total of 130 infections from Peruvian Amazon cohort collected during 2003 to 2008. *P. vivax* infection was detected by using nested PCR and then employed for restriction length polymorphism analysis using HhaI and AluI enzymes, respectively. Turnover from the first of pair-infection symptomatic case into second of pair-infection being asymptomatic was observed in 38/130 cases whereas turn over from asymptomatic to asymptomatic was observed in 28/130 cases. Each turnover cases had the highest number of prior infections before enrollment of the study, 18/38 and 20/28, respectively. Interestingly, turnover from an asymptomatic

case into symptomatic case was 14/130 and therefore showing that there is double the chance of being asymptomatic on the second infection if the person was asymptomatic at the prior infection. Across all first or second of pair-infections, MSP3 A_6_4 genotype was the most frequently observed. However in addition to previously known *P. vivax* genotypes in Peruvian Amazon community (as reported previously), there are 54 new *P. vivax* genotypes were detected. Individuals who had A_6_4 in the first and second infection were significantly less likely to be febrile in their second infection (5/6). High frequency of different genetic types in the first infection versus the second infection in the infection-pairs suggested a limitation of genetic diversity in the second infection. Moreover, individuals with a repeating A_6_4 infection were less likely symptomatic suggests allele specific immunity within this community.

965

UNIVERSAL COVERAGE OF ITNS: RELATIONSHIP BETWEEN HOUSEHOLD OWNERSHIP AND USE - TANZANIA

Manoj P. Menon[1], Mac Otten[2], Steven S. Yoon[1], David M. Gittelman[1], Robert D. Newman[2]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]World Health Organization, Geneva, Switzerland

Insecticide-treated bednets (ITNs), a mainstay of malaria control efforts, offer both personal protection against malaria infection and community-wide protection given high levels of community use. ITN distribution schemes which aim for universal coverage (UC) may result in a higher level of use and broader community impact than targeted campaigns. However, UC has not been clearly defined and may reflect ITN ownership rather than use. We explored the relationship between ITN ownership and use through analysis of survey data from Tanzania to help inform ITN quantification for UC campaigns. The 2007-2008 Tanzania HIV/AIDS and Malaria Indicator Survey (THMIS), utilizing a two-stage sample design, is a nationally representative household survey which collected data on household ownership and use of ITNs. We defined universal household ITN "use" as the percentage of households in which all residents slept under an ITN the night prior to a survey. We created new "ownership" variables to yield proportions of households which possessed 2 ITNs per household, 3 ITNs per household, 1 ITN per 2 residents, and 1 ITN per sleeping room. We compared these household ITN "ownership" indicators with household ITN "use". A total of 8,497 households were interviewed. All residents slept under an ITN in 970 (11%) of households. Among households possessing either 2 or 3 ITNs, 34% and 32% of households contained residents in which all members slept under an ITN respectively. If ownership was defined as 1 ITN per 2 residents or 1 ITN per sleeping room, a larger percentage of all residents in a household slept under an ITN, 53% and 44% respectively. In conclusion, to achieve the transmission reduction potential of ITNs and consequent community protection, scale up of ITN distribution is needed. Among the currently used definitions for UC of ITNs, data from Tanzania suggest that 1 ITN per 2 residents best correlated with every member of a household sleeping under an ITN. These results can assist program managers in quantifying needs and distributing ITNs during UC campaigns.

967

SEASONALITY AND AGE SPECIFIC MALARIA MORBIDITY IN DIDIENI, DISTRICT OF KOLOKANI, MALI

Alassane Dicko[1], Amadou Barry[1], Mohamed Dicko[1], Issaka Sagara[1], Christophe Rogier[2], Ogobara K. Doumbo[1]

[1]MRTC/FMPOS, University of Bamako, Bamako, Mali, [2]Institut de recherche biomédicale des armées-IRBA, Marseille, France

In Mali like most of sub-saharan African countries, most of the health centers do not have capacity to confirm the diagnosis of malaria and reported malaria cases are essentially based on presumptions. This results to a lack of accurate measure of the malaria burden essential for the control and prevention strategies. To assess the place the malaria in the overall morbidity and its variation by age and season, a prospective survey was carried out in the community of Didieni from April 2007 to March 2008 including all the consultations of the resident population at the community health center in Didieni. Malaria rapid diagnostic test (Optimal®) was performed in all suspected cases of malaria all the times during the study period. Data were recorded in special records books, doubled entered and analyzed. Of the 5565 cases of consultations, 1501 (27.0%) were due malaria representing the first cause of consultations in the resident population. The frequency of malaria varied significantly with age and the season. Most of cases 91.6% (1375/1501) occurred between August and December and mainly in children under 5 years of age 43.2% (648/1501). Severe malaria represented 0.8% (47/5565) of the consultations. About 95.7% (45/47) of them occurred between August and December and mostly in children of under 5 years of age 78.7% (37/47). In conclusion, malaria continued to be the first cause of morbidity in Mali. Controls measures should target the period of August to December for impact.

968

MALARIA TRENDS AMONG GOLD MINERS IN SURINAME

Hedley Cairo[1], Ferdinand Refos[1], Loretta Hardjopawiro[1], Marjolein Jubithana[2], Leopoldo Villegas[1]

[1]Malaria Program, Ministry of Health, Paramaribo, Suriname, [2]Department of Epidemiology, Bureau of Public Health, Ministry of Health, Paramaribo, Suriname

Suriname has achieved the Millennium Development Goals (MDGs) in malaria in 2008 with a significant reduction of cases (>90%) and mortality (0 deaths since 2007). This was obtained scaling up integrated, locally-adapted highly effective interventions in the populations living in the interior of the country. However, gold mining has boomed in Suriname. Malaria transmission in Suriname is strongly related to population movements, being gold mining activities the main reason. This study aimed to describe the epidemiological trends of malaria among gold miners in Suriname from 2004 through 2009. The malaria surveillance system report cases among gold miners mainly in the capital Paramaribo (Tourtone clinic - TC) and malaria service deliveries working in situ in the mining areas under the coordination/supervision of the malaria program (MP). Estimated populations of 15000 - 20000 individuals have been working in gold mining areas in Suriname and additionally, 10000 in the neighboring French Guiana. Between 2006-2009, 11988 malaria diagnoses were performed and 4352 malaria cases were diagnosed. The MP diagnosed 2041 malaria cases and TC, 2311 cases. *Plasmodium falciparum* was diagnosed in more than 40% of the cases. A six fold increase in malaria cases was observed in 2009 compared with 2004. Overall 40-50%% of the malaria cases are imported from French Guiana. Eighty two percent of the individuals infected were between 20 and 50 years of age. An increased number of malaria cases in the Suriname were detected in gold mining areas between 2004 and 2009. This was due to the increased availability and accessibility of rapid diagnostic and treatment facilities in gold mining areas and an aggressive Active Case Detection policy implemented by the malaria program. Malaria prevalence in mining areas ranged from 1.1% to 4.5%. In order to control the remaining malaria foci in gold mining districts in the country, an integrated comprehensive malaria control approach will be used including long lasting insecticidal nets, IEC/BCC, aggressive active case detection and media awareness campaign. Further details of the strategy will be presented and discussed.

969

HEALTH FACILITY SURVEILLANCE: AN ASSESSMENT OF DATA QUALITY AND UTILITY

Jessica Butts, Manoj Menon, Steven Yoon

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

In sub-Saharan Africa, health facility (HF) data are used to monitor progress in malaria control in addition to other data sources, such as population-based surveys. Malaria case data from HFs, however, are often of uncertain quality. The U.S. President's Malaria Initiative (PMI) supports a number of HFs in 10 countries to strengthen their capacity to collect, analyze, and report on 4 core indicators: total number of outpatients, total number of suspect malaria cases, total number of suspect cases tested, and total number of lab-confirmed cases. Patient-level data are collected in 7countries and aggregate data are collected in 3 countries. PMI-supported HFs were assessed to determine the quality and utility of their data. Data were collected from 3 randomly selected HFs in each of 10 countries. Data consistency was assessed for all sites, and completeness and accuracy were assessed for those sites collecting patient-level data. Consistency was measured as the difference between HF reported summary data and the recalculated result from the assessment. Completeness was measured by counting the number of case report forms (CRF) with missing data. Accuracy was measured as agreement between the individual CRF and the HF database record. Data utility was assessed by reviewing how data were used for decision making by the HF and the National Malaria Control Program. Although 90% of the countries reported all core variables, only 30% reported data that were consistent with assessment findings. Slightly more than half (57%, 4/7) of countries captured all CRF data elements, and 71% (5/7) of countries' data were accurate. The majority of countries were able to provide examples of how data are used. In conclusion, despite the availability of dedicated PMI resources to improve HF data quality, HF data quality remains problematic, primarily due to inconsistent and incomplete data, in most of the assessed countries. Although data are being used, quality issues threaten their validity. Further efforts to improve data quality and increase data use are warranted.

970

MALARIA LONG INCUBATION PERIOD, WITHOUT THE USE OF PROPHYLAXIS, IN PATIENTS DIAGNOSED OUTSIDE THE TRANSMISSION AREAS IN BRAZIL

Patricia Brasil[1], Anielle de Pina Costa[1], Renata Saraiva Pedro[1], Clarisse da Silveira Bressan[1], Sidnei da Silva[1], Pedro Luiz Tauil[2], Cláudio Tadeu Daniel-Ribeiro[1]

[1]Fiocruz, Rio de Janeiro, Brazil, [2]Universidade de Brasilia, Brasilia, Brazil

Brazil is a tropical country with the largest number of malaria cases in the Americas (WHO). In 2009, approximately 306,000 cases were recorded, with 99.8% concentrated in the Amazon region. In the same year, 708 cases were recorded in the extra-Amazon region, where transmission does not occur, and they are largely imported from the states of the Amazon or African countries. Prolonged incubation period in Malaria was first described by Korteweg (1902) in Holland and was subsequently observed in infections caused by some strains of *Plasmodium vivax* in temperate areas. The diversity in essential biological characteristics with the existence of two strains of *P.vivax*, or the different number of sporozoites inoculated by the bite of infectious *Anopheles* were the explanations to the differences in the duration of the incubation period and emergence of relapse in malaria. Nowadays it is mainly related to the use of prophylactic malaria treatment. The opportunity to study some cases of P. vivax malaria in Rio de Janeiro where there is no vector transmission has made it possible to study certain aspects of natural history of the disease in man, without the interference of new infections. In our study, prolonged periods of incubation (ranged from 90 to 360 days), occurred in seven (14%) patients with malaria by P.vivax as well as in one individual infected by P. malariae. The average and median of the latent period (125 days) in P.vivax infections were about nine times larger than the classical period (14 days) described in the literature. No patient had used malaria prophylaxis nor had received blood transfusions. They all came from Amazonia in Brazil, except for the patient infected by P. malariae who came from Indonesia. The disclosure of this occurrence for the first time in the tropics is particularly important because in theory, it raises changes in biological and evolutionary concepts; and in practice, because malaria is one of the most common infectious diseases among travelers and long incubation period is among the main causes of failure in malaria diagnostic suspicion.

971

THE IMPACT OF MALARIA ON THE UNITED STATES MILITARY: SEPTEMBER 2001 TO PRESENT

Cindy Tamminga[1], Judith E. Epstein[1], Karl Kronmann[2], Natalie Wells[3], Christian F. Ockenhouse[4], Thomas L. Richie[1]

[1]United States Military Malaria Vaccine Program, Naval Medical Research Center, Silver Spring, MD, United States, [2]Naval Medical Research Unit No. 3, Ghana Detachment, Legon, Ghana, [3]Navy Environmental Preventive Medicine Unit No. 2, Norfolk, VA, United States, [4]United States Military Malaria Vaccine Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States

Malaria has had a significant impact on U.S military operations throughout history. It was responsible for greater loss of manpower than enemy fire in all conflicts occurring in tropical regions during the 20th century. Malaria continues to present a major challenge to force health protection during operations in any environment where malaria is endemic. This includes 108 countries spanning the tropical and subtropical regions of the world, including most of subSaharan Africa and large regions of South Asia, Southeast Asia, Oceania, Central Asia, the Middle East, Central and South America and the Caribbean. The U.S military is either currently deployed or has the potential to deploy on short notice to any of these regions, making malaria a leading infectious threat to mission success. In our malaria-naïve military population, an infection with any of the five *Plasmodium* species infecting humans can severely degrade performance, result in missed duty, and may lead to prolonged hospitalization and, in some cases, death. The measures used to avoid malaria frequently compromise military performance and, given the difficulties of implementing control measures and chemoprophylaxis in combat, cannot be completely relied upon to prevent infection. Recent events in a number of endemic locations including Liberia in 2003 and 2009-10, Benin in 2009, and Haiti in 2010 underscore the DoD's critical need for a malaria vaccine for deployed military personnel. This presentation will discuss the impact of malaria upon U.S. forces abroad, highlighting recent events, and will provide an introduction to the ground-breaking strides being made by military researchers in the development of a successful malaria vaccine to protect deployed forces.

972

A COST ANALYSIS: A MALARIA OUTBREAK AMONG MILITARY PERSONNEL DEPLOYED TO LIBERIA

Anne Roberts[1], Cindy Tamminga[2], Eyako Wurapa[3], Judith Epstein[2], Patrick Malone[1], Timothy Whitman[4], Gail Levine[2], Thomas Richie[2]

[1]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]United States Military Malaria Vaccine Program, Naval Medical Research Center, Silver Spring, MD, United States, [3]95th CA BDE Surgeon, Fort Bragg, Fayetteville, NC, United States, [4]National Naval Medical Center, Bethesda, MD, United States

"The history of malaria in war might almost be taken to be the history of war itself" wrote Col. C. H. Melville of the Royal Army Medical College, London in 1910. A century later, malaria continues to be a major threat to every military operation which occurs in malaria-endemic areas. The lost work hours, impact on force readiness, risk to mission accomplishment, financial toll, suffering and occasional tragic deaths of young able troops despite the availability of effective insecticides, repellants and

chemoprophylactic drugs are constant reminders that the problem of malaria in the military remains to be solved. This ongoing threat to deployed personnel was highlighted in 2003 when 28% of a 290 person Joint Task Force was stricken with *Plasmodium falciparum* malaria infection despite being prescribed mefloquine for prophylaxis, necessitating the airlift evacuation of 44 U.S Marines to Germany or the U.S., five of whom required admission to an intensive care unit (ICU). A total of 41 Marines were evacuated to the National Naval Medical Center (NNMC) in Bethesda, MD including three of the five individuals requiring ICU support. A retrospective record review was performed to assess the costs associated with the evacuation and hospitalization of the 41 Marines. The results of this review will be presented as a 21st century benchmark of the magnitude of costs associated with malaria outbreaks in deployed troops. It is our intent that this estimate of the financial impact of a malaria outbreak will serve to assist with the calculation of a return on investment (ROI) for resources invested in new products to protect military personnel against malaria, support military planning for future deployments in endemic areas and inform funding decisions based on disease impact.

973

PREVALENCE OF *PLASMODIUM VIVAX AND P. FALCIPARUM* IN PREGNANT WOMEN OF THE PERUVIAN AMAZON, A LOW TRANSMISSION ZONE

Kimberly F. Faldetta[1], Jean N. Hernandez[2], OraLee Branch[1]

[1]Department of Medical Parasitology, New York University, New York, NY, United States, [2]Laboratorio de Investigaciones Productos Naturales Anti-parasitos, Universidad Nacional de la Amazonia Peruana, Iquitos, Peru

The Amazonian communities surrounding Iquitos, Peru are hypoendemic for *Plasmodium vivax* and *P. falciparum*. In recent years, the prevalence of infection of both parasites has been decreasing in the general population; however, the prevalence in pregnant women is unknown. Malaria in pregnancy (MIP) poses serious risks to a pregnant woman and her fetus, increasing risks of maternal anemia and infant low birth weight. The objectives of this study were to determine the regional burden of MIP, assess whether or not pregnant women are at increased risk for either or both species of malaria, and ascertain whether the trend in pregnant women is congruent with the decreasing incidence of malaria in the general population of the Peruvian Amazon basin. From 2004-2008, 950 women were enrolled in the study at the time of delivery. *P. falciparum* and *P. vivax* parasites were detected using both standard microscopy techniques and polymerase chain reaction (PCR). Enzyme-linked immunosorbent assays (ELISAs) were performed against recombinant merozoite surface protein1-19 (MSP1-19), an erthyrocytic stage protein for both *P. falciparum* and *P. vivax* to determine the existences of IgG and IgM in patient serum. Most women (*P. vivax* 58.7%, N=917; *P. falciparum* 22.2% N=898) had a previous lifetime report of malaria (clinical, symptomatic). Preliminary data indicates that the prevalence of *P. vivax* (pregnant 15.2%, N=204; non-pregnant 9.2%, N=2098) and *P. falciparum* (pregnant 7.8%, N=204; non-pregnant 4.3%, N=2098) were higher in our pregnant than non-pregnant cohort from 2003-2008. Unlike previous studies (in high transmission zones), our low transmission study found that primigravids were not more likely than multigravids to have *P. vivax* (ages 15-19 p=0.4366; ages 20-30 p=0.9562) or *P. falciparum* (ages 15-19 p=0.1421 chisquared; ages 20-35 p=0.8232 chisquared) when controlled for age. Analysis on ELISA data is pending. The current analysis indicates that MIP in low transmission zones may behave differently than in high transmission zones. This has implications for health policy and control methods in hypoendemic areas.

974

MALARIA EPIDEMIOLOGY IN A SUB-URBAN AREA OF THE PERUVIAN AMAZON REGION

Veronica E. Soto-Calle[1], Juan J. Contreras[1], Annette Erhart[2], Alejandro Llanos-Cuentas[1], Dionicia Gamboa[1], Niko Speybroeck[3], Umberto D'Alessandro[2]

[1]Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Department of Parasitology, Institute of Tropical Medicine, Antwerp, Belgium, [3]Department of Animal Health, Institute of Tropical Medicine, Antwerp, Belgium

Vivax malaria is endemic in the Peruvian Amazon region where it represents up to 80% of all malaria. Intensive and regular malaria control activities have been carried out since the end of 2006 leading to a decrease in the overall malaria prevalence. However, malaria remains a public health problem, and in addition, recent publications from the Amazon region have reported high numbers of asymptomatic and sub-patents infections. The objective of this study was to estimate the prevalence of patent and sub-patent malaria infections and their epidemiological characteristics in a peri-urban community of Iquitos city the capital of the Peruvian Amazon Region. A cross-sectional survey was conducted in Ex Relleno, a newly settled community with low socioeconomic status. Villagers were invited to be examined and treated for malaria infections by a medical doctor, on a voluntary and free of charge basis. After clinical examination, a finger prick blood sample was taken for microscopic and molecular examination (species-specific PCR). A total of 169 individuals were examined, among which only 4 (2.4%) were found positive for *Plasmodium spp* by microscopy (patent infections), while another 20 infections were identified when using PCR, leading to a malaria prevalence of 24 (14.2%). *P.vivax* prevalence was 2.4% (4/169) and 3.6% (6/169), respectively by microscopy and PCR technique, whereas for *P.falciparum* these figures were respectively, 0% (0/169) and 10.7% (18/169). Patent infections were mainly symptomatic (3/4) while all *P.falciparum* PCR positive individuals were asymptomatic. In conclusion, most of the malaria infections in this sub-urban community were sub-patent and asymptomatic. Even though sub-patent infections might be less infectious to malaria vectors as compared to patent infections, this reservoir, by its size and its hidden nature, is likely to be a play a major role in malaria transmission and will complicate further elimination strategies in the Amazon region. Further research is necessary to assess the extent of this phenomenon and the long term impact of different interventions.

975

LOW PARASITE MULTIPLICATION RATE WAS OBSERVED IN LESS COMPLEXITY OF INFECTION COMMUNITY PERUVIAN AMAZON

Jean Hernandez[1], OraLee Branch[2]

[1]New York University, Iquitos, Peru, [2]New York University, New York, NY, United States

The most dangerous type of malaria specie is *Plasmodium falciparum*, however this infection is fast in the human is hard try to culture *in vitro*, and with this obstacle difficult to do assay and gain more knowledge about the pathways for the invasion to red blood cells and what is the selectivity that has the parasite to enter the RBC and what are the alternatives ways to infect the cells. For this study we included 50 samples from persons with malaria *Falciparum* from Iquitos Nauta road, Loreto. Communities with low transmission for malaria but constant all the year, these samples were collected since 2005-2008. All these samples were entered to the culture to see the SI in the day 0 and day 2 and the impact in the success and the relation with the clinical manifestations. The samples were entered to the culture was from people who had 26 in average years old, strains showed geometric mean SI at day cero 2.30 and day two geometric mean 3.69, also the age average for the SI > 1 29 years old, PMR mean 0.06. There was no difference between SI>1 (2/14) and SI<1 (4/36) in terms of patients presenting more than 5

symptoms. However there was significant difference observed between patients presenting less than symptoms (2/14) as compared to 5 and more symptoms (12/14) when SI was less than one.

976

OVERWHELMING MALARIA PREVALENCE IN CAMEROONIAN SCHOOLCHILDREN

Helen Kimbi Kuokuo[1], Herve Nyabeyeu Nyabeyeu[2], Hilda Ajeagah Uforka[1], Frederick Keka Chi[1], Humphrey Asaah Gah[2], Christian Ngouadjio Nguetse[2], **Leopold Gustave Lehman**[2]

[1]University of Buea, Buea, Cameroon, [2]University of Douala, Douala, Cameroon

Malaria remains the main children killer in sub-Saharan countries in 2010 despite numerous efforts to tackle the disease. Control programs including distribution of ITNs to pregnant women and delivery of IPT has widely been adopted in endemic regions. Fetching parasitological data is crucial for any control strategy and this has become easier with the use of battery-operated fluorescence microscopes on the field. Nevertheless, school children who constitute one of the main targets are not yet focused enough for malaria control interventions. The objective of this study was to conduct a cross-sectional survey to assess malaria prevalence in schools of semi-urban and rural areas in south-west Cameroon in order to propose integrated control measures. 542 primary school children in rural and relatively urbanized areas aged 6 to 14 years were screened in 4 schools during 4 days in April 2010. The inclusion criterion was the handing over of an informed consent form signed by parent/legal guardian. Demographic and clinical data were recorded. Blood was collected by finger prick. Parasitaemia was assessed on the spot using Partec Rapid Malaria Tests slides and 3 CyScope® fluorescence LED microscopes operated with built-in rechargeable batteries (PARTEC, Görlitz, Germany). There were 1367 total school children with 542 participants (participation rate = 39.65%). The number of positive cases among the participants was 313 or a rate of 56.7%. Prostration, fever, headache, abdominal pains were the most common symptoms for children with high parasitaemia (7.7 % of tested children). Malaria prevalence remains extremely high in semi-urban and rural schools of the Buea Health District. The low adhesion rate may be due to superstitious believes of many parents who link children's blood collection to witchcraft. The results can be biased because consenting parents may have more frequent malaria cases in their family. In conclusion, sensitization should be intensely conducted and rapid malaria screening encouraged in schools for a better control of malaria.

977

PLASMODIUM FALCIPARUM MULTIPLICATION RATE AND SURVIVAL OF PARASITES IN *IN VITRO* CULTURE IS ASSOCIATED WITH *IN VIVO* INFECTION CLINICAL FACTORS, BLOOD GROUP, AND PARASITE GENETIC DIVERSITY

Thomas M. Zervos[1], Jean N. Hernandez[2], Patrick L. Sutton[1], Lindsay P. Torres[2], OraLee H. Branch[1]

[1]New York University, New York, NY, United States, [2]Universidad Nacional de la Amazonia Peruana, Iquitos, Peru

To better understand the factors influencing *Plasmodium falciparum* success in *in vitro* culture, a study was designed to investigate epidemiologic variables on culture success over time. Host variables considered upon collection of infected blood were: age, sex, temperature, hematocrit, and blood group. Parasite variables considered, included: parasite density, complexity of infection (COI), and genotype. In this study, 306 isolates were collected from 2003-2009, within Amazonian villages near Iquitos, Peru, though passive and active case detection. Parasites from vacutainer blood were cultured *in vitro*. Parasite growth over time was divided into 5 categories based on the %Δ in parasitemia. Host and parasite variables were analyzed independently and stratified by age and COI. Growth rate in first 48hrs (multiplication rate: "MR") and culture success over time was studied in relation to host and parasite variables. *P. falciparum* cultures with the greatest success were cultured from individuals with a higher parasite density ($p<0.0001$). Complex infections successfully adapted to culture at a higher frequency than single infections ($p<0.032$) and COI was also correlated to increased disease severity in children ($p<0.001$). Genotype analysis indicated that the Mad20 allele-type of *Pf*msp1-block 2, correlated to increased disease severity, which then translated into increased culture success. Patient isolates with blood groups other than O (A, B, AB) had a greater MR in the first 48hrs of culture ($p<0.016$). In conclusion, results from this study indicate variables that might reflect host immunity or pathology of parasitemia *in vivo* are related to the MR and growth *in vitro*. Individuals of the O blood group might induce a strong selective pressure that limits parasite growth. Finding a relationship between genetic diversity and MR might suggest associated parasite factors that impact growth or an ability to invade host RBCs. This study will be further investigated using other genetic markers and more detailed analyses of clinical symptoms.

978

BIOPHYSICAL AND IMMUNOLOGICAL STUDIES WITH A RECOMBINANT *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN (PFCSP)

Jennifer Carter[1], Christopher Pool[1], Brooke A. Bozick[1], Particia De la Vega[1], Evelina Angov[1], Roberta Spaccapelo[2], Andrea Crisanti[3], Jittawadee R. Murphy[1], Christian F. Ockenhouse[1], Sheetij Dutta[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Università degli Studi di Perugia, Perugia, Italy, [3]Imperial College London, London, United Kingdom

A highly purified preparation of a near full length recombinant circumsporozoite protein (PfCSP) was analyzed by SDS-PAGE and shown to migrate ~10 kDa higher than its predicted 30 kDa molecular weight under both reducing and non-reducing conditions. Blue-native non-denaturing PAGE and analytical size exclusion chromatography further confirmed that the rCSP monomer had a highly extended molecular structure and its size was equivalent to a ~60 kDa globular protein. Vaccination of PfCSP along with an adjuvant Montanide ISA720 induced high titer antibodies in mice and this vaccination conferred sterile protection in two strains of mice against challenge with a transgenic *Plasmodium berghei* parasite line that expressed the *P. falciparum* CSP gene. The anti-PfCSP antibodies recognized the native CSP on sporozoites by IFA and inhibited the invasion of NF54 strain sporozoites into hepatocytes. Availability of a high quality near full-length rCSP and the transgenic mouse protection model will allow us to develop novel strategies to enhance the protective efficacy of CSP based vaccines in humans.

979

A PHASE 1B DOUBLE-BLIND RANDOMIZED CONTROLLED AGE-DEESCALATING TRIAL OF TWO VIROSOME FORMULATED ANTI-MALARIA VACCINE COMPONENTS ADMINISTERED IN COMBINATION TO HEALTHY SEMI-IMMUNE TANZANIAN ADULTS AND CHILDREN

Blaise Genton[1], Thomas Aebi[1], Mwanajaa S. Abdallah[2], Mpina Maximillian[2], Ester B. Machunda[1], Thabiti A. Mbaga[2], Juma Mpuruti[2], Patrick Cech[1], Bettina Ley[1], Marcel Tanner[1], Nicole Westerfeld[3], Sabine Stoffel[3], Rinaldo Zurbriggen[3], Gerd Pluschke[1], Claudia Dauenberger[1], Salim Abdulla[2]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [3]Pevion Biotech Ltd., Bern, Switzerland

Influenza virosomes represent an innovative antigen delivery system that has already proven its suitability for vaccine design. The aim of this trial was to demonstrate the safety and immunogenicity of the combination of two virosome formulated malaria peptidomimetics (PEV3B: 50 µg AMA-1

and 10 µg CSP) in semi-immune subjects. The design was a prospective randomized, double-blind, controlled, age-deescalating study. PEV3B was injected i.m. on days 0 and 90. Control vaccine was Inflexal V (virosomal influenza vaccine). Specimens for humoral response were obtained at screening, and on days 30, 90, 120, 180, 365. 10 adult males and 40 children aged 5-9 years living in a malaria endemic area were recruited, 8 adults and 32 children were injected with PEV3B, 2 and 8 respectively with Inflexal V. No serious or severe adverse events related to the vaccine were observed. The only local solicited adverse event reported was pain at the injection site. The incidence of pain was higher in the Inflexal V group compared with the PEV3B group (50% vs 10%, p=0.01). General solicited adverse events reported were headache and elevated temperature, with comparable rates between groups. For immunogenicity, antibody titers after vaccination were always higher in the PEV3B group than in the Inflexal V one at all sample days for both antigens ($p<0.05$), except at day 120 for CSP in adults, and day 365 for AMA-1 in children. In children the proportion of responders using either antigen was significantly higher in PEV3B than in Inflexal V ($p<0.05$), except for AMA-1 at Day 365. Incidence rate of clinical malaria from day 120 (30 days post second vaccination) until day 365 was half in children injected with PEV3B than with Inflexal V (0.00342 vs 0.00178, p=0.09). The safety data demonstrated that 2 vaccinations with PEV3B are safe and well tolerated. PEV3B elicited long-lived humoral responses to both target antigens with the strongest response generally observed 30 days after second immunization. This study confirms that virosomes are a suitable delivery system for malaria peptide antigens in malaria semi-immune subjects, including children.

980

PROGRESS ON THE DEVELOPMENT OF A SECOND GENERATION PAN-REACTIVE APICAL MEMBRANE ANTIGEN-1 VACCINE

Sheetij Dutta[1], Lisa Dlugosz[1], John D. Haynes[1], Christopher D. Pool[1], Robin F. Anders[2], Mick Foley[2], Adrian Batchelor[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]La Trobe University, Bundoora Victoria, Australia

Apical Membrane Antigen-1 (AMA1) based vaccines have shown promising parasite inhibitory effects against malaria in Phase 2a/b clinical trials. The major hurdle in the path of AMA1 vaccine development remains the diversity of its field isolates and the strain-specificity of its immune response. The availability of the crystal structure of AMA1 has allowed us to follow a two-pronged strategy to design a second generation pan-reactive AMA1 vaccine. First, we are using growth inhibition assay with polyclonal and monoclonal antibodies to map the cross-reactive inhibitory epitopes of AMA1. We hypothesize that cross-reactive epitopes displayed on an immunologically silent scaffold can form the basis of an engineered vaccine that will induce broadly inhibitory antibodies against AMA1. Alternatively we are also using the available structural and phylogenetic data on AMA1 to choose alleles that can be included in a rationally designed polyvalent vaccine. Results of cross-reactive epitope mapping, rabbit immunogenicity and parasite sero-typing using growth inhibition assays will be presented. Although AMA1 is being used here as a model to test strategies to broaden immune responses, similar approaches may be applicable to other infectious diseases where diversity remains a major hurdle to vaccine development.

981

REVIEW OF THE HUMAN MALARIA CHALLENGE MODEL AT THE WALTER REED ARMY INSTITUTE OF RESEARCH FROM 1995-2007

Michele Spring[1], Ilin Chuang[1], Robin Nielsen[2], Kari Hunter[3], Marla Brunell[3], Paige Waterman[4], Mark Polhemus[5], Christian Ockenhouse[1]

[1]United States Military Malaria Vaccine Program, Silver Spring, MD, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [4]Walter Reed Army Medical Center, Washington, DC, United States, [5]United States Medical Research and Materiel Command, Fort Detrick, MD, United States

The seminal paper first describing the success of the human malaria challenge method was published in 1986 by Chulay et al. In this model, female *Anopheles* mosquitoes fed on *in vitro* cultures of *Plasmodium falciparum* driven to gametocyte production, and then fed on six malaria-naïve volunteers. The Walter Reed Army of Research has been conducting the human malaria challenge since the Chulay study, and the Department of Entomology has provided such mosquitoes for challenges involving over 1000 volunteers. The safety and clinical outcomes of the malaria challenge model in clinical trials have been reviewed in two publications. Church et al reviewed records of 18 malaria challenge studies between 1985 and 1992, and Epstein et al discussed studies conducted from 1996-2002. This study adds to the data available collecting data from 12 studies conducted at Walter Reed Army Institute of Research from 1995 to 2007 not previously reviewed. This is thr largest review to date and involves approximately 550 human volunteers who were experimentally challenged by the bite of *Anopheles stephensi* mosquitoes infected with *P. falciparum* sporozoites including volunteers after receiving one or more malaria vaccinations or control volunteers. Our goals will be to summarize the safety data of the malaria challenge model by delineating the signs and symptoms reported. We will report the frequency, severity and duration of these symptoms as well as describe the laboratory results and identify any abnormalities wherein associated with malaria challenge. In addition we will define parasitologic infections in challenge volunteers by prepatent, patent and incubation period, any relationship of the clinical disease and time of parasitemia, and further quantify the details of malaria infection in vaccine-protected, non-protected, and non-vaccinated volunteers. With the increasing cost and complexity of overseas trials to assess vaccine efficacy, the challenge model is of increasing importance in evaluating which malaria vaccines should go on to field trials.

982

FORMULATION AND PRE-CLINICAL EVALUATION OF TRANSMISSION BLOCKING POTENTIAL OF PLANT-PRODUCED *PLASMODIUM FALCIPARUM* SEXUAL STAGE PFS25 AND PFS230 VACCINE CANDIDATES

Vidadi Yusibov[1], Christine E. Farrance[1], Jessica A. Chichester[1], R. Mark Jones[1], Vadim Mett[1], Konstantin Musiychuk[1], Moneim Shamloul[1], Satish Sharma[1], Stephen Streatfield[1], Robert W. Sauerwein[2], Will Roeffen[2], Yimin Wu[3], Olga Muratova[3], Patrick Duffy[3], Janice Culpepper[4]

[1]Fraunhofer-CMB, Newark, DE, United States, [2]Radboud University, Nijmegen, The Netherlands, [3]National Institutes of Health, Rockville, MD, United States, [4]The Bill and Melinda Gates Foundation, Seattle, WA, United States

Malaria is a serious and sometimes fatal mosquito-borne disease caused by a protozoan parasite. There are hundreds of millions of cases of malaria occurring each year around the world, and nearly one million people are killed. Malaria is transmitted by the female *Anopheles* mosquito which takes up the sexual stage of the parasite during a blood meal. The parasite completes the sexual stages in the mosquito before being transmitted to a

subsequent host. Vaccines directed against the mosquito parasitic stages are designed to halt development into oocysts and thus are transmission-blocking vaccines. We are targeting different antigens for development of an effective transmission blocking vaccine and have successfully produced multiple versions of the Pfs25 and Pfs230 antigens in our plant-based launch-vector system and have shown them to generate strong transmission blocking activity. We are currently evaluating multiple versions of our antigens in dose ranging and adjuvant studies. Results of these studies will determine a candidate vaccine for clinical development.

983

PROTECTIVE IMMUNE RESPONSES ELICITED BY IMMUNIZATION WITH A CHIMERIC BLOOD-STAGE MALARIA VACCINE PERSIST BUT ARE NOT BOOSTED BY *PLASMODIUM YOELII* CHALLENGE INFECTION

James R. Alaro, Michele M. Lynch, James M. Burns, Jr.

Drexel University College of Medicine, Philadelphia, PA, United States

Plasmodium falciparum merozoite surface protein 1 has emerged as the lead blood-stage vaccine candidate with *Pf*MSP142 the most advanced. Preclinical studies showed that protection afforded by r*Pf*MSP142 depended on the induction of high levels of neutralizing antibodies against epitopes in the C-terminal EGF-like domains of MSP119. Disappointingly, *Pf*MSP142 vaccine has failed to confer acceptable protection in humans, partly due to, T and B cell epitope polymorphisms and overall poor immunogenicity. Moreover, the likelihood that a subunit vaccine will successfully control this complex parasite is being questioned. We have investigated some of these issues using the murine malaria parasite, *Plasmodium yoelii*. We focused on improving the design of the vaccine construct by coupling the protective *Py*MSP119 with the conserved and relatively immunogenic epitopes of *Py*MSP8 to generate chimeric *Py*MSP1/8. We previously reported that immunization with r*Py*MSP1/8, formulated in Quil A adjuvant, protected against lethal *P. yoelii* 17XL, well beyond that achieved by single or combined immunizations with the component antigens. Here, we continue the evaluation of the chimeric *Py*MSP1/8 vaccine and show that immunization with r*Py*MSP1/8 elicited an MSP8-restricted T cell response that was sufficient to provide help for both *Py*MSP119 and *Py*MSP8 specific B cells to produce high and sustained levels of protective antibodies. The enhanced efficacy of immunization with r*Py*MSP1/8, in comparison to combined formulation of r*Py*MSP142 and r*Py*MSP8, was not due to an improved conformation of protective B cell epitopes or creation of novel protective epitopes. Unexpectedly, r*Py*MSP1/8 vaccine-induced antibody responses were not boosted by exposure to *P. yoelii* 17XL infected RBCs. However, r*Py*MSP1/8 immunized and infected mice mounted robust responses to a diverse set of blood-stage antigens. The data support the further development of *P. falciparum* MSP1/8 chimeric vaccine but also suggest that vaccines that prime for responses to a diverse set of parasite proteins will be required to maximize vaccine efficacy.

984

PHASE 1/2A CLINICAL TRIAL ON SAFETY, TOLERABILITY, IMMUNOGENICITY AND EFFICACY OF PRIME BOOST REGIMEN OF DNA- AND ADENOVIRUS-VECTORED MALARIA VACCINES ENCODING *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN (CSP) AND APICAL MEMBRANE ANTIGEN (AMA1) IN MALARIA-NAÏVE ADULTS

Ilin Chuang[1], Martha Sedegah[1], Susan Cicatelli[2], Michele Spring[3], Cindy Tamminga[1], Jason Bennett[3], Melanie Guerrero[2], Mark Polhemus[4], James Cummings[2], Evelina Angov[3], Joe Bruder[5], Noelle Patterson[1], Keith Limbach[1], Elke Bergmann-Leitner[3], Jittawadee Murphy[6], Lorraine Soisson[7], Carter Diggs[7], Christian Ockenhouse[3], Thomas Richie[1]

[1]United States Military Malaria Vaccine Program, Naval Medical Research Center, Silver Spring, MD, United States, [2]Clinical Trials Center, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]United States Military Malaria Vaccine Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [4]United States Army Medical Research and Materiel Command, Ft. Detrick, MD, United States, [5]GenVec, Gaithersburg, MD, United States, [6]Department of Entomology, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [7]United States Agency for International Development, Washington, DC, United States

Malaria causes approximately 515 million cases and 1 million deaths annually. Genetically-based vaccines such as DNA plasmids and adenovirus vectors induce strong CD8+ T cell-mediated immunity, believed to be important in protection against the hepatic stage malaria. Heterologous prime-boost regimens may overcome the effects of pre-existing immunity to viral vectors. This trial assessed the safety, immunogenicity and efficacy of a prime-boost malaria vaccine in healthy, malaria-naïve adults. Three doses of a DNA vaccine consisting of two plasmids (Vical Inc.), encoding CSP and AMA1 (1 mg each), were delivered intramuscularly by jet injection (Biojector 2000 Inc.) at four-week intervals. Sixteen weeks later, a boosting dose of an adenovirus-vectored vaccine (AdCA) was given intramuscularly by needle. The AdCA consisted of two serotype-5 adenovectors (GenVec Inc.) encoding CSP and AMA1 (1 x e10 pu each). Four weeks following the AdCA boost, 15 immunized subjects and six unimmunized controls were challenged with homologous *Plasmodium falciparum* sporozoites via five infected-mosquito bites. Both the DNA and AdCA vaccines were found to be safe and well-tolerated. There were no vaccine-related serious adverse reactions. All controls and eleven immunized subjects developed parasitemia. There was no significant delay in parasitemia between groups. Four immunized subjects (26.7%) remained asymptomatic and sterilely protected 28 days post-challenge. Three protected subjects had a strong cell-mediated immune response to AMA1 or CSP + AMA1 as determined by ELISpot. Preliminary results from CD4/CD8 depletion study and flow cytometry study will be presented. In conclusion, the results from this trial demonstrate proof of principle regarding the efficacy of a DNA-prime, Adenovirus 5-boost vaccine regimen against *falciparum* malaria. Further study is needed to compare efficacy of the full vaccine regimen to that of the individual vaccine components.

985

INTERFERON GAMMA ELISPOT RESPONSES IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM VOLUNTEERS IMMUNIZED WITH A METABOLICALLY ACTIVE, NON-REPLICATING PLASMODIUM *FALCIPARUM* SPOROZOITE VACCINE

Martha Sedegah[1], Sumana Chakravarty[2], Kirsten E. Lyke[3], Judith E. Epstein[1], Charles Anderson[2], Harini Ganeshan[1], Jennylynn Lejano[1], Estaban Abot[1], Glenna J. Banania[1], Renato Sayo[1], Maria Belmonte[1], Christine Park[1], Thomas L. Richie[1], Stephen L. Hoffman[2]

[1]United States Military Malaria Vaccine Program, Naval Medical Research Center, Silver Spring, MD, United States, [2]Sanaria Inc., Rockville, MD, United States, [3]Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States

Protection of mice by immunization with radiation-attenuated sporozoites is dependent on T cells and interferon gamma (IFNγ). The aim of this work was to determine if immunization of volunteers with the PfSPZ Vaccine, a metabolically active, non-replicating *Plasmodium falciparum* (Pf) sporozoite (SPZ) vaccine, might likewise induce PfSPZ-specific IFNγ responses, and if these responses might correlate with protection. We developed an ELISpot assay that utilizes live PfSPZ (produced and cryopreserved in an identical fashion to the vaccine itself), instead of individual proteins or peptides, to stimulate peripheral blood mononuclear cells (PBMCs) in culture. The rationale behind using whole sporozoites as antigen in the ELISpot as opposed to individual peptides or proteins was that hundreds to thousands of proteins may be expressed by sporozoites, and it is not known which ones are responsible for protective immunity after immunization with radiation attenuated sporozoites and thus which could be selected for use in a more traditional ELISpot assay. Fresh PBMCs collected prior to immunization and 2 weeks after the fourth, fifth, and sixth doses of PfSPZ Vaccine were incubated with irradiated (150 Gy) PfSPZ for 36 hours in culture, and the IFNγ spot forming cells were enumerated. Post-immunization PBMCs from more than 50% of volunteers satisfied the criteria set forth for a positive response, demonstrating that immunization of the volunteers with the PfSPZ Vaccine induced PfSPZ-specific T cell responses. The magnitude of the PfSPZ-specific IFNγELISpot responses increased with increasing doses of the PfSPZ Vaccine and there was no significant difference between responses in volunteers immunized by the intradermal or subcutaneous routes. Surprisingly, the highest responses were noted after the fourth dose of the vaccine and did not increase after the fifth and sixth doses. The use for the first time of sterile, irradiated, purified PfSPZ as antigen in an IFNγ ELISpot assay has generated clear data regarding T cell responses in humans immunized with the PfSPZ Vaccine.

986

THE EFFECTS OF ROUTE OF DELIVERY ON PROTECTIVE EFFICACY OF RADIATION ATTENUATED *PLASMODIUM YOELII* SPOROZOITES IN THE MURINE MODEL FOR THE PFSPZ VACCINE

Charles F. Anderson, Solomon Conteh, Sumana Chakravarty, Stephen L. Hoffman

Sanaria, Rockville, MD, United States

Immunization of humans with attenuated *Plasmodium falciparum* sporozoites is the gold standard for the induction of sterile immunity against malaria and is the scientific rationale for commercial development of the PfSPZ Vaccine, currently in phase I clinical trials. While the routes of vaccine administration and dose size for the current trial had to be empirically chosen for safety and pragmatic reasons, the optimal dose, dosing regimen and route of delivery for high-level protection are still unknown. We have conducted studies using the murine model for malaria in order to identify a method that provides a high degree of protection with the lowest number of sporozoites. Mice were immunized with radiation attenuated *P. yoelii* sporozoites (irrPySPZ) by various routes and the most efficacious route of delivery of sporozoites was found to be intravenous; 3 doses of just 750 irrPySPZ each usually conferred 90%-100% protection. When irrPySPZ were administered subcutaneously (SC) or intradermally (ID), a 6-7 fold increase in dose was required for comparable protection. When irrPySPZ that had been cryopreserved using a method similar to that for the PfSPZ Vaccine were used for immunizations, a 2-3 fold increase in irrPySPZ administered by the intravenous route was required for comparable protection to that of fresh irrPySPZ administered by the same route, and a 3-5 fold increase in numbers of irrPySPZ administered by ID or SC routes as compared to fresh irrPySPZ. Thus, compared to non-cryopreserved irrPySPZ administered intravenously, a >20-fold increase of cryopreserved irrPySPZ administered ID or SC was required for a high degree of protection. These results suggest that the protective potential of the PfSPZ Vaccine can be best demonstrated with intravenous inoculation, while at the same time parenteral administration for mass immunizations can be targeted to closely mimic the intravenous route using variations in injection techniques.

987

DEVELOPMENT OF A *PLASMODIUM VIVAX* RECOMBINANT CS PROTEIN VACCINE

B. Kim Lee Sim[1], Richard E. Stafford[1], Adriana Ahumada[1], Minglin Li[1], Rana Chattopadhyay[2], Martha Sedegah[3], Emily Chen[1], Ray Xu[1], Maria Orozco[1], Bing Jiang[1], Ruobing Wang[4], Harini Ganeshan[3], Steve Abbot[3], Socrates Herrera[5], Stephen L. Hoffman[1]

[1]Protein Potential, Rockville, MD, United States, [2]Sanaria, Rockville, MD, United States, [3]United States Military Malaria Vaccine Program Naval Medical Research Center, Silver Spring, MD, United States, [4]Seattle Biomedical Research Institute, Seattle, WA, United States, [5]Instituto de Immunologia, Universidad del Valle Cali, Colombia

Plasmodium vivax (Pv) causes 80-250 million cases of malaria annually, as many cases of malaria in travelers as *P. falciparum* (Pf), severe morbidity and mortality, and substantial economic burden. There is a huge potential market for a Pv vaccine in travelers and military from the developed world, and among populations in countries with endemic Pv. The fact that Pf and Pv co-exist in most malaria endemic areas presents technical and ethical constraints for deployment of a vaccine effective only against Pf, and malaria cannot be eradicated without eliminating Pv. The only subunit malaria vaccines that have been reproducibly shown to prevent Pf malaria in humans are based on the Pf circumsporozoite protein (PfCSP). These vaccines elicit antibodies against the central repeat region of the molecule, which is conserved in all isolates of Pf. Development of a PvCSP vaccine has been complicated by the fact that there are 2 major alleles 210 and 247, based on variation in sequence of the central repeats. We constructed PvCSP recombinant proteins that combined 3 copies of PvCSP 210 repeats and 3 copies of PvCSP 247 repeats with N-terminus, C-terminus or N- and C-termini (full length) of the PvCSP. All were expressed in Pichia pastoris as secreted proteins. All induced antibodies in mice that recognized PvCSP as well as native protein on air-dried Pv sporozoites expressing PvCSP 210 (India) or 247 (Thai) in IFAs. All were recognized by sera from individuals from Pv-endemic areas. The induced antibodies were biologically active as they inhibited invasion and development of Pv sporozoites in hepatoma cells. At a 1:20 dilution, these antibodies had a range of 78 - 84% inhibition as compared to 95% inhibition with 100 µg/mL of the protective mAb, NVS3. Further, mice immunized with the recombinants had good T cell responses against the PvCSP as measured by IFNγ ELIspot assays. Comparative assessments selected the full length PvCSP as the vaccine candidate. Progress on process development, manufacturing and characterization in compliance with cGMPs will also be presented.

988

DEVELOPMENT OF *PLASMODIUM FALCIPARUM* CELTOS AS A MALARIA VACCINE IMMUNOGEN

Nuvjeevan S. Dosanjh[1], Richard E. Stafford[1], Adriana Ahumada[1], Emily Chen[1], Minglin Li[1], Soundarapandian Velmurugan[2], Solomon Conteh[2], Lixin Gao[1], Martha Sedegah[3], Maria Belmonte[3], Tao Li[2], Yonas Abebe[2], Abraham Eappen[2], Joseph T. Bruder[4], Steven G. Reed[5], Stephen L. Hoffman[1], B. Kim Lee Sim[1]

[1]Protein Potential, Rockville, MD, United States, [2]Sanaria, Rockville, MD, United States, [3]United States Military Malaria Vaccine Program, Naval Medical Research Center, Silver Spring, MD, United States, [4]Genvec, Gaithersburg, MD, United States, [5]Infectious Disease Research Institute, Seattle, WA, United States

The conserved *Plasmodium* protein CelTOS (cell traversal protein for ookinetes and sporozoites) mediates the invasion of sporozoites through the liver sinusoidal cell layer and the migration of ookinetes through mosquito mid-gut epithelial cells. Thus, it has the potential to induce protective immune responses against pre-erythocytic and mosquito stages of the malaria parasite, thereby preventing infection and transmission. To characterize the biological activity of immune responses against CelTOS and to move toward development of a *P. falciparum* (Pf) CelTOS immunogen we expressed, purified and characterized Pf and P. yoelii (Py) CelTOS in Pichia Pastoris. We next studied in Balb/c and/or CD1 mice the immunogenicity of the recombinant proteins alone with the following adjuvants, GLA-SE emulsions (with TLR4 and 9 agonists), Montanide 720, TiterMax and Freunds, and as part of a prime boost strategy using attenuated adenovirus serotype 5 (Ad). IgG responses to the antigen in mice immunized with recombinant protein alone revealed OD 1 titers of greater than > 100,000 by ELISA and end point titers of 12,800 in IFAs of air-dried Pf and Py sporozoites showing that antibodies recognized native CelTOS. Antibodies against PfCelTOS were biologically active and inhibited the development of liver stage parasites in ILSDA assays (49% reduction in liver invasion compared to adjuvant control sera). The antibodies against PfCelTOS recognized the protein in Pf retorts and ookinetes by IFA, and are now being studied for transmission blocking activity. Further, Interferon Gamma (IFNγ) ELIspot data showed increased T-cell responses from spleen cells isolated from mice immunized with PfCelTOS in TLR9 emulsion (207 Net SFCs/106 against 14-mer peptides of PfCelTOS, 93 net SCFs/106 against recombinant PfCelTOS) compared to those immunized with emulsion alone. The protective efficacy of recombinant PyCelTOS in mice is being assessed alone and as part of the prime-boost strategy with adenovirus expressing PyCelTOS. The recombinant PfCelTOS is now in process development to be used as an immunogen alone or in combination with other pre-erythrocytic immunogens like PfCSP in clinical trials.

989

BIOASSAY TESTING WITH PERMANET-2 LONG LASTING INSECTICIDAL NET SAMPLES COLLECTED AFTER 3 TO 32 MONTHS OF USE IN ETHIOPIA DEMONSTRATES PERSISTENCE OF INSECTICIDE ON NETS BUT REDUCED KILLING EFFECT IN WILD TYPE *ANOPHELES ARABIENSIS*

Gedeon Yohannes Anshebo[1], Stephen C. Smith[2], Aprielle Brackery[3], Damtew Yadeta[4], Patricia M. Graves[3], Tekola Endeshaw[1], Teshome Gebre[1], Paul M. Emerson[3]

[1]The Carter Center, Addis Ababa, Ethiopia, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]The Carter Center, Atlanta, GA, United States, [4]Oromia Regional Health Bureau, Addis Ababa, Ethiopia

After large-scale distribution of PermaNet2 long-lasting insecticidal nets (LLIN) in Ethiopia in early 2007, nets were collected from households to assess insecticide concentration and effectiveness at killing *Anopheles* mosquitoes at 3-6 months (150 nets, 3 sites), 17-21 months (200 nets, 10 sites) and 28-32 months (220 nets, 11 sites) after distribution. Nets for bioassays were randomly selected from these samples. Deltamethrin concentration was assessed by X-ray fluorescence spectroscopy. Bioassays used the CDC modified WHO cone method, with a 3 minute exposure of 30-52 mosquitoes per sample in 8 replicates, followed by a 24 hour holding period. For the 3-6 month samples, bioassays were done in Atlanta using the deltamethrin susceptible *An. gambiae* Kisumu strain. Subsequent bioassays were done at Adama, Ethiopia against the susceptible *An. arabiensis* Nazareth strain and adults reared from wild caught *An. arabiensis* larvae collected at Sodere, Oromia Region. Results were adjusted for control tests (untreated netting) run simultaneously. In tests with *An. gambiae* after 3-6 months of use, the average adjusted 24 hour mortality was 99.4% (N=24 nets, range 92.3-100%). After 17-21 months of use (N=40 nets), the average mortality was 92.7% (range 33.3-100%) with the susceptible Nazareth *An. arabiensis* and 90.4% (range 61.9-100%) with wild caught mosquitoes. After 28-32 months (N=44 nets), the average mortality was 94.6% (range 68.4-100%) with the susceptible strain but had declined to 46.1% (range 0-90%) with wild caught mosquitoes. The mean (range) deltamethrin concentration on the tested nets was 61.5 mg/m2 (range 8.6-97.3) after 3-6 months, 43.3 (1.4-93.7) after 17-21 months, and 44.3 (13.1-85.5) after 28-32 months of use. The results demonstrate that the residual insecticide on the nets after up to 32 months of use was sufficient to kill the majority of susceptible *An. arabiensis* but not the wild caught mosquitoes. This suggests that there is some resistance to deltamethrin in wild mosquitoes in Ethiopia, enabling about half to survive in the bioassay tests.

990

ADAPTIVE CHANGES IN AMINO ACID AND CODON BIASES OF THE MOSQUITO SODIUM CHANNEL IN THE EVOLUTION OF PERMETHRIN SELECTION

Nannan Liu, Qiang Xu, Lee Zhang, Lan Zhang, Ting Li

Auburn University, Auburn, AL, United States

Target site insensitivity resulting from point mutations within the voltage-gated sodium channel of the insect nervous system is known to be of primary importance in the development of resistance to pyrethroid insecticides. Here, we report a systematic analysis of nucleotide polymorphisms through the entire sodium channel cDNAs among susceptible, intermediately resistant parental, and highly resistant offspring mosquitoes *Culex quinquefasciatus* and the dynamics of the synonymous and nonsynonymous nucleotide composition of the mosquito sodium channel under insecticide selection pressure. Three nonsynonymous and 6 synonymous mutations were found in the *Culex* mosquito sodium channel. A comparative framework was used to examine adaptive changes in the patterns of amino acid and codon usage in the 3 *Culex* mosquito strains under permethrin selection pressure, revealing the frequency of both nonsynonymous and synonymous mutations in the mosquito sodium channel underwent a rapid population expansion following permethrin selection. This finding suggests permethrin selection is the prevailing selective force in the evolution of the amino acid and codon usage of the mosquito sodium channel, thus affecting the sensitivity of the sodium channel to insecticides and enabling the mosquito to survive.

991

REDUCING SELECTION FOR RESISTANCE BY LOWERING PYRETHROID CONCENTRATION: THE EFFECTS OF AGE AND EXPOSURE HISTORY

Katey Glunt, Matthew B. Thomas, Andrew F. Read

Pennsylvania State University, University Park, PA, United States

Malaria control campaigns usually involve widespread use of chemical insecticides to eliminate the mosquito vector. The fast-killing action of these compounds imposes strong selection for the evolution of resistance. It has recently been proposed that current delivery methods might be refined to target, rather than all potential vectors, only the immediately-

dangerous, infected mosquitoes and, therefore, we could reduce the pressure on the insects to evade chemical control as well as interrupt disease transmission. We investigated one possible selection-reducing manipulation of insecticide application, lowering the concentration to preferentially kill older mosquitoes. Specifically, we examined the effects of single and repeated exposure to low doses of the pyrethroid, permethrin, on survival of adult female *Anopheles* stephensi mosquitoes of different ages. Mosquitoes were exposed to permethrin at days 4, 8, 12 and 16 days post adult emergence using standard WHO resistance assay protocols and subsequently monitored for 24 days. Permethrin concentrations were less than half of the lowest recommended insecticide-treated net dose. We found that age at exposure had a greater impact on survival than number of times previously exposed, with older mosquitoes surviving less than younger ones at all concentrations. Though there was no increase in lethality with repeated exposure to low doses of permethrin, the overall reduction in survival, combined with a degree of age-discrimination, is consistent with the idea that doses of insecticide that do not kill 100% of all mosquitoes shortly after contact could still lead to effective disease control, if females are killed before they become old enough to contribute to transmission.

992

THE EFFECT OF MOSQUITO AGE ON INSECTICIDE RESISTANCE STATUS

Mouhamadou C. Seidou[1], Georgina V. Bingham[2], Tessa B. Knox[3], Helen V. Pates Jamet[2]

[1]Vestergaard Frandsen-CSRS Project Laboratory, Abidjan, Côte D'Ivoire, [2]Vestergaard Frandsen, Lausanne, Switzerland, [3]Vestergaard Frandsen, Nairobi, Kenya

Appropriate monitoring of insecticide resistance in mosquito vectors is a key component of vector control programs. A standard WHO protocol is routinely used to monitor insecticide resistance. The protocol uses 2-5 day old unfed mosquitoes exposed to filter papers impregnated with a discriminating dose of insecticide. There is some discussion as to whether mosquitoes become more susceptible as they age, although evidence for this is limited. This study investigated the effect of age on insecticide resistance status in a field collected strain of *Anopheles gambiae s.s.* from the Ivory Coast. Unfed females aged between 1 to 14 days were exposed to standard papers with deltamethrin, permethrin, DDT, bendiocarb and malathion following the standard procedure as described by the WHO. Using probit analysis the results of each tested age group were compared to show the time of exposure causing 50% and 90% knockdown. The 24 h post-exposure mortality of each different age group was also recorded. The laboratory standard susceptible strain KISUMU was used for control purposes. The significance of the results will be discussed in relation to current monitoring methods and the interpretation of data generated using this standard test.

993

THE INCREASE IN THE UTILITY AND IMPORTANCE OF BIOMOLECULAR TECHNIQUES IN RESISTANCE MONITORING IN INSECT VECTORS

IRAC Public Health Team

CropLife International, Brussels, Belgium

The main problem associated with the onset of resistance is the failure to reduce vector populations and the potential for an increase in disease transmission. Monitoring will allow a timely change in strategy and various bioassays, biochemical and molecular methods exist that can be used to test and monitor resistance development. Identification of the resistance mechanisms involved gives an indication of which alternative compounds should be used. Molecular assays are an ideal complement to bioassays, and are especially useful to monitor trends in resistance gene frequency over time. Molecular assays can detect resistance at very low frequency, can indicate the presence of heterozygous individuals with recessive resistance genes that are not detected through bioassays and require fewer mosquitoes than bioassays. Their use is currently restricted to research labs since field test kits are still in development. Molecular techniques are now routinely used for identification of sibling species using the multiplex PCR assay and molecular M and S forms within the *Anopheles gambiae* complex using a restriction fragment length polymorphic (RFLP) PCR assay. The frequency of *kdr* alleles is detected using allele specific RT-PCR (Reverse Transcription Polymerase Chain Reaction); all of these assays can be carried out on phenotyped samples (survivors and non-survivors) of WHO susceptibility tests and departure from Hardy-Weinburg proportions can be examined. Microarrays have been developed and have demonstrated increases in RNA levels associated with oxidase (P450), Glutathione-S-Transferase (GST) and exterase (COE) activity in resistant mosquitoes. There is currently no field method to test for the presence of resistance associated P450s, COE and GSTs, other than bioassays with synergists. The development of these methods is essential to enable complete characterization of resistant populations. This will assist control programmes in making the most informed decision they can regarding appropriate insecticide choice and will contribute to the development and introduction of new insecticide classes needed for vector control.

994

MORTALITY RATES AND INSECTICIDE RESISTANCE IDENTIFICATION FOR *ANOPHELES ALBUMANUS* (DIPTERA: CULICIDAE) IN NORTHERN PERU

Kirk D. Mundal

Navy Medical Research Center Detachment, Lima, Peru

Mortality Rates and Insecticide Resistance Identification for *Anopheles albumanus* (Diptera: Culicidae) in Northern Peru. Insecticide resistance and cross-resistance in vector populations is a growing problem worldwide. Insecticide resistance by malaria vectors in Peru is largely unidentified. Peru possesses several malaria vectors to include *Anopheles albumanus*. An. albumanus populations were sampled using human landing collection within the department of Piura Peru and colonized to the F 42 generation. The CDC bottle assay method was used in this study. Approximately 20, three to six day old adult female mosquitoes were placed in 250ml Wheaton® bottles coated with the CDC's recommended diagnostic dosages for Anopheline mosquitoes. DDT, malathion, fenitrothion, etophenprox, permethrin, propoxur, lambda cyhalothrin, deltamethrin, alpha cypermethrin, cypermethrin, and bendiocarb were evaluated in this study. Eight replications were done for this study. Of the insecticides tested, mortality rates ranged from 100 percent down to 2%. Four insecticides had less than 90% mortality: deltamethrin (76%), alpha cypermethrin (60%), cypermethrin (19%), and bendiocarb (2%) indicating insecticide resistance to these compounds is present within this population of mosquitoes.

995

DELIVERING INSECTICIDAL CRAB BAITS (ICB) AS A TOOL AGAINST *AEDES POLYNESIENSIS* BREEDING SITES IN LOW-ISLAND ENVIRONMENTS IN FRENCH POLYNESIA

Jérôme Marie[1], Tuterarii Paoaafaite[1], Marc Faaruia[1], David R. Mercer[2], Richard C. Russell[3], Hervé C. Bossin[1]

[1]Institut Louis Malardé, Papeete, French Polynesia, [2]University of Kentucky, Lexington, KY, United States, [3]University of Sydney, Westmead, Australia

Burrows from the crab Cardisoma carnifex, found in atolls of French Polynesia, are one of the principal breeding sites for the mosquito Aedes polynesiensis, primary vector of Lymphatic Filariasis in the South Pacific. A novel method of mosquito control is the use of Insecticide-laiced Crab Baits (ICB). This technology is based upon the principal that crabs will harvest and deliver the treated bait into the crab burrow/mosquito breeding site. A semi-natural system mimicking crab burrows was developed in the laboratory allowing the assessment of both Bti (Mosquito

Bits®) and Methoprene (Altosid® pellets) on mosquito larvae mortality and the potential acute and long-term toxicity on non-target species Cardisoma carnifex under near-natural conditions. Both insecticides were selected for their environment friendly action. We evaluated their impact on juvenile crabs as well as on male and female adult crabs.

In the presence of alternative food, most ICB were taken up and eaten within one hour of their placement into the buckets. Exposure of mosquito larvae to BTI-treated "crab burrow" water resulted in high survival. Increasing the BTI dose 8-fold did not increase mortality. Methoprene bioassays by comparison generated very encouraging results with high mortality rates observed over a period of 4 months. These results indicate the potential suitability of Altosid®-based ICB formulation. Control of Ae. polynesiensis immatures developing in crab burrows was recorded without measurable adverse effects on the crab population. No mortality, acute or long-term toxic effects amongst juvenile or adult crabs was observed. Exposure to ICBs does not appear to impact crab development. All exposed juvenile crabs underwent at least one molt. ICBs impregnated with methoprene appeared to have no impact on non-target mosquito species (Culex pipiens and Toxorhynchites amboinensis) that utilized the semi-natural crab burrows as breeding sites during the experiment. If successful in the field, this ICB strategy could help suppressing the vector of LF in low islands of French Polynesia.

996

CONTACT IRRITANCY RESPONSES IN *AEDES AEGYPTI* USING SUBLETHAL DOSES OF PYRETHROID CHEMICALS IN IQUITOS, PERU

Fanny Castro-Llanos[1], Hortance Manda[2], Kirk Mundal[1], John Grieco[2], Amy Morrison[3], Edwin Requena[1], Hugo Jaba[1], Victor Lopez[1], Nicole Achee[2]

[1]Naval Medical Research Center Detachment, Lima, Peru, [2]Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [3]University of California Davis, Davis, CA, United States

Dengue, transmitted by *Aedes aegypti*, is one of the most important viral diseases world-wide. Current methods for the reduction of disease transmission includes controlling adult vector populations using chemical applications at toxic doses; however, issues of insecticide resistance, adverse health effects and environmental concerns have driven the need to redesign our currently available tools. The use of chemicals at sublethal doses to modify vector behavior is one possible novel strategy. We have shown in previous studies that pyrethroids have irritant effects on *Ae. aegypti* at dosing levels below current field application rates (i.e., LD90). This irritant action induces movement of the insect away from a chemical source and can be exploited to promote exit from a space occupied by human hosts prior to biting thereby reducing the probability of disease transmission. This study, conducted in part within a larger research program to field-validate a Push-Pull strategy to reduce *Ae. aegypti* inside homes, quantified contact irritancy behavior of *Ae. aegypti* at sublethal doses for two pyrethroids insecticides: alpha-cypermethrin and deltamethrin using an experimental hut study design in Iquitos, Peru. Chemicals were applied to textile material at varying doses and surface area coverage. Laboratory reared female *Ae. aegypti* adults were released inside each experimental hut and exit movement patterns quantified every 30 min from 0600-1800h using interception traps to capture escaping mosquitoes. Results compared escape density rates over time among dose, surface area coverage and chemical variables. This information will be used to design the optimum contact irritant treatment scheme for experimental Push-Pull trials.

997

IDENTIFICATION OF CANDIDATE GENES ASSOCIATED WITH DELTAMETHRIN RESISTANCE IN THE LATIN AMERICAN MALARIA VECTOR *ANOPHELES ALBIMANUS*

Mónica Stein, Pamela M. Pennington, Mabel Taracena, **Norma R. Padilla**

Universidad del Valle de Guatemala, Guatemala, Guatemala

Insecticide resistance is a wide spread phenomenom that undermines the efforts to control malaria. Identifying the genetic basis of insecticide resistance may lead to the development of novel tools for early detection of resistant populations. New tools include applications of transcriptomics and genomics that detect genes associated with resistant phenotypes. Although *Anopheles albimanus* is the predominant malaria vector in Central and South America, there have been few research efforts to study the response of this vector to insecticide exposure at the molecular level. Adult females of colonized *An. albimanus* were exposed to deltamethrin in an effort to detect transcripts associated with resistance. We used Suppressive Subtractive Hybridization (SSH) to identify differentially expressed genes in a two-pronged approach. First we identified genes over-expressed after exposure to a lethal dose 90 of 0.3 ug/ml deltamethrin (LD90). Additionally, we subjected a population to an LD90 during 18 generations, at which point unexposed females were taken from the colony under selection and compared with females from the unselected colony in a second SSH analysis. We expect that an overlap between the over-expressed transcripts in both experiments will reveal *An. albiman*us genes potentially associated with resistance to deltamethrin, such as P450 family members. We propose that this approach will increase the probability of selecting true candidates. Three transcripts were over-expressed in the colony under selection as compared with the unselected colony. These transcripts, as well as those differentially expressed between exposed and non exposed susceptible populations, are being sequenced to determine their identity. Further work will verify that over-expression of these transcripts is associated with resistance and may contribute to improving monitoring and detection techniques.

998

INSECTICIDE SUSCEPTIBILITY OF *AEDES AEGYPTI* IN CARTAGENA (COLOMBIA)

Doris E. Gómez Camargo[1], Ronald Y. Maestre[2], Ibeth Pisciotti[3], Dacia I. Malambo[1], Claudio J. Gómez Alegría[4]

[1]University of Cartagena, Cartagena, Colombia, [2]University of Cartagena-Secretaria de SaludAtlántico, Cartagena, Colombia, [3]Departamento Administrativo Distrital de Cartagena, Cartagena, Colombia, [4]Universidad Nacional de Colombia, Bogotá, Colombia

Dengue fever keeps an endemic behavior in Cartagena (Colombia), a location in the northern Caribbean coast of Colombia where insecticides have played an important role in actions towards the control of this disease during the last four decades. However, it is not known if the selective pressure on Aedes aegypti population in this location has induced some resistance to insecticides. Here, the susceptibility of Aedes aegypti in Cartagena was evaluated against organophosphorus, organochlorine and pyrethroid insecticides during a year (2009). Biological assays were carried out with adults (F2) and third-instare larva of A. aegypti collected in different urban districts including Los Alpes, Zaragocilla, and Pasacaballos. For adults, the CDC-Atlanta method was applied using diagnostic doses for malathion (100 µg/ml), fenitrothion (75 µg/ml), DDT (150 µg/ml) and lambdacyhalotrine (6,25 µg/ml). For larva, the World Health Organization method was applied, with a diagnostic dose of Temephos (0,012ppm). Each insecticide was tested three times, with four replicates each time, and a control with no insecticide was also included.

All three populations showed susceptibility to malathion, fenitrothion, deltametrine and propoxur, with a 100% mortality in all cases. Concerning to Lambdacyhalotrine (47-70% mortality) and Temephos (95 a 99%

mortality), variation was found in the susceptibility/resistance. In contrast, resistance was found in all three populations with 3-6% mortality In conclusion, our results suggest some degree of resistance to insecticides in three populations of A.aegyptis in Cartagena-Colombia. This might indicate a growing phenomenon of insecticides resistance in this location.

999

ASSESSING THE EFFICACY OF DELTAMETHRIN-IMPREGNATED LETHAL TARGETS FOR THE CONTROL OF THE LYMPHATIC FILARIASIS VECTOR IN TAHITI, FRENCH POLYNESIA

Charles Jeannin[1], Tuterarii Paoaafaite[1], Henri Frogier[1], Albert Tetuanui[1], Marc Faaruia[1], Jérôme Marie[1], Eric W. Chambers[2], Richard C. Russell[3], Scott A. Ritchie[4], Stephen L. Dobson[2], Hervé C. Bossin[1]

[1]Institut Louis Malardé, Papeete, French Polynesia, [2]University of Kentucky, Lexington, KY, United States, [3]University of Sydney, Sydney, Australia, [4]James Cook University, Cairns, Australia

Lymphatic filariasis (LF) is one of the world's leading causes of disability. The ongoing Pacific Program to Eliminate Lymphatic Filariasis (PacELF) is based on a mass drug administration program to reduce human LF prevalence. A supplemental method to eliminate LF is the control of its main vector, Aedes polynesiensis, to reduce host-vector contact. The use of insecticide impregnated materials is showing great efficacy for the control of various mosquito-borne diseases, particularly malaria. We have been evaluating the efficacy of pyrethroid impregnated outdoor visual resting targets (Lethal Targets) to control this exophilic diurnal mosquito in the field. Following preliminary laboratory attractiveness tests of different colors, a navy blue 100% cotton fabric was selected. Effective impregnation with deltamethrin was verified using a standard WHO cone bioassay. Preliminary sampling was conducted in four different villages along the west coast of Tahiti to identify potential experimental field sites. Twice monthly collections were undertaken with BG Sentinel traps (Biogents, Regenwald) to derive baseline data on the vector populations and likely-use blocks (treatment and control) were selected in the village of Toahotu on the Tahiti peninsula. Permission was obtained from property owners to place a lethal target and to use a BG trap for weekly collections. Mosquito sampling during the month preceding deployment of the LTs is underway and will provide an estimate of the mosquito density prior to the treatment. A similar sampling regime will be used following placement of LTs to assess their overall impact on the mosquito population. Results of the ongoing trials will be presented.

1000

LARVAL HABITAT SEGREGATION BETWEEN THE MOLECULAR FORMS OF THE AFRICAN MALARIA MOSQUITO, *ANOPHELES GAMBIAE* IN A RICE FIELD AREA OF BURKINA FASO, WEST AFRICA

Geoffrey Gimonneau[1], Marco Pombi[2], Marc Choisy[1], Serge Morand[3], Roch Dabire[4], Carlo Costantini[5], Frederic Simard[6]

[1]Institut de Recherche pour le Développement, Montpellier, France, [2]Sezione di Parassitologia, Università di Roma "Sapienza", Rome, Italy, [3]Institut des Sciences de l'Evolution de Montpellier, Montpellier, France, [4]Institut de Recherche en Science de la Santé, Bobo Dioulasso, Burkina Faso, [5]Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale/Institut de Recherche pour le Développement, Yaoundé, Cameroon, [6]Institut de Recherche en Science de la Santé/Institut de Recherche pour le Développement, Bobo Dioulasso, Burkina Faso

Disruptive selection acting on alternative eco-phenotypes can promote the evolution of reproductive isolation between populations, a process known as ecological speciation. In West Africa, lineage splitting between the M and S forms of the major Afro-tropical malaria mosquito, *Anopheles gambiae* is thought to be driven by ecological divergence, occurring mainly at the larval stage. Here, we will present evidences for habitat segregation between these two cryptic species in and around irrigated rice-fields located within the humid savannas background of western Burkina Faso, West Africa. Longitudinal sampling of adult mosquitoes emerging from a range of larval development sites was conducted from June to November 2009. Every other week, emergence traps were set up above larval development sites distributed along a 15km-long transect, from the heart of the rice-fields area into the surrounding savannas. In total, eighty larval development sites were georeferrenced and characterized (distance to the rice fields and to the nearest house, surface, depth, presence of standing vegetation, algae and/or debris, presence of predators and other culicine species, water origin, turbidity and general surrounding). A null model analysis revealed that the two molecular forms are non-randomly distributed (p=0.003). Canonical correspondence analysis was used to explore the spatial pattern of occurrence of the two sibling species and their relation to environmental variables. A major ecological gradient was extracted, in relation to the rice field perimeter (p=0.002). The M form was associated to larger breeding sites, which were mainly represented by rice field paddies. On the opposite, the S form was found to depend upon temporary, rain-filled breeding sites. These results support hypotheses about larval habitat segregation and confirm that both forms have different larval habitat requirement. Segregation appears clearly linked to anthropogenic permanent habitat and the community structure and diversity cascades they support.

1001

HOUSEHOLD-LEVEL PREDICTORS OF *AEDES AEGYPTI* PRESENCE AND ABUNDANCE IN IQUITOS, PERU: IMPLICATIONS FOR DENGUE CONTROL

Peter J. Boldenow[1], Mark L. Wilson[1], Arthur Getis[2], Helvio Astete[3], Moises Sihuincha[4], Jeffrey D. Stancil[5], Thomas W. Scott[3], Amy C. Morrison[3]

[1]University of Michigan, Ann Arbor, MI, United States, [2]San Diego State University, San Diego, CA, United States, [3]University of California, Davis, Davis, CA, United States, [4]Loreto Regional Health Department, Iquitos, Peru, [5]Naval Medical Research Center Detachment, Lima, Peru

Understanding what factors affect *Aedes aegypti* presence and abundance in/around houses enhances opportunities to target high-risk sites for mosquito control efforts. Using extensive *Ae. aegypti* surveillance data collected throughout Iquitos, Peru during 1999 through 2002, we evaluated associations between household characteristics and presence of adult mosquitoes. Houses with at least one adult *Ae. aegypti* captured with backpack aspiration (cases) were compared with mosquito-free houses (controls). Matching of case and control houses for space (<100 m distance) and time (same day) was performed to eliminate possible confounding from non-house factors and changes in mosquito abundance over time. Vegetation coverage surrounding houses was estimated from NDVI values (Landsat satellite images). Adjusted odds ratios were calculated using conditional logistic regression. Significantly more houses with adult *Ae. aegypti* had open soffit and room partitions (OR = 1.46, p = <0.0001), more manually filled containers (OR1 Container Increase = 1.02, p = <0.0001), more naturally rain filled containers (OR1 Container Increase = 1.05, p = <0.0001), more containers filled via roof runoff (OR1 Container Increase = 1.12, p = <0.0001), more residents (OR1 Person Increase = 1.03, p = <0.0001), and greater vegetation (OR = 1.05, p = <0.0016). These results demonstrate a complex pattern of household-level biophysical and social factors that are associated with transmission risk in this region of endemic dengue, and suggest contexts where interventions might be more effective.

1002

EFFECTS OF BACTERIAL GROWTH ON THE HATCHING OF *AEDES AEGYPTI* EGGS

Loganathan Ponnusamy[1], Katalin Böröczky[1], Dawn M. Wesson[2], Coby Schal[1], **Charles S. Apperson**[1]

[1]North Carolina State University, Raleigh, NC, United States, [2]Tulane, University, LA, United States

It is well established that eggs of *Aedes aegypti* will hatch when the water in which they are submerged is contaminated by microbes. Effects of bacteria on egg hatch is commonly thought to result from a decline in dissolved oxygen (DO) concentration during microbial growth. Notably, there have been no studies to establish effects of bacterial age and other factors associated with bacterial growth on egg hatching. We hypothesized that metabolites associated with bacteria growth or bacteria themselves would stimulate hatching. To test these hypotheses, we exposed eggs of *Ae. aegypti* to bacterial cultures of varying age and assessed subsequent percentage hatch over a time course of one to 4-h. The bacterial cultures were comprised of a mix of 14 species that were originally cultured from an experimental plant infusion constructed from senescent leaves of the bamboo plant *Arundinaria gigantea*. Levels of DO were measured concurrently. In 24-h old stationary phase cultures (cell density = 3.5X109 CFU/ mL), 95% of eggs hatched in 1-h, whereas for 8-d old dead phase cultures (5.9X107 CFU/ mL) only 9% of the eggs hatched in 1-h. DO in the 24-h and 8-d old cultures averaged 0.81 mg/L and 2.8 mg/L, respectively. Surprisingly, 92% of eggs hatched within 1-h after exposure to a 6-h old log phase bacterial culture (7.4 X106 CFU/ mL) at 5.0 mg/L DO. Additionally, an average of 93% of eggs exposed to bacteria cells suspended in 0.85% NaCl solution hatched within 4-h at an average DO of 7.6 mg/L. In comparison, from 0 to 3% of eggs hatched in deionized water or the saline solution even after an exposure period of 5-d. These results suggest that the hatch of *Ae. aegypti* eggs is mediated by bacteria and/or bacteria-associated factors irrespective of DO concentration.

1003

FEEDING PATTERNS AND ARBOVIRUS DETECTION IN *CULEX (MELANOCONION) TAENIOPUS* (DIPTERA: CULICIDAE) FROM GUATEMALA

Oscar F. de Leon[1], Ana L. Ramirez[1], Ana S. Gonzalez[1], Silvia M. Sosa[1], Maria L. Müller[1], Barbara W. Johnson[2], Ann Powers[2], Nicholas A. Panella[2], Celia Cordón-Rosales[1], Nicholas Komar[2], **Maria Eugenia Morales-Betoulle**[1]

[1]Universidad del Valle Guatemala, Guatemala, Guatemala, [2]Centers for Disease Control and Prevention, Fort Collins, CO, United States

Several mosquito-borne arboviruses including members of the *Flavivirus*, *Alphavirus* and *Orthobunyavirus* genera are important zoonotic agents that may cause febrile and encephalitic illness in vertebrate hosts. *Culex (Melanoconion) taeniopus* mosquitoes were associated with transmission of *Venezuelan equine encephalitis virus* (VEEV; alphavirus) and *Nepuyo virus* (bunyavirus) in Guatemala in the late seventies, and have been shown in experimental studies to be efficient vectors of VEEV. During an arbovirus ecology study conducted in the department of Izabal, Guatemala from 2007-2009, we collected 5171 *Cx. taeniopus* using CO2-baited CDC light and gravid traps. To better define the role of *Cx. taeniopus* as a vector of arboviruses, engorged mosquitoes were removed from the collections for blood meal host determination by PCR tests using mitochondrial *Cyt b* and *COI* primers followed by amplicon sequencing. Species-specific DNA sequences were determined from comparison to known sequences in GenBank and the Barcode of Life database (BOLD). All mosquitoes were tested (in pools or individually) by RT-PCR and Vero plaque assays to detect arboviruses. As a result, viruses of all three genera indicated above were detected. Vertebrate hosts were identified from 262 blood meals (85% of 308 engorged mosquitoes tested). Mammals and birds comprised 28% and 31%, respectively, of the identified blood meals. Dog and cow were among the most common mammalian hosts and chicken was the most common avian host. Our results indicate that *Cx. taeniopus* carries several arboviruses in Guatemala and would probably serve a bridging function between sylvatic vertebrates and peridomestic hosts including people and their domestic animals.

1004

NATURAL INFECTION RATES BY *PLASMODIUM* SPP. OF ANTHROPOPHILIC ANOPHELINES FROM LOCALITIES OF NORTHWESTERN COLOMBIA

Doris A. Rosero[1], Giovan F. Gómez[1], Erika C. Higuita[1], Lina A. Gutiérrez[1], Shirley Luckhart[2], Jan E. Conn[3], **Margarita M. Correa**[1]

[1]Grupo Microbiologia Molecular, Escuela de Microbiologia, Universidad de Antioquia, Medellin, Colombia, [2]Department of Medical Microbiology and Immunology, University of California, Davis, CA, United States, [3]Griffin Laboratory, Wadsworth Center, New York State Department of Health, Albany, NY, United States

In Colombia, malaria remains an important public health problem. The assessment of natural infection by *Plasmodium* spp. in anopheline mosquitoes is an important aspect to determine the role of different *Anopheles* species in malaria transmission. We evaluated natural infection rates (IR) by *Plasmodium* spp. in 6,463 anophelines collected using human landing catches. The collections were realized between January and November 2009 in six localities of two malaria regions of Colombia: Urabá - Bajo Cauca-Alto Sinú and the Pacific Coast. Nine *Anopheles* species were detected in three subgenera: *An. nuneztovari* s.l. (58.42%), *An. darlingi* (36.80%), *An. albitarsis* s.l. (1.73%), *An. albimanus* (1.70%), *An. triannulatus* s.l. (1.12%), and *An. punctimacula, An. neivai, An. pseudopunctipennis* and *An. neomaculipalpus* at <0.25%. ELISA and molecular confirmation by nested PCR showed that only two species were naturally infected by *Plasmodium* spp.: *An. nuneztovari* s.l. and *An. darlingi*. Two *An. nuneztovari* s.l. from Buenaventura-Valle del Cauca were found naturally infected with *Plasmodium vivax* VK247 (IR=1.8%), and three specimens from Puerto Libertador-Córdoba were infected, one with *P. falciparum* (IR=0.05%) and two with *P. vivax* VK210 (IR=0.1%). One specimen of El Bagre-Antioquia was infected with *P. vivax* VK247 (IR=0.62%). The *An. darlingi* infected with *P. falciparum* (IR=0.09%) was from Vigía del Fuerte-Antioquia and the other with *P. vivax* VK210 (IR=0.28%) from El Bagre-Antioquia. These findings suggest that *An. nuneztovari* s.l. and *An. darlingi*, two major malaria Colombian vectors continue to play an important role in malaria transmission in these regions. These results constitute the most recent known reports of anophelines naturally infected by *Plasmodium* spp. in these localities and contribute to the understanding of the species involved in the transmission in these regions, information that is useful for the design of selective vector control strategies.

1005

VECTOR BIOLOGY OF ANOPHELINE MOSQUITOES IN LA CAPILLA-EL BAGRE, ANTIOQUIA, COLOMBIA

Nelson J. Naranjo[1], Mariano Altamiranda[1], Juan C. Marin[1], Astrid V. Cienfuegos[1], Shirley Luckhart[2], Jan E. Conn[3], **Margarita M. Correa**[1]

[1]Grupo de Microbiología Molecular, Escuela de Microbiología, Universidad de Antioquia, Medellin, Colombia, [2]Department of Medical Microbiology and Immunology, University of California, Davis, CA, United States, [3]Griffin Laboratory, Wadsworth Center, New York State Department of Health, Albany, NY, United States

Antioquia is one of the departments most affected by malaria transmission in Colombia, and El Bagre (Ant) was the municipality reporting the highest number of cases in 2009 (8,909). Characterization of the bionomic aspects of anophelines involved in malaria outbreaks provides important information for targeted control strategies. We determined the abundance, biting behavior and entomological inoculation rate-EIR of the

anopheline species present in La Capilla, El Bagre from January-December 2009. Six-day collections, every three months, were conducted using human landing catches, outdoors and indoors from 18:00-0:00 h with a one-day collection from 18:00-06:00 h. A total of 2,459 anophelines belonging to six species were identified, *Anopheles darlingi* (49%), *An. nuneztovari* s.l. (42%), *An. albitarsis* s.l. (5%), *An. triannulatus* s.l. (3.6%), *An. punctimacula* (0.24%) and *An. pseudopunctipennis* (0.12%). Mostly all species were more abundant at the onset and the end of the rainy season. Only *An. nuneztovari* and *An. darlingi* were infected by *Plasmodium vivax*. *An. nuneztovari* presented a marked endophagic behavior and *An. darlingi* had variable behavior. There was a significant difference between the mean numbers of *An. nuneztovari* indoors and outdoors (t=4.53, P≤0.001, n=180), while the difference was not significant for *An. darlingi* (t= -0.18, P≤0.85, n=180). Both species were active throughout the night; *An. nuneztovari* presented three main peaks at 21:00-22:00, 23:00-00:00 and 03:00-04:00 and *An. darlingi* showed higher activity at 19:00-00:00. Their EIR values did not differ significantly. The results demonstrate that *An. nuneztovari* and *An. darlingi* have an important role in malaria transmission in this locality. Even though less abundant species collected were not infected, further studies will help to clarify their potential roles in malaria transmission at the local level, since all the species identified have been incriminated as vectors in other regions.

1006

VEGETATION CHARACTERISTICS AND WEST NILE VIRUS TRANSMISSION POTENTIAL IN SUBURBAN NEIGHBORHOODS

Marilyn O. Ruiz[1], Bethany Krebs[1], Gabriel Hamer[2], Allison Gardner[1], William M. Brown[1], Jeffrey Brawn[1], Christopher Small[3], Edward D. Walker[4]

[1]University of Illinois - Urbana, Urbana, IL, United States, [2]University of Wisconsin - Madison, Madison, WI, United States, [3]Columbia University, Palisades, NY, United States, [4]Michigan State University, East Lansing, MI, United States

Transmission of West Nile virus (WNV) occurs primarily among vector mosquitoes and avian reservoir hosts. Prevalence of infection in mosquitoes, birds or other hosts varies temporally and spatially with virus quantity and replication rate, host-seeking behavior of mosquitoes, and the proximity of infectious vectors and hosts. Vegetation affects transmission as forage and cover for vectors and hosts and mediates the effects of temperature, humidity and rainfall. We examined how vegetation measured in several ways provides innovative and scale-appropriate characterization of suburban landscapes to gain insight into transmission risk. Risk related to vegetation is assessed as the association of landscape characteristics with abundance of hosts and vectors and WNV infection. We interpret those in the context of infection risk in a region of suburban Chicago, Illinois, where West Nile virus has been observed since 2001. Vegetation characteristics included size, abundance, species, and spatial distribution and arrangement. We measured characteristics of microhabitats by combining methods involving digital processing of remotely sensed data, visual interpretation of image features, and analysis of direct observations. We created landscape metrics from these observations with FRAGSATS software and other spatial techniques. Transmission risk was estimated from field observations of infection and density of vectors and avian hosts from suburban neighborhoods, including numbers of *Culex* mosquitoes from light traps, WNV infection rates in mosquito pools and sampled birds, and locations of American robins (*Turdus migratorius)*, a species of interest in WNV transmission in the Chicago area. Multivariate statistics were used to test the statistical significance of relationships. We present novel measures of regional urban vegetation, an analysis relating these to the habitats of *Culex* species mosquitoes and American Robins, and an interpretation in light of risk of infection. Mosquito infection was higher in sites with taller and larger trees, while mosquito abundance was associated with more canopy cover, density of stems, presence of contiguous vegetation, and fewer trees in the Rosaceae. These methods to characterize vegetation are useful to elucidate transmission dynamics of WNV at a fine spatial scale and may be applied to other peri-urban arboviruses, such as Dengue virus and Ross River virus.

1007

TRAPPING STUDIES WITH THE MALARIA VECTOR *ANOPHELES DARLINGI* IN SURINAME AND THE RELATION WITH BITING PREFERENCES

Helene Hiwat[1], Marjolein de Rijk[2], Rob Andriessen[2], Constantianus J. Kroenraadt[3], Willem Takken[2]

[1]Malaria Program, Ministry of Health, Paramaribo, Suriname, [2]Laboratory of Entomology, Wageningen University and Research Center, Wageningen, The Netherlands, [3]Wageningen University and Research Center, Wageningen, The Netherlands

The effectiveness of CO2-baited and human-baited mosquito traps at sampling An. darlingi mosquitoes was evaluated and compared to human landing collections (HLC). Biting preferences of this mosquito on a human host were studied and related to the trapping data. Traps used were the CDC Miniature Light Trap (with and without light), the BG Sentinel Mosquito Trap (without BG Lure), the Mosquito Magnet ® Liberty Plus Mosquito Trap (MM-Plus) (without octenol) and a custom design trap. Carbon dioxide or humans were used as bait. The number of An. darlingi collected was greater with the HLC, than with all other collection methods. None of the traps correlated with the HLC in number of An darlingi captured over time. Of the traps evaluated the BG Sentinel Mosquito Trap with CO2 or human bait and the MM-Plus, proved most efficient in collecting An. darlingi. In the field study on An. darlingi biting preferences the females showed directional biting behaviour ($p<0.001$) with a majority of females (93.3 %) biting the (lower) leg and feet region when confronted with a human host sitting down. Higher efficiency of the closer-to-the-ground collecting MM-Plus and BG Sentinel Mosquito Trap may be a result of this biting preference of the vector.

1008

ENVIRONMENTAL AND SOCIOECONOMIC FACTORS LIMITING THE DISTRIBUTION OF *AEDES AEGYPTI* IN THE VENEZUELAN ANDES

Sarah Guagliardo[1], Meagan Fitzpatrick[1], Juan Carlos Navarro[2], Javier Bastidas[3], Jose Carlos Gonzalez[3], **Maria Diuk-Wasser**[1]

[1]Yale University, New Haven, CT, United States, [2]Universidad Central de Venezuela, Caracas, Bolivarian Republic of Venezuela, [3]Direccion de Salud Ambiental, Merida, Bolivarian Republic of Venezuela

Dengue fever is a serious acute illness caused by infection with one of four viral strains. It is spread through the bites of infected mosquitoes, the primary vector in Venezuela being *Aedes aegypti*. The objective of this study was to determine whether temperature and other environmental factors limit the distribution of *Ae. aegypti* in high altitude areas, while controlling for socioeconomic determinants. During the summer of 2009, 24 randomly selected sites distributed across three cities (El Vigia, Ejido and Merida) ranging in altitude from 70 to 1950m were surveyed for mosquito breeding habitats in and around the homes. Occupant interviews were conducted to assess socioeconomic status and access to relevant infrastructure, such as trash collection and water services. For the highest and largest city (Merida), environmental variables were derived from an Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) satellite image. These included land surface temperature, elevation, the mean and standard deviations for each ASTER spectral band, the Normalized Difference Vegetation Index (NDVI) and the Normalized Difference Built Index (NDBI). El Vigia, the town at the lowest elevation, had a higher proportion of positive containers (17.7%) than Merida and Ejido (10.5% and 10.7%, respectively). Water holding containers were more common in El Vigia, while flower pots and vases - linked to better socioeconomic conditions, were more frequent in the

other two towns. In Merida, the proportion of positive containers was higher in warmer and less vegetated areas (lower NDVI). Mean house size and number of inhabitants were also positive predictors, while access to water and trash services was not found to be predictive, in contrast with previous studies in coastal Venezuela. In conclusion, environmental factors derived from remotely sensed data show a closer association with the distribution of *Ae. aegypti* larvae in high altitude areas than socioeconomic factors found to be strongly predictive in other dengue endemic areas.

1009

MANSONIA SPECIES AS POTENTIAL VECTORS OF LYMPHATIC FILARIASIS IN GHANA

Josephine C. Ughasi[1], Hilaria Berkard[1], Delphina Gomez[2], Maxwell Appawu[1], Michael D. Wilson[1], Daniel A. Boakye[1]

[1]Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana, [2]Ghana Atomic Energy Commission, Kwabenya, Accra, Ghana

Wuchereria bancrofti, the causative agent of lymphatic filariasis (LF) is transmitted by mosquito species belonging to *Anopheles, Aedes, Culex* and *Mansonia*. In East Africa, *Anopheles*, *Culex* and *Mansonia* species are known vectors but in West Africa including Ghana only *Anopheles* species are reported vectors. *Anopheles gambiae* s.l and *An. funestus* are the main vectors with An. pharoensis playing a minor role. The mosquito species involved in the transmission of LF is important to achieve the goal of elimination using only mass drug administration (MDA) with ivermectin/ DEC and albendazole. It is recognized that it may be difficult to eliminate LF when the vectors are culicines because they exhibit "limitation" while anophelines show "facilitation". Collections of mosquitoes in LF endemic areas in Ghana have shown large numbers of *Culex* and *Mansonia* species and while it has been established that *Culex* does not transmit the disease in Ghana, the status of *Mansonia* is not known. Recent data from Ghana has shown that after 6 rounds of annual MDA there is still a high prevalence of the disease in some areas where An. melas, *An. gambiae* s.s., *Mansonia* and *Culex* species are the main biting mosquitoes. It has therefore become necessary to determine whether *Mansonia* species play a role in the transmission of LF in these areas. Indoor mosquitoes were collected once a month for three months using pyrethrum spray catches between the hours of 0500-0800 GMT in six communities (Atabadze, Anyinase, Bandor, Epoano, Ponkrom and Sanka) in the KEEA district of the Central Region of Ghana. A total of 824 mosquitoes composed of 500 *Anopheles* species, 240 *Mansonia* species and 84 *Culex* species were caught, dissected and examined for the presence of *W. bancrofti*. Four infected *Mansonia africanus* were found at Sanka with all developmental stages (L1, L2 and L3) of *W. bancrofti*, one *M. africanus* with L2 at Anyinase and one *An. gambiae* s.s with an L3 at Epoano. This is the first report indicating *Mansonia* species as possible vectors of LF in Ghana and West Africa.

1010

POTENTIAL YELLOW FEVER VECTORS AND THEIR ECOLOGY IN THE PAGA COMMUNITY IN THE KASSENA-NANKANA DISTRICT, UPPER EAST REGION OF GHANA

Millicent C. Hall[1], Charles A. Kyorku[2], Maxwell Appawu[1], Samuel Dadzie[1], Daniel A. Boakye[1]

[1]Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana, [2]University of Development Studies, Navrongo, Ghana

Yellow fever (YF) is an acute, infectious, haemorrhagic viral disease with significant public health impact in the tropics transmitted mainly by Aedes mosquitoes which also transmit dengue haemorrhagic fever. Epidemics of yellow fever have previously occurred in Ghana particularly from the 1970s to the 1990s. Recently there have been YF outbreaks in Burkina-Faso which shares borders with Ghana and unconfirmed reports in certain localities in Ghana. Unfortunately, the last detailed work on the vectors in Ghana was published in 1975. There is therefore the need to update the data on yellow fever vectors in Ghana especially within communities along the Ghana-Burkina Faso border. We studied potential yellow fever vectors and their ecological characteristics in Paga at the Ghana-Burkina Faso border in the Kassena-Nankana District (KND). Mosquitoes were collected from 40 households in the Paga community from human landing catches, larval collections and ovitraps for two months each in the rainy and dry seasons. A total of 1197 mosquitoes were collected and morphologically identified. Of these, 609 (50.9%) were *Aedes aegypti*, 423 (35.3%) were *Ae. vittatus* while the remaining 13.8% consisted of *Ae. africanus, Ae. simpsoni*, *Anopheles gambiae*, *Culex* and *Mansonia* species. *Ae. aegypti*, *Ae. vittatus, Ae. africanus* and *Ae. simpsoni* were found breeding in different water holding containers. *Ae. aegypti* larvae were found prevalant in earthenware pots (65.6%), which is the most common water holding container in the area followed by car tyres (34.4%) while Ae. vittatus bred mostly in rock pools (98.6 %). Most of the breeding occurred in the rainy season. Also *Ae. aegypti* and *Ae. vittatus* were the most common biting mosquitoes (50.9% vrs 35.3%) with *Ae. aegypti* biting mostly outdoors (59.2%, N=201) in the rainy season. A high proportion of *Ae. aegypti* (45.3%) was found laying eggs in the ovitraps during the rainy season compared to 4.1% during the dry season. These observations have implication for yellow fever transmission in the community.

1011

FIRST REPORTED HUMAN CASE OF JAMESTOWN CANYON VIRUS INFECTION IN MONTANA, 2009

Jennifer L. Lowell[1], Denise Higgins[1], Michael A. Drebot[2], Robert S. Lanciotti[3], Erin Staples[3], Bonnie Barnard[1]

[1]Montana Department of Public Health, Helena, MT, United States, [2]Public Health Agency of Canada, Winnipeg, MB, Canada, [3]Centers for Disease Control and Prevention, Division of Vector-borne Infectious Diseases, Fort Collins, CO, United States

Jamestown Canyon virus (JCV) is a mosquito-borne zoonotic pathogen belonging to the California (CAL) group viruses and the Bunyaviridae Family. Although JCV is widely distributed and has been detected in >20 mosquito species throughout temperate North America, reports of human JCV infections in the U.S. are rare, and generally confined to the Midwest and eastern states. We report the first detected case of human JCV infection in Montana. On May 26, 2009, a 51-year-old male resident of MT with no travel history presented to the emergency room (ER) with severe acute frontal headache, fever, dizziness, and left unilateral numbness and tingling. Initial testing in the ER revealed normal blood chemistries, electrocardiogram, CT scan, and MRI, and was released. Six days post-onset, the patient visited his primary care physician exhibiting headache, muscle pain, and muscle weakness consistent with encephalitis. Acute- and convalescent-phase sera were tested for West Nile (WN), Saint Louis Encephalitis (SLE), LaCrosse (LAC), and JC viruses. Positive IgM and IgG enzyme linked immunosorbent assays (ELISA), static neutralization titres, and high WN virus IgG avidity results indicated a previous WN virus infection. However, plaque reduction neutralization tests (PRNT) revealed a four-fold rise between samples in JCV titers while an acute-phase sample IgM ELISA test result was equivocal for LAC virus antibody using a LAC antigen preparation. Because CAL group virus antibody cross reactivity may occur to various degrees in IgM ELISAs that utilize only one kind of CAL group antigen, IgM ELISA assays incorporating JCV antigen were subsequently performed. Positive JCV IgM ELISA values were documented for the patient's sera, and the presence of JCV specific IgM and the observed diagnostic rise JC specific antibody by PRNT confirmed JCV infection. This finding may represent a previously unrecognized JCV focus in MT and a need for MT clinicians to consider JCV infection in differential diagnoses for patients with unexplained febrile or encephalitic illness. As well, incorporation of both LAC and JC virus antigens in screening ELISAs should be considered when performing CAL serogroup virus serology.

1012

RE-EVALUATING THE LINK BETWEEN MALARIA AND CLIMATE

Krijn Paaijmans, Simon Blanford, Andrew Bell, Andrew Read, Matthew Thomas

Pennsylvania State University, University Park, PA, United States

Malaria transmission is strongly influenced by environmental temperature but the biological drivers remain poorly quantified. Most studies analyzing malaria-temperature relations, including those investigating malaria risk and the possible impacts of climate change, are based on mean temperatures and extrapolate from functions determined under unrealistic laboratory conditions. Here we show how the influence of temperature fluctuations and extreme events can be as, or more important than changes in mean conditions for malaria transmission. We investigated the effects of mean temperature and temperature fluctuation on key aspects of mosquito and parasite life history using a combination of novel empirical and theoretical approaches. We find that, in general, temperature fluctuation reduces the impact of increasing mean temperatures. Specifically, we show that diurnal temperature fluctuation around warmer mean temperatures slows processes such as larval development and parasite incubation, whereas fluctuation around cooler mean temperatures speeds up these processes, compared with constant temperatures. These effects suggest that by ignoring fluctuation, we may currently be overestimating malaria risk in warmer environments, and underestimating risk in cooler environments. This role of daily variation has rarely been considered in the dynamics and distribution of malaria. If we are to optimize control efforts and develop appropriate adaptation or mitigation strategies for future climates, we need to incorporate into predictive models the effects of daily temperature variation and how that variation is altered by climate change.

1013

FLORAL-BASED ATTRACTION OF *CULEX* MOSQUITOES

Sandra A. Allan, Ulrich Bernier, Eric Schmelz, Erin Vrzal

United States Department of Agriculture/ARS, Gainesville, FL, United States

Feeding on plant sugars and nectar is essential for providing energy for flight and enhanced longevity of *Culex* mosquitoes. Relatively little is known about the cues used for location of plant sugars and nectars and this study focused on volatile cues associated with flowers. Discovery of attractants associated with flowers may provide the basis for development of novel surveillance methods for *Culex* mosquitoes. Flowers of several common plant species of plants in north-central Florida were discovered to effectively attract mosquitoes of several *Culex* species in olfactometer assays. Day-old males and females with no prior exposure to sugar or flowers responded equally well to flowers. Responses of day-old females and 7-10 day old mosquitoes (previously sugar-fed but with no flower exposure) were similar. These responses increased with duration of sugar-starvation. Volatile compounds from flowers were collected by grab sampling of headspace with vacuum silonite-lined bottles and by solvent (hexane, dichloromethane) elution from solid phase adsorbents (i.e. Porapak Q, Hay-Sep). Compounds in these samples were identified by GC/MS using 3-stage trap and purge or direct injection. Several identified compounds were effective in attraction of sugar-starved day-old mosquitoes when evaluated in an olfactometer. The addition of flowers or chemicals effective in the olfactometer enhanced collections of *Culex quinquefasciatus* and *Cx. nigripalpus* in MMX traps under field conditions.

1014

THE IMPACT OF HOUSE SCREENING ON BITING BEHAVIOR OF *ANOPHELES* SPECIES AND TRANSMISSION OF LYMPHATIC FILARIASIS AND MALARIA IN GOMOA MAMPONG, A RURAL COMMUNITY IN GHANA

Ellen J. Biney, Hilaria Berkard, Kojo Y. Sakyi, Maxwell Appawu, Michael D. Wilson, Daniel A. Boakye

Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana

Malaria and lymphatic filariasis (LF) account for the majority of mortalities and morbidities due to parasitic infections worldwide. While chemotherapy is one of the strategies for malaria control, it is the mainstay for LF elimination. Vector control using bednets which has been an integral part of malaria control has been intensified recently with the inclusion of indoor residual spraying (IRS) with insecticides. These vector control interventions are known to impact LF transmission especially in areas where the same *Anopheles* species transmit both diseases. The interventions target indoor biting and/or resting mosquitoes thus leaving outdoor biting mosquitoes mostly unaffected. There is therefore a critical need to determine the role of outdoor biting mosquitoes in the transmission of malaria and LF. We examined the effect of a vector control intervention on *Anopheles* biting density and infection with *Plasmodium* species and *W. bancrofti* at Gomoa Mampong in the Central Region, Ghana where mass drug administration with ivermectin and albendazole against LF has been going on for 7 years. Mosquitoes were collected indoor and outdoor from two houses one of which has been screened against indoor biting mosquitoes and the other without any intervention. Sampling was done for five months using human landing collection and the mosquitoes identified morphologically and with PCR-RFLP. *Anopheles* species were dissected for *W. bancrofti* and examined with ELISA for infections with *Plasmodium* species. A total of 4563 *Anopheles* species were collected of which the house with screens had 2339 (51.3%) outdoors and only 80 (1.8%) indoors. Comparative biting density for the house without screens were 1575 (34.5%) outdoors and 569 (12.5%) indoors. Thus, screening appears to have shifted the biting from predominantly indoor to outdoors. All the mosquitoes dissected were negative for *W. bancrofti* reflecting the impact of the MDA in the area. None of the 334 indoor biting *Anopheles* was infected with *Plasmodium* while those collected outdoors gave a sprozoite rate of 0.42% (2/475).

1015

ROLE OF AN INVASION-INDUCED HEME PEROXIDASE FROM *ANOPHELES GAMBIAE* DURING *PLASMODIUM* DEVELOPMENT

Giselle A. Oliveira, Carolina Barillas-Mury

National Institutes of Health, Rockville, MD, United States

Invasion of *Anopheles gambiae* midgut epithelium by *Plasmodium berghei* ookinetes causes severe damage to the invaded cells which ultimately leads to apoptosis. Invaded cells induce nitric oxide synthase (NOS) expression which catalyses the formation of nitric oxide (NO). This highly reactive NO is quickly converted into nitrite (NO2-) and triggers an extensive protein nitration in ookinete-invaded cells. Our group has demonstrated that there is a lapse in time between the process of protein nitration in invaded cells and the induction of NOS. Also, the process seems to require peroxidase activity capable of catalyzing tyrosine nitration in the presence of nitrite and hydrogen peroxide, as reported previously. A search of the *An. gambiae* genome revealed the presence of 16 different peroxidase genes; ookinete invasion of midgut cells induces the expression of 5 of them. Studies using double-stranded (ds) RNA-mediated knock down of these candidates in susceptible G3 mosquitos showed that only one of them, a heme peroxidase named HPX2 (AgHPX2), resulted in a significant increase in *P. berghei* oocyst numbers compared to dsLacZ-injected controls. Using colorimetric assay with a peroxidase-specific substrate, TMB, in the presence of hydrogen peroxide, we observed

that AgHPX2 silencing decreased the peroxidase activity induced by *Plasmodium* invasion in midguts. A corresponding decrease in tyrosine nitration levels occurred when AgHPX2 is silenced in infected midguts. These findings indicate that AgHPX2 is responsible for *Plasmodium* induced peroxidase activity in the midgut and it is an important part of the protein nitration process observed in ookinete-invaded cells. Immunofluorescence microscopic studies are underway to localize AgHPX2 in midgut cells.

1016

LIFE-HISTORY OF *AEDES ALBOPICTUS* ADULTS UNDER DIAPAUSE CONDITIONS

Dina M. Fonseca, Laran Kaplan

Center for Vector Biology and Entomology Department, Rutgers University, New Brunswick, NJ, United States

Vector-born infectious diseases are experiencing resurgence in temperate regions of the World, even in developed countries. The weather in such regions often includes a period of cold weather deadly to most adult or larvae mosquitoes. *Aedes albopictus* the Asian tiger mosquito is an important vector of dengue and yellow fever and most recently of chikungunya fever. This species of mosquito is thought to be originally tropical but it has adapted to temperate conditions by producing diapausing eggs and has expanded widely worldwide both in tropical and temperate regions. Diapausing eggs are cold tolerant and dormant and are produced by adult females after they experience levels of daylight below a locally adapted critical photoperiod. We quantified the life-history traits of adults reared under summer and fall light conditions, and compared female oviposition behavior in laboratory choice tests. We compared the effects of food and access to oviposition sites on the number of eggs deposited. We also examined the effect of presence of eggs on oviposition behavior and on egg retention by females under both diapause and non-diapause conditions. Our objective is to develop a predictive model of the both the rise and fall of abundance of *Ae. albopictus* during the active season and to identify putative costs and weaknesses of the adaptation to temperate climates of this and other critical nuisance mosquitoes and disease vectors.

1017

ESTABLISHMENT OF POPULATION MALARIA SURVEILLANCE IN KAGERA REGION, TANZANIA, BY ROUTINE TESTING FOR PARASITEMIA AT TIME OF MEASLES VACCINATION AND FIRST ANTENATAL CARE ATTENDANCE TO EVALUATE IMPACT OF MALARIA INTERVENTION

Claudia Anderegg[1], Franky Chacky[2], Esther Green[1], Renata Mandike[2], Peter McElroy[3], Fabrizio Molteni[1]

[1]Research Triangle Institute, Dar es Salaam, United Republic of Tanzania, [2]National Malaria Control Programme, Dar es Salaam, United Republic of Tanzania, [3]United States President's Malaria Initiative, CDC Tanzania, Dar es Salaam, United Republic of Tanzania

As malaria interventions are scaled up, urgency exists to provide data to demonstrate impact on the disease. The most accepted impact measures are from large, infrequent, nationally representative population surveys. Programmatic decisions need more frequently measured impact data from lower administrative levels. To evaluate the real-time impact of Indoor Residual Spraying (IRS) in Kagera Region (40,838 km^2) in north-western Tanzania, we introduced a surveillance system to test for parasitemia in children and pregnant women attending reproductive and child health (RCH) clinics. All children attending for regular measles vaccinations (9 months of age) and all pregnant women attending their first antenatal care visit were tested using routinely available HRP2-based Rapid Diagnostic Tests (RDTs) in 17 health centers (2nd level facilities) located in IRS (operations begun within past two years) and non-IRS areas of Kagera. The RCH clients came from a catchment of 71 villages. In Jun-Oct 2009, 77% of children and pregnant women attending RCH clinics were tested with an RDT. Among clients tested, 3,884 and 2,916 came from IRS or non-IRS areas, respectively. The overall positivity rate was 2.8% (95% CI: 2.5% to 3.5%) and 9.6% (95% CI: 8.9% to 11.1%) in IRS and non-IRS villages, respectively. No parasitaemia was detected in 29% of IRS villages (n=28) compared with 12% of non-IRS villages (n=43). Low positivity rates (<5%) were detected in 54% of IRS villages compared to 9% of non-IRS villages. In 49% of non-IRS villages the positivity rate exceeded 10% compared to 4% of IRS villages. In conclusion, children receiving measles immunization and pregnant women attending RCH clinics in Kagera represent a readily accessible population for directly monitoring the impact of various malaria interventions. This system is providing data over time and will allow us to evaluate the impact of single or multiple vector control strategies, particularly IRS alone or combined with insecticide treated bednets (recently distributed to all children <5 years).

1018

MOSQUITO FEEDING AND POST-BLOOD MEAL FLIGHT BEHAVIOR IN BERNALILLO COUNTY, NEW MEXICO: IMPLICATIONS FOR TRANSMISSION AND CONTROL OF WEST NILE VIRUS

Jacob A. Greenberg[1], Mark DiMenna[2], Bruce Hofkin[1]

[1]University of New Mexico, Albuquerque, NM, United States, [2]City of Albuquerque, Department of Environmental Health, Albuquerque, NM, United States

West Nile virus (WNV) causes annual mortality and morbidity in animals, including humans, across North America. In Bernalillo County, New Mexico, potential mosquito vectors have been identified (*Culex quinquefasciatus, C. tarsalis,* and *Aedes vexans*), but little is known about which species is most important in maintaining the natural, sylvatic transmission cycle or in transmitting WNV to mammals of interest. Since 2004, mosquitoes have been collected weekly, identified to species, and tested for WNV. Since 2006, mosquito blood meals have been analyzed to determine which, if any, species is most likely to feed on known avian reservoir hosts or on mammals of interest. Of the potential mosquito vectors, *C. quinquefasciatus* derived >85% of its blood meals from avian hosts; of these >20% were American robins, known to be competent WNV reservoir hosts. *Aedes vexans* fed almost exclusively on mammals (>95%), while *C. tarsalis* fed on mammals and birds (62% and 38%, respectively). Bernalillo County has a zoo, and because zoo animals are kept in enclosures, mosquito flight distance can be estimated, post-feeding from enclosure to trap site. Analysis indicated that mosquito species generally took blood meals less than 140 meters from the trap site. For the 2004-2008 seasons, prevalence of WNV was higher in *C. quinquefasciatus* than *C. tarsalis* and *A.vexans*, 6.56%, 3.99%, and 0.83%, of pools tested respectively. Preliminary data suggest that *C. quinquefasciatus* with its strong preference for avian hosts and high infection rate it is important in maintaining the sylvatic cycle of the virus as well as in transmission to mammals.

1019

AEDES AEGYPTI INFECTION BARRIER MAY LIMIT URBAN TRANSMISSION OF MAYARO VIRUS IN IQUITOS, PERU

Kanya C. Long, Sarah A. Ziegler, Nicole Hausser, Stephen Higgs, Robert B. Tesh

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

Mayaro virus (MAYV) is a forest-associated, mosquito-borne alphavirus circulating in South America. Although current evidence suggests a sylvatic transmission cycle maintained by canopy-dwelling mosquitoes such as *Haemagogus janthinomys*, the ability of MAYV to emerge and to be transmitted by an urban vector could have significant implications for public health. In Iquitos, a city of approximately 350,000 people in the Amazon region of Peru, an average of six cases of Mayaro fever are identified annually through a clinic-based surveillance network established

by the U.S. Naval Medical Research Center Detachment-Lima. Due to the identification of Mayaro fever cases in Iquitos clinics, and because *Aedes aegypti* is well-established in the city, we assessed the potential for *Ae. aegypti* to serve as an urban vector for MAYV. To investigate laboratory vector competence, infection, dissemination and transmission rates of MAYV in an F1 generation of *Ae. aegypti* from Iquitos, Peru were determined following artificial and viremic mouse feedings. Infection rates ranged from 0% (0/22) at a blood meal titer of 1.3 x 105 pfu/ml of MAYV to 84% (31/37) at 2.2 x 107 pfu/ml. The rate of dissemination varied from 60% to 100% of infected mosquitoes, independent of dose. Importantly, transmission of MAYV from 70% (21/30) of infected mosquitoes was demonstrated by capillary tube feeding and by bite on suckling mice. These data suggest that a midgut infection barrier, rather than midgut escape or salivary gland barriers, may be a limiting factor to *Ae. aegypti* serving as a vector in Iquitos. Human viremias may be below the threshold of infectivity for MAYV in this strain of A*e. aegypti*, explaining the limited number of cases of MAYV seen in urban Iquitos and the strong rural bias to human transmission in the region.

1020

ENGINEERED STERILE MOSQUITOES FOR DENGUE CONTROL - FROM LAB TO FIELD

Luke Alphey

Oxitec Limited, Oxford, United Kingdom

Recent advances in insect genetic engineering have opened new possibilities for the control of mosquitoes and hence of mosquito-borne diseases. Oxitec has developed strains of *Aedes aegypti* and *Aedes albopictus* which are homozygous for one or more dominant lethal genes and are "genetically sterile" unless provided with the repressor molecule tetracycline in the diet. Use of such strains for mosquito control, a method known as RIDL, is based on the Sterile Insect Technique (SIT) which has been used successfully for the suppression or local elimination of several insect species in agriculture. Sterile male mosquitoes are released continually over a wide area to mate with the target pest population; no progeny result from these matings and the target population declines. Mathematical modeling indicates RIDL-SIT would be effective against *Aedes* mosquitoes. The first engineered strains with the necessary genetic properties ('RIDL strains') have been successfully tested in confined conditions for mating competitiveness with wild-type mosquitoes, suppression and a range of life history and behavioural traits in a range of locations and conditions. Preparations are underway for field trials to demonstrate suppression of wild populations. This presentation will summarise the results of experiments to date and discuss the options for testing and programmatic use of such technology.

1021

DIFFERENCES IN THE CLINICAL FEATURES ACCORDING TO GENOTYPES OF *ORIENTIA TSUTSUGAMUSHI*

Dong-Min Kim[1], Seung-Hyun Lee[2]

[1]Chosun University College of Medicine, Gwangju, Republic of Korea, [2]Seonam University College of Medicine, Namwon, Republic of Korea

Scrub typhus is an acute febrile illness caused by *Orientia tsutsugamushi* (*O. tsutsugamushi*) transmitted by bites of thrombiculid mites. The aim of this study was to investigate whether there are any differences in clinical features and severity between the Boryoung and Karp genotypes. Nested polymerase chain reactions (PCR) were performed with the blood buffy coats or eschars of patients with suspected scrub typhus who visited six hospitals from September to December 2006. We compared the clinical features and severity of illness in patients confirmed by nested PCR to have the Boryoung and Karp genotypes. Of 191 patients definitively diagnosed with scrub typhus, 168 were positive for nested PCR. Of these 168 patients, 133 were clustered as having the Boryoung genotype and 19 as having the Karp genotype. In this prospective study, the eschar detection rate was extremely high because of the thorough physical examinations carried out. Eschars and rashes were observed in 97% and 94% of the patients in the Boryoung group, but in only 73.7% and 68.4% of the patients in the Karp group, and the differences were statistically significant. However, there were no significant differences in complication rates, need for intensive care or mean lengths of hospital stay. In conclusion, our data indicate that the frequency of occurrence of eschars and rashes may depend on the genotype of *O. tsutsugamuchi*.

1022

MUTANS STREPTOCOCCI: ANTIBIOTIC SUSCEPTIBILITY, CO-RESISTANCE AND SELECTION OF COTRIMOXAZOLE RESISTANCE AMONG PARTICIPANTS IN MULAGO DENTAL AND TASO CLINICS IN UGANDA

William Buwembo[1], Rwenyonyi Mugisha[1], Gote Swedberg[2], Fred Kironde[1]

[1]Makerere University, Kampala, Uganda, [2]Uppsala University, Uppsala, Sweden

The objective of this study was to evaluate the antibiotic susceptibilities of cariogenic mutans streptococci isolated from participants attending a dental and an HIV clinic in Kampala-Uganda, the antibiotic co-resistance and whether cotrimoxazole prophylaxis selects for resistance in these isolates. *In vitro* susceptibility to seven antibiotics were evaluated for 84 mutans streptococci (one isolate per person) from oral bacterial flora of 171 participants with dental caries. Isolates were confirmed by DNA analysis while the antimicrobial susceptibilities were assessed by E test and Kirby Bauer disc diffusion methods. Resistance to cotrimoxazole was also compared to 64 mutans streptococci (one isolate per person) isolated from 204 HIV positive participats taking cotrimoxazole prophylaxis. In the non prophylaxis group, 14.3% and 23.8 % of the isolates were resistant to cotrimoxazole and amoxicillin, respectively. Resistance to ceftriaxone, vancomycin, Chloramphenical, erythromycin and tetracycline was found in 46.4, 27.3, 14.3, 11.9 and 54.8% of the isolates, in that order. Isolates which were resistant to ceftriaxone were resistant to tetracycline (Wilcoxon Signed Ranks Test, Z=0.990, P=0.332) while strains which were resistant to erythromycin were also resistant to chloramphenical (Wilcoxon Signed Ranks Test, Z=-0.611, P= 0.541). The antibiotic resistance patterns of Streptococcus. mutans and Streptococcus sobrinus were similar (Kruskal-Wallis Test, P<0.005). Cotrimoxazole resistance was higher among the group on prophylaxis (54.7% Vs. 14.7%, Odds ratio: OR 7.24, 95%(3.10-17.21) (P is 0.000)) as compared to the non prophylaxis group. In conclusion, there was a high rate of resistance to Tetracycline and Ceftriaxone. The high frequency of non-susceptibility to tetracycline and Ceftriaxone among the mutans streptococci limits their use as therapeutic or prophylactic agents for diseases caused by these organisms. Also noted was that Cotrimoxazole prophylaxis selects for resistance in these isolates and thus a need for effective periodic surveillance of antibiotic susceptibility of the tooth decay causing streptococci.

1023

A PILOT STUDY OF THE EPIDEMIOLOGY OF NEISSERIA MENINGITIDIS CARRIAGE IN CHILDREN IN BAMAKO, MALI

Nicole E. Basta[1], Samba O. Sow[2], Mahamadou M. Keita[2], James Stuart[3], Musa Hassan-King[4], Boubou Tamboura[2], Julia Bennett[5], M. Elizabeth Halloran[1], Martin Maiden[5], Brian Greenwood[6]

[1]University of Washington, Seattle, WA, United States, [2]Center for Vaccine Development-Mali, Bamako, Mali, [3]University of Bristol, Bristol, United Kingdom, [4]MenAfriCar Consultant, London, United Kingdom, [5]University of Oxford, Oxford, United Kingdom, [6]London School of Hygiene and Tropical Medicine, London, United Kingdom

Meningococcal meningitis outbreaks caused by *Neisseria meningitidis* are responsible for significant morbidity and mortality in the African meningitis belt. Asymptomatic pharyngeal colonization with *N. meningitidis*, known as carriage, is more common than invasive disease and the source of transmission. Yet, the epidemiology of carriage in this region is not

well understood. The African Meningococcal Carriage Consortium (MenAfriCar) aims to define the epidemiology of *N. meningitidis* carriage and evaluate a newly developed conjugate meningococcal serogroup A vaccine. Here, we describe the epidemiology of *N. meningitidis* carriage in children in Mali prior to the vaccine's introduction. We estimate the prevalence of carriage, assess potential risk factors, and evaluate two methods for determining carrier status. We conducted a cross-sectional pilot study of 250 children in Bamako, Mali. Eligible children were enrolled in school, 5-15 years old, healthy, and not vaccinated against meningitis for two years. Two oropharyngeal swabs were collected from each child; one by swabbing the posterior pharynx behind the uvula and the other by swabbing the posterior pharynx and one *tonsillar fossa*. Samples were processed using standard bacteriologic methods and 16S RNA sequence analysis to identify *N. meningitidis.* A questionnaire provided information about potential risk factors such as household size, smoking, and recent respiratory symptoms. The prevalence of *N. meningitidis* carriage among the 250 children (at least one swab positive based on 16S RNA analysis) was 21.2% (95% CI 16.3-26.8). The average age of carriers and non-carriers was similar (10.4 vs. 10.6 years). Carriers were more likely to be male (55% vs 49% of non-carriers), to live with < 8 people (36% vs. 27%), and to live in a house where no one smokes (51% vs. 36%). The swabbing methods had a high concordance (0.9) and a *kappa* of 0.61, denoting substantial agreement beyond chance. In conclusion, we found a high prevalence (21.2%) of *N. meningitidis* carriage among children in Bamako, Mali. Risk factors associated with carriage in Europe and the US did not appear to be associated with carriage in children in Mali, though further investigation is needed. Our results highlight the need for interventions that prevent or reduce meningococcal carriage, thus reducing transmission and preventing invasive disease.

1024

DETECTION OF Q-FEVER SPECIFIC ANTIBODIES UTILIZING COM-1 ENZYME-LINKED IMMUNOSORBENT ASSAYS

Erin Glennon, Hua-Wei Chen, Zhiwen Zhang, Wei-Mei Ching

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

Coxiella burnetii, the causative agent of Q-fever, is a Gram-negative, obligate intracellular, and dimorphic bacteria. Acute Q-fever presents itself with flu-like symptoms, hepatitis, or pneumonia, and is usually a self-limiting disease with a low mortality rate. Chronic Q-fever, while less prevalent, often results in endocarditis, which has a much higher mortality rate. Since the symptoms of acute Q-fever are highly nonspecific, diagnosis can prove very difficult. The currently accepted method is indirect immunofluorescence assay (IFA) using the whole cell antigen. However, the process of isolating and purifying this whole cell antigen involves working with the highly dangerous *C. burnetii* in a Biosafety Level 3 lab (BSL-3), and the quality of the purified antigen is often inconsistent. Previously, six immunodominant antigens were identified by immunoblotting using two-dimensional gel separated whole cell antigens against patient sera. One of them, a 27 kDa outer membrane protein (Com-1) is *C. burnetii* specific. The recombinant Com-1 was purified and refolded to develop an enzyme-linked immunosorbent assay (ELISA). In this report the conditions for IgM and IgG detection using Com-1 in ELISA were optimized, and it was found that amplification using biotin tagged anti-human IgG and IgM along with steptavidin-HRP polymer could increase the signal and improve the sensitivity of the assay. Results from the optimized ELISA were very consistent with IFA data, indicating that the recombinant Com-1 could be used to replace the whole cell antigen for detection of Q-fever specific antibodies.

1025

SEROSURVEY OF LEPTOSPIROSIS AMONG PATIENTS WITH ACUTE FEBRILE ILLNESS IN ACCRA

Janice A. Tagoe[1], Naiki Puplampu[1], Shirley Cameron Odoom[1], Bassem Abdul-Rahman[2], Engy Emil Habashy[2], Billy Pimentel[2], Karl Kronmann[3], Kwadwo Koram[1], Michael Wilson[1], Moustapha Abdel[2], Edward Nyarko[4], Prince Agbenohevi[4]

[1]Noguchi Memorial Institute for Medical Research, Greater Accra, Ghana, [2]Naval Medical Research Unit No. 3, Cairo, Egypt, [3]Naval Medical Research Unit No. 3, Ghana Detachment, Greater Accra, Ghana, [4]37th Military Hospital, Greater Accra, Ghana

The disease burden of some infectious diseases such as leptospirosis in Ghana has not been well defined because of difficulties in disease diagnosis and also because Malaria, which can have similar presentation and is hyper endemic in Ghana, may be mistakenly diagnosed instead. The Ghana Detachment of NAMRU-3 has recently completed a year-long study of acute febrile illness patients in Accra. 166 patients were enrolled in this study. Patients presenting at the hospital with fever lasting 2 days or more and a temperature of >38°C were examined. Those meeting enrollment criteria were informed of the study and signed an informed consent. Parents or guardians signed the consents for their children and assent was obtained from the children. Patients with obvious focal clinical diagnosis and children under the age of 4 were excluded. Patients, who met the AFI case definition, completed a case record form and blood samples were collected for malaria thick and thin film, blood cultures and serology. Positive results were confirmed at NAMRU-3, Cairo. The *Leptospira* IgM ELISA (PanBio Diagnostic's kit) was used as screening test for the diagnosis of acute leptospirosis. A value of 1.1 (according to manufacturer) was used as cut-off for further testing by MAT (Microscopic agglutination test). The MAT was performed on ELISA-positive sera to determine the most reactive *Leptospira* serogroups. A reactive MAT was determined by titer ranges, 1:200-1:25600. Of the 166 patients, 13 (7.8%) cases showed seroreactivity to *Leptospira* IgM by ELISA, 8 have so far been screened by MAT, 1 was reactive for serovar *Georgia* (1:3200) and 2 were reactive for the genus *L. Biflexa* but one showed reactivity for serovars *(Andamana*; 400) and *Bratislava* (400) and the other, serovars (*Andamana; 6400*); Bratislava (400) and Bataviae (400). The hospital diagnosis for 7 of these cases was malaria, even though the malaria smear results were all reported as negative. This suggests that almost 8% of patients that are diagnosed with malaria in our hospitals could have Leptospirosis. Leptospirosis in Ghana is currently underreported and more extensive study has to be conducted. This information is important to determine the disease burden of AFI etiologies, other than malaria, and to provide better treatment to patients in Ghana.

1026

TEMPORAL TRENDS OF BURULI ULCER DIAGNOSES IN ANANEKROM, GHANA

Julie A. Clennon[1], William Opare[2], Sara Vrabec[1], Ellen A. Whitney[1], Edwin Ampadu[2], Lance A. Waller[1]

[1]Emory University, Atlanta, GA, United States, [2]National Buruli Ulcer Control Programme, Ghana Health Service, Accra, Ghana

Buruli ulcer (BU), caused by the bacterium *Mycobacterium ulcerans*, is a devastating skin disease that can result in significant morbidity. It is endemic in tropical and sub-tropical regions in Western Africa including Ghana, where it is found in the southern part of the country. Within Ghana, the village of Ananekrom, located in Asante Province, is known to have the greatest number of reported cases (N = 34) during 2009. Using data collected by the Ghana Health Service, National Buruli Ulcer Control Programme during 2009, we examined the monthly temporal trends of diagnosed BU cases by clinical manifestation (nodule, plaque, edema, ulcer) and compare them with weather data (minimum average temperature, maximum average temperature, precipitation, normalized difference vegetation index). Clinical features were considered separately

and together given possible differences in the lag period between the development of clinical symptoms and diagnosis due to healthcare seeking behavior. From July to November, there was a gradual increase in the number of diagnosed cases. During the peak month of November (N = 8), half the cases were nodules and the other half were ulcers. Cross-correlation analyses allowed a comparison of lags between case counts and environmental variables through the year. A significant 2 month temporal lag was detected between plaque cases and ulcer cases. There was no correlation detected between any BU clinical manifestations and rainfall. A correlation between minimum average temperature and non-ulcerative cases was detected. Given the small numbers of cases, it is possible that elevated case counts may have been influenced by BU educational activities in the area. Understanding the temporal dynamics of the appearance of BU cases will help enable better identification of possible factors influencing patterns of infection, treatment, and reporting.

1027

COMBINATION OF DNA VACCINE PLASMIDS CARRYING THE GENE CODING FOR THE TRUNCATED 47 KDA ANTIGEN AND THE CODON OPTIMIZED GENE OF 56 KDA ANTIGEN CAN PROVIDE EXCELLENT PROTECTION AGAINST THE HOMOLOGOUS CHALLENGE OF *ORIENTIA* KARP STRAIN IN A MOUSE MODEL

Wei-Mei Ching, Chien-Chung Chao, Teik-Chye Chan, Zhiwen Zhang, Erin Huber, Hua-Wei Chen

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

Scrub typhus is an acute, febrile disease caused by infection with *Orientia tsutsugamushi*. At the present time there is no vaccine for scrub typhus. Western blot analysis of whole cell antigen with naturally infected patient sera revealed several potential antigens, including 56 kDa, 47 kDa and 110 kDa proteins. The 56 kDa protein appeared to be the most immunodominant protein which account for 10- 15% of the total amount of expressed proteins, making it one of the vaccine candidates. Recombinant protein r56Kp has been shown to provide excellent homologous protection of immunized mice. However, the protection provided by DNA plasmid VR1012 carrying the full ORF of 56Kp is poor. One of the possible reasons could be the low expression level of the 56 kDa antigen gene in mammalian system. In order to increase the expression level, the full length of 56 kDa antigen gene with optimized codon for mammalian expression was cloned into VR1012 (p56OptKp). Previously we have shown that the plasmid carrying the gene of 47 kDa antigen also provided very good homologous protection and some heterologous protection. However this 47 kDa antigen belongs to the family of HtrA and exhibits a very high sequence homology (46% identical, 70% semi-conserved, and 81% similar) with human protease HtrA1 in its central portion (aa 85-235). To avoid the concern of autoimmune responses for this vaccine candidate, we have successfully cloned a truncated fragment e (coding aa 236-477) into the VR1012 (p47eKp). The combination of p56OptKp and p47eKp at 1:1 ratio was evaluated for protective efficacy at two different doses (100 ug and 50 ug). Mice were immunized twice at four weeks interval and challenged at four weeks after the last immunization. The morbidity and mortality were monitored daily for 21 days post challenge. Close to 90% of the immunized mice were protected against the lethal challenge at different doses. These results strongly suggested that a successful vaccine formulation can be achieved by combining these two modified DNA plasmids.

1028

A PREDOMINANT CLONAL COMPLEX OF VANCOMYCIN-RESISTANT *ENTEROCOCCUS FAECIUM* IS ASSOCIATED WITH THE LARGE VRE OUTBREAK IN RIO DE JANEIRO, BRAZIL

Vânia L. Merquior[1], Filomena S. Rocha[2], Jaqueline M. Morais[2], Adriana R. Faria[1], Beatriz N. Monteiro da Silva[1], Ludmilla D. Teixeira[2], **Lucia M. Teixeira**[2]

[1]Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil, [2]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Vancomycin-resistant *E. faecium* (VREF) strains have been worldwide reported among the leading causes of nosocomial infections. Since the emergence of VREF in Brazil, very few studies have been conducted to investigate phenotypic and molecular diversity of these strains in this country. We report the phenotypic and molecular characterization of VREF isolates from different Brazilian hospitals located in Rio de Janeiro state. VREF isolates, obtained from clinical sources or rectal screening in hospitalized patients seeking medical care at 15 hospitals over an 8-year period (2002 to 2009), were included in the study. Phenotypic characterization was based on conventional physiological tests. Antimicrobial susceptibility was determined by the disk diffusion method. MICs of vancomycin were determined by the E-test and the presence of *van* genes and *esp* gene was investigated by PCR. Genetic diversity was evaluated by pulsed-field gel electrophoresis (PFGE), using *Sma*I as the restriction enzyme, and by analysis of multiple-locus-variable number of tandem repeat (MLVA) for 6 genomic loci. *E. faecium* isolates harbored the *vanA* gene and expressed high-level resistance to both vancomycin and teicoplanin. All the isolates were resistant to ampicillin, erythromycin, imipenem, teicoplanin and showed high-level resistance to streptomycin and gentamicin. The majority of the isolates (>80%) was also resistant to ciprofloxacin and norfloxacin. All the isolates were susceptible to linezolid, nitrofurantoin and about 90% of the isolates were susceptible to fosfomycin. Resistance to tetracycline (about 6%) was due to the presence of the *tetO* and *tetM* genes. Both typing schemes were highly concordant and they identified a prevalent clonal complex (CC) designed as profile A by PFGE and MT12 by MLVA. The isolates belonging to the prevalent CC harboured the *esp* gene. These data strongly suggest the epidemic dissemination of a single CC of VREF in the area investigated.

1029

CLINICAL MANIFESTATIONS OF SYPHILIS IN A RURAL UGANDAN HOSPITAL: ARE WE DOING ENOUGH TO DETECT SYPHILIS AMONG HIV PATIENTS IN AFRICA?

Peterson Stephen Kyebambe

Kabale Regional Hospital, Kabale, Uganda

Syphilis is still an important problem worldwide. With the advent of HIV/AIDS, syphilis became overshadowed and neglected resulting in its being missed in many patients yet the two diseases tend to coexist. Many people who are tested for HIV are not screened for syphilis, with the consequence of them suffering the complications of the disease undetected. For instance, 2 patients at our HIV clinic nearly lost their sight to previously undiagnosed syphilis manifesting as 'idiopathic chorioretinitis'. Objectives of this study were: 1) to re-awaken awareness of syphilis as a still important infection in Africa especially in HIV patients; and 2) to find if there is a difference in systemic symptoms and signs between HIV positive and HIV negative patients at our site. We randomly checked through case files of patients who had a final diagnosis of syphilis at discharge or death over the years 2002-2009.We looked at their age, gender, HIV status, syphilis status as well as symptoms and signs pertaining to the different organ systems-comparing the HIV positive with the HIV negative. Sixty-five subjects had both an HIV test and a confirmatory syphilis test. It is these 65 whose symptoms and signs that we compared. Of the 102 case files we reviewed, 85 had both a screening test result- RPR/VDRL plus the confirmatory TPHA. Eight had only a screening test- RPR/VDRL and 9 had

no evidence of a syphilis test in their charts. Seventy had an HIV test result, with 36 being HIV negative and 34 HIV positive while 32 had no HIV test result. Sixty-five subjects had both an HIV test and confirmed syphilis test with the summary as noted:Systemic symptoms/signs found:

Skin : 18.2% of HIV+s,18.8% of HIV-s Oral : 9% of HIV +s, 0% of HIV-s Musculoskeletal :18.18% of HIV+s,9.38% of HIV-s Respiratory:12.12% of HIV +s,18.75% of HIV-sCardiovascular :15.15% of HIV +s,15.63% of HIV-s.Neurological :51.5% of HIV+s, 31.25% of HIV-s Ocular : 0.0% of HIV+s,6.25% of HIV-s Abdominal :36.36% of HIV+s, 25% of HIV-s Genitourinary:3.03% of HIV+s,15.63 %of HIV-s,ENT :0% of HIV+s,and 0% of HIV-s. In conclusion: 1) syphilis is still a common and important disease, therefore routine testing for it is necessary by all clinicians; and 2) there were some differences in the frequency of different body systems affected in syphilis patients who are HIV positive versus those who are HIV negative. However with our data, the differences were not statistically significant. A bigger study is needed.

1030

MOLECULAR EPIDEMIOLOGY OF GROUP A STREPTOCOCCUS AMONG CHILDREN AGED 5 TO 15

Mahamadou M. Keita[1], Abdoulaye Berthe[1], Mariam Samake[1], Boubou Tamboura[1], Milagritos Tapia[2], Samba O. Sow[1]

[1]Center for Vaccine Development Mali, Bamako, Mali, [2]Center for Vaccine Development Baltimore, Baltimore, MD, United States

Group A Streptococcus (GAS) is one of the most common and versatile human pathogens, causing superficial invasive infections as well as rheumatic fever and other immunological sequelae. Recently, multivalent M type-specific vaccines have shown promising results in trials. To use such vaccines in developing countries, the GAS burden and distribution of *emm*-types must be characterized. Four public elementary schools in two low -income quartiers (Djicoroni -Para and Sébénicoro) in Bamako Mali were identified and a census of the students was performed at the beginning of the study and at the beginning of the school year. Study personnel were present in each school infirmary to identify 5- to 15 year old children with pharyngitis and complete a clinical history and physical exam. A throat swab was obtained and processed to culture GAS according to standard procedures. *Emm*-typing was performed according to the Centers for Diseases Control and Prevention Protocol. All children with GAS pharyngitis were treated with a 10- day course of penicillin or erythromycin (if allergic to penicillin). From 30 May 2006 to 29 September 2009 of 12.500 students under surveillance per year 1757 presented with pharyngitis, 614 from Sébénikoro and 1143 from Djicoroni-Para. Of these 468 (26%) were positives for GAS. Almost half of the cases (61%) were over 10 years of age and most were female (60%). In addition to the classic symptoms which are pain (99. 8%) and difficulty swallowing (99.1%) others predominants symptoms such as fever (61. 5%), abdominal pain, (25. 4%), nasal running (38.5%), hypertrophied tonsil (74.6%), hypertrophy of anterior cervical lymph nodes (75.2%) were also present. Most of the children with GAS positive were sharing the bed with others children respectively 335 cases (71.5%) for 0 to 2 children in the same bed, 126 (27%) for 3 to 5 and 7 cases (1.5%) for those who were more than 6 children in the same bed. These data suggest that GAS is an important cause of pharyngitis in Malian schoolchildren. *Emm* -type distribution of pharyngitis cases appears to cover a broader age range. More data is needed to determine the burden of GAS infections and potentially introduce a vaccine.

1031

LEPTOSPIROSIS-ASSOCIATED SEVERE PULMONARY HEMORRHAGE SYNDROME IS ASSOCIATED WITH ELEVATED IL-10 AND INCREASED CD19+ B CELLS AND ΓΔ T CELLS POPULATION

Eliana A. Reis[1], Elsio A. W, Jr.[1], Guilherme S. Ribeiro[1], Andrea C. Santos[1], Alcineia O. Damião[1], Jorge Clarencio S. Andrade[1], Nilzelene S. Silva[1], Albert I. Ko[2], Mitermayer G. Reis[1]

[1]Fundação Oswaldo Cruz, Salvador-Bahia-Brazil, Brazil, [2]Cornell, Ithaca, NY, United States

Leptospirosis, a spirochete zoonotic disease, is increasingly recognized as an important cause of hemorrhagic fever. The objective of this study was to determine the cytokine profile and cell phenotype surface markers of patients with leptospirosis-associated severe pulmonary hemorrhage syndrome (SPHS), infected with *Leptospira interrogans*. Peripheral blood samples of leptospirosis patients with SPHS (n=5) and without (n=17) were examined for cytokine production using Cytometric bead array to measure the levels of IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IFN-γ and TNFα. Cell phenotype population of lymphocytes γΔ, CD3, CD4, CD8, CD19 surface markers were performed by FACSAria analysis. The major cell population of patients consisted of approximately γΔ T lymphocytes (5.8%), CD3+ (44.4%), CD4+ (21.43%), CD8+high (8%) and CD19+ (12.4%) cells. Additionally, patients with SPHS showed increased expression of CD86+ on CD19+ B cells. Production of anti-inflammatory cytokines, such as IL-10 and IL-4 was prominent in patients with SPHS. Interestingly, IFN-γ, IL-12 and TNF-α production was suppressed in the absence of SPHS. Furthermore, bacteremic load had no association with outcome response. In conclusion, our preliminaries results indicated that leptospirosis patients with SPHS presented Th2-like immune response while those without SPHS showed type-1 response.

1032

ESTIMATE OF RISK FACTORS FOR LEPTOSPIROSIS IN CARTAGENA DE INDIAS - COLOMBIA

Guy Mejia[1], Enrique Ramos[1], Enrique Mazenett[2], Jorge Morelos[2], Dacia Malambo[3], Ronald Maestre[1], Gustavo Mora[1], Doris Gomez[4]

[1]Universidad de Cartagena, Cartagena, Colombia, [2]Departamento Administrativo Distrital de Salud de Cartagena, Cartagena, Colombia, [3]Universidad de Cartagena, Departamento de Biologia, Cartagena, Colombia, [4]Programa de Doctorado en Medicina Tropical, Universidad de Cartagena-SUE CARIBE, Grupo UNIMOL, Cartagena, Colombia

Leptospirosis is a zoonosis of worldwide distribution, focused on the tropics, which can occur in both rural and urban centers whose transmission is integrated into the ecology of wild mammals and pets, as well as to the availability of water sources, neutral or alkaline soils and abundant rainfall in tropical countries. The aim of this study was to estimate the risk for transmission of leptospirosis based on the mortality in Cartagena de Indias - Colombia. The study was performed during the period November 2009 - March 2010, and applied on a sample of 20.003 dwellings, distributed in three locations of the city, by random sampling by cluster (MACO) multistage. It was designed a survey to identify risk factors for leptospirosis in the urban cycle, which included both socio-demographic variables, as the variables associated with risk of exposure and morbility. The main findings of this research are: The percentage distribution of employment shows that 60% of the population corresponds to students and housewives. Less than 3% of the inhabitants of the dwellings surveyed play occupations characterized as hazardous for leptospirosis. 40% of the population was in contact with animals. The analysis of risk factors based on morbidity, in terms of administrative zoning of the city, shows that living in the locality 3 is a risk factor in the transmission of leptospirosis, the town submitted an odd ratio of 3.4 (95% CI: 1,3 - 8,7). On the other hand living in the site 2 can be considered as a protective factor against disease (OR = 0.14, 95% CI:

0.019 to 1.0). The untreated water consumption appears to be a risk factor for leptospirosis (OR = 8.2, 95% CI: 1.1 - 61.6), the widespread practice of storing water for drinking is also associated with a failure to deal with it, even in locations where there is a continuous service. The risk associated to the environment showed two important factors: first, established a OR: 36.6 for contact with cattle with 95% CI 5.1 to 306.8. This finding is suggestive of the possible mode of transmission and risk of this infection in the city.

1033

DIFFERENT OUTCOMES OF EXPERIMENTAL LEPTOSPIRAL INFECTION IN MOUSE STRAINS WITH DISTINCT GENOTYPES

Cleiton S. Santos[1], Julio O. Macedo[1], Mauricio S. Bandeira[2], Adenizar D. Chagas Jr[1], Alan J. McBride[1], Flavia W. McBride[2], Mitermayer G. Reis[1], **Daniel A. Athanazio**[2]

[1]Oswaldo Cruz Foundation, Salvador, Brazil, [2]Federal University of Bahia, Salvador, Brazil

The mouse disease model has the advantage of a broad array of immunologic and genetic manipulation tools available for basic research. Some studies on transgenic and/or mutant mouse strains as models for experimental leptospirosis have been reported, however, the wider use of such models is hampered by our poor understanding of the outcome of experimental leptospiral infection among the different mouse strains available. We studied the outcome of infection by a virulent strain of Leptospira interrogans serogroup Icterohaemorrhagiae strain Cop in four commonly used wild-type mouse strains: A, CBA, BALB/c and C57BL/6. All infected animals received an intraperitoneal low inoculum (1.0 x 10e3) or high inoculum (1.0 x 10e6) in two independent experiments with 5-15 animals per group. Controls were inoculated with 1ml of sterile EMJH medium. The outcome endpoints evaluated in this study were survival, presence of kidney lesions, leptospire load in kidney samples, microscopic agglutination test (MAT) titre and anti-leptospiral IgG antibody levels. None of the mice strains were susceptible to lethal leptospirosis. However, several strains developed specific outcomes associated with sub-lethal leptospirosis. The difference of anti-Leptospira IgG levels between mice strains were significant in animals infected with lower inoculums with lower IgG levels observed in strain A. In both experiments and regardless of inoculum size, BALB/c mice produced lower levels of anti-Leptospira agglutinating antibodies, had a lower leptospiral load in kidney tissue and did not develop interstitial nephritis. Mouse strain A exhibited a high load of leptospires in kidney samples indicating that it may be the strain of choice for studies requiring large amount of leptospires recovered from murine renal tubules. Mouse strains CBA and C57BL/6 developed inflammatory lesions suggesting their use in studies on interstitial nephritis.

1034

INTER-LABORATORY AGREEMENT OF PULSED-FIELD GEL ELECTROPHORESIS IDENTIFICATION OF *LEPTOSPIRA* SEROVARS

Katrin Mende[1], Renee L. Galloway[2], Sara Becker[1], Miraim L. Beckius[1], Clinton K. Murray[1], Duane R. Hospenthal[1]

[1]Brooke Army Medical Center, Fort Sam Houston, TX, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States

Leptospirosis is a worldwide zoonosis caused by any of more than 250 *Leptospira* serovars. Serovar classification occurs through cross-absorption agglutination testing, a complex task which most laboratories cannot perform. The Centers for Disease Control and Prevention (CDC) developed a pulsed-field gel electrophoresis (PFGE) technique to identify *Leptospira* serovars. We measured its inter-laboratory reproducibility. A blinded exchange of 93 Leptospiraceae strains occurred between Brooke Army Medical Center (BAMC), who exported 36 strains, and Centers for Disease Control and Prevention, who exported 57 strains. Each strain was assigned a unique code at the providing institution. Exchanged strains included reference, clinical, and uncharacterized isolates. PFGE was performed with *NotI* using *Salmonella* Braenderup H9812 (digested with *XbaI*) as a standard. Gel images were analyzed with BioNumerics software and compared to patterns in each laboratory's database. The CDC database contained patterns of more than 800 strains; the BAMC database, more than 300. CDC identified 31 of 36 strains; 3 were misidentified (misID) and 2 did not match (noID) serovars in their database. BAMC identified 43 of 57 strains; 2 were misID and 12 were not in their database (noID). Overall, 93.7% (74 of 79) of strains present in each receiving laboratory's database were correctly identified. Exchange of gel images for noID isolates revealed most of them matched the pattern in the providing lab's database. Of the 5 misID isolates, 4 were not identified correctly as the reference strains were named differently, although patterns produced were the same. For the fifth isolate, the wrong serovar was sent to CDC. In conclusion, identification of leptospiral serovars by conventional methods is not readily available or practical. Molecular typing methods and equipment have become much more common in both research and clinical labs. The PFGE methodology showed good inter-laboratory reproducibility, but the integrity of reference databases is an issue with errors in *Leptospira* serovar identification.

1035

COMMUNITY PREVALENCE RATE OF METHICILLIN RESISTANT *STAPHLOCOCCUS AUREUS* (MRSA) ASSOCIATED WITH PVL AMONG QATAR UNIVERSITY STUDENTS

Adil Makkiya, Luna Badsha, RafaatSaba Rashidnoor

Department of Health Sciences, The University of Qatar, Doha, Qatar

Staphylococcus aureus (MRSA).can cause infections ranging from mild to severe diseases that can be fatal. These infections occur between people works in hospitals, healthcare, and even the visitors of the hospitals. It is continuing to be the major nosocomial pathogen of Hospitals and community acquired. As a consequence, it was divided into two types either: Hospital Associated (HA-MRSA) or Community Associated MRSA (CA-MRSA) that may occur among healthy people in the wider community. Nasal swabs were obtained from female students at Qatar University. Swabs were inoculated into chromagel agar plates and incubated for 24 hours, pink coloured colonies were selected and appeared, for confirmation of identity *Staphylococcus aureus*. Final confirmation of MRSA, was done by susceptibility testing to cefoxitin through disk diffusion method. Out of the 514 samples, only 1 sample was positive for MRSA. The above isolate was sensitive to Ampicillin-Sulbactam, Cifazolin, Cefexime,Cefoxitin, Ceftriaxone, Oxacillin, Penicillin G, and Trimethomprim-Sulfamethoxazole. However its resistance was noticed to Ampicillin-Sulbactam, Cifazolin, Cefexime,Cefoxitin, Ceftriaxone, Oxacillin, Penicillin G, and Trimethomprim-Sulfamethoxazole. Now it is essential for hospital staff to follow the safety rules when they are dealing with patients, because the MRSA infections are transmitted to the community by contacting with hospital environment. This depends on maintaining their self hygiene because they can be the source of spreading MRSA among patients. The resistance of MRSA to antibiotics in Qatar is lower in comparing with Kuwait and Saudi Arabia. The sensitivity of MRSA to antibiotics is decreasing worldwide, and it is spreading between the people, becoming epidemic especially between school students, colleges, hospitals and any other places that people can gather. For this reason it is essential to do screening for schools in general, nurseries, civil workers and other universities in Doha and to do more genetic and molecular researches about MRSA in Qatar.

1036

SPOTTED FEVER GROUP AND TYPHUS GROUP RICKETTSIOSES AMONG HOSPITALIZED FEBRILE PATIENTS IN NORTHERN TANZANIA, 2007-2008

Malavika Prabhu[1], William L. Nicholson[2], Aubree J. Roche[2], Gilbert J. Kersh[2], Kelly A. Fitzpatrick[2], Lindsay D. Oliver[2], Robert F. Massung[2], Anne B. Morrissey[1], John A. Bartlett[3], Jecinta J. Onyango[4], Venance P. Maro[4], Grace D. Kinabo[5], Wilbrod Saganda[6], John A. Crump[1]

[1]Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Durham, NC, United States, [2]Rickettsial Zoonoses Branch, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Duke Global Health Institute, Duke University, Durham, NC, United States, [4]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania, [5]Kilimanjaro Christian Medical College, Tumaini University, Moshi, United Republic of Tanzania, [6]Mawenzi Regional Hospital, Moshi, United Republic of Tanzania

The importance of spotted fever group rickettsiosis (SFGR) and typhus group rickettsiosis (TGR) as causes of febrile illness in sub-Saharan Africa is unknown. In the first sub-Saharan Africa study of its type, we investigated the prevalence and correlates of SFGR and TGR in northern Tanzania. We identified febrile patients among consecutive admissions to two hospitals in Moshi, Tanzania, from September 2007 to August 2008, recorded standardized clinical data, and collected acute and convalescent sera. Acute SFGR and TGR were defined as a >=4-fold increase in IgG immunfluorescence assay titer to *R. conorii* or to *R. typhi*, respectively; a titer of >=1/64 defined SFGR or TGR exposure. Predictors and clinical management of SFGR and TGR were examined. Among 870 febrile patients, 449 (51.6%) had paired sera tested for acute SFGR and TGR; 828 (95.2%) had sera tested for SFGR and TGR exposure. Results suggested acute SFGR and TGR among 36 (8.0%) and 2 (0.5%) patients, respectively; 193 (23.3%) and 23 (2.8%) patients had results suggesting SFGR and TGR exposure, respectively. Among acute SFGR cases, the median (range) age was 15 (1, 77) years; clinical features included headache (66.7%), rigors (66.7%), and cough (61.1%). Acute SFGR was associated with leukopenia (OR 4.3, p=0.002) and serologic evidence of other zoonoses (OR 2.2, p=0.046). SFGR and TGR were never clinically diagnosed; the most common diagnoses among subsequently identified cases of acute SFGR were pneumonia in 14 (38.9%) and malaria in 12 (36.6%); 3 (8.3%) received antimicrobials active against SFGR. There was a protective effect of HIV against SFGR exposure (OR 0.36, p<0.001) and a trend toward the same for acute SFGR among those >=18 years (OR 0.32, p=0.071). No patients with SFGR or TGR died. SFGR but not TGR appears to be an important cause of febrile illness among inpatients in northern Tanzania; SFGR is likely endemic in this region. Clinical presentation of SFGR is nonspecific and appropriate antimicrobial treatment rare. The possible protective effect of HIV against SFGR warrants investigation.

1037

DETECTION OF *LEPTOSPIRA* FROM SPIKED BLOOD AND URINE SAMPLES DRIED ONTO WHATMAN FTATM MATRIX CARDS

Ashley M. Maranich, Clinton K. Murray, Katrin Mende, Xin Yu, Miriam L. Beckius, Duane R. Hospenthal

Brooke Army Medical Center, Fort Sam Houston, TX, United States

Diagnosing leptospirosis is challenging and testing is often not available in remote settings where the disease is commonly contracted. Diagnostic testing is usually performed in reference laboratories, hindering the collection of samples early in the course of illness when testing may be more reliable. We evaluated the use of diagnostic PCR testing of serum and urine samples collected on Whatman FTA™ matrix cards (Piscataway, NJ) as a tool for remote diagnosis of acute clinical samples. If successful, this would allow for improved epidemiology data in addition to providing a means for delayed or remote diagnosis. 4 pathogenic and 1 saprophytic strains of *Leptospira* were grown in EMJH media at 30°C. Serial dilutions (1x107-1x102 organisms/mL) were prepared with 65uL of each dilution added to FTA™ cards. Identical dilutions of the 5 *Leptospira* strains spiked in human blood and urine samples were applied to FTA™ cards. 1 and 4 weeks after application, 2x2 mm squares were cut from each card, DNA extracted using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA), and conventional and real-time PCR testing performed using 16S and LipL32 primers. Pathogenic *Leptospira* strains were detected with both primers using both techniques. The lowest detection in pure culture was 1x104 with reliable detection at 1x106 and higher. In spiked blood and urine samples, positive results were seen 1x105 with consistent positivity at and above 1x106. Similar recovery rates were noted at 4 weeks. Good correlation was seen between pure culture and blood or urine samples. In conclusion, our results support the use of FTA™ cards as a convenient way to collect acute clinical samples from patients without access to care, providing maintenance of sample integrity for diagnostic PCR up to 4 weeks after collection. Our PCR technique required a large burden of organisms for detection, potentially limiting its use in patients without severe disease. If PCR sensitivity is improved, FTA™ cards could be a successful point-of-care collection technique for diagnosing leptospirosis.

1038

EVASION STRATEGIES OF *ECHINOCOCCUS GRANULOSUS* TO TH1 HOST PROTECTIVE RESPONSE DURING HUMAN INFECTION

Manel Amri, Chafia Touil-Boukoffa

University of Sciences and Technology Houari Boumediene (USTHB), Algiers, Algeria

Human cystic hydatid disease constitutes a major health problem in Algeria. More recently, we have highlighted an evident role of IFN-γ (Th1 cytokine) in parasite killing by NOS2 (Nitric oxide Synthase2) pathway. Moreover, IL-10 (Treg cytokine) production seems to be an evasive mechanism taken by the parasite to establish in the host by Arginase pathway, as reported previously. Of note, NOS2 and Arginase are known to compete for the common substrate, L-Arginine. Moreover, IL-10 downregulates IFN-γ production. Indeed, more researches are required to identify factors present in parasite cyst which affect protective Th1 response in *Echinococcus granulosus* human infection. We investigate the effect of laminated-layer (accelullar layer of hydatic cyst) extract (LLs) on Th1/Treg and NOS2/Arginase balance in culture performed with mononuclear cells (PBMC) of hydatid patients and healthy donors. Furthermore, we have investigated the effect of LLs on parasite viability in PBMC-parasite cocultures. Our results demonstrated that LLs reduced IFN-γ/NO production and enhanced IL-10 production and Arginase activity. In addition, LLs enhanced parasite survival *in vitro*. Similar findings are observed in cultures and cocultures performed with PBMC of patients and healthy donors. Moreover, the major antigenic fraction in LLs: the fraction 4 (12kDa, purified by chromatography) has the same effect as LLs. In conclusion, collectively, the present study provides evidence that *Echinococcus granulosus* laminated layer impairs Th1 protective response and allow the parasite to survive. Inhibition of these mechanisms seems to be important issue to address during the design of anti-hydatic treatment.

1039

MUSCULAR HYDATIDOSIS IN TWO YOUNG MALES

Gurkan Mert, Hanefi Cem Gül, Bulent Karaman, Ozgur Koru, Ismail Yasar Avci, Can Polat Eyigun

GATA, Ankara, Turkey

Hydatid cyst, caused by *Echinococcus granulosus* is a worldwide occurring infectious disease. Although diffuse internal localization of hydatid cysts is common, intramuscular localization has rarely been reported. In this text, we present two cases. In case 1, ultrasonography (US) of a 22 years

old male revealed a cystic mass in the paraspinal muscles. He didn't allow for surgical intervention. After the magnetic resonance imaging, PAIR (Puncture-Aspiration of cyst contents_Injection of hypertonic saline solution_Respiration) was used as a percutaneous treatment of hydatid cysts US guidance to the patient. Albendazole was given to patient before (7 days) and after (28 days) drainage. The cysts, examined after 7 days, a progressive shrinkage and solidification displayed. No allergic reactions or dissemination of cyst contents were discovered. In case 2, the hydatid cysts were localized in the lateral abdominal wall region. The patient was operated and treated with albendazole.In both cases, albendazole-induced elevations of liver enzymes were not determined.The cystic lesions with rare anatomic localizations require differential diagnosis, especially particularly in the endemic regions of hydatid disease. Following a positive diagnosis, a less invasive method can be applied. Percutaneous drainage treatment was efficient for both diagnosis and treatment in case 1.

1040

THE WHO-CLASSIFICATION SYSTEM OF ALVEOLAR ECHINOCOCCOSIS OFFERS GUIDANCE FOR STRUCTURED TREATMENT

Peter Kern[1], Niels P. Krochmann[1], Wolfgang Kratzer[2], Klaus Buttenschoen[3], Beate Gruener[1]

[1]Comprehensive Infectious Diseases Center, Ulm University, Germany, [2]Department of Medicine I, Ulm University, Germany, [3]Department of Surgery, Division of General Surgery, University of Alberta, AB, Canada

Human alveolar echinococcosis (AE) is caused by the metacestode of Echinococcus multilocularis. The parasite forms multi-vesiculated lesions embedded in a dense fibrous tissue, infiltrates the primarily affected liver like a malignant tumor, and ultimately spreads into neighbouring or distant organs. The WHO-PNM-classification system encompasses the wide clinical spectrum by using 4 "P" categories for the distribution of lesions (P for parasite in the liver), 2 "N" and 2"M" categories for the presence or absence of local infiltration including lymph nodes (N) and metastasis (M), respectively. Stages I to IV are derived from those categories. The purpose of this study is to validate the classification system for surgically treated patients, and its usefulness to guide treatment for newly diagnosed patients. 144 patients (58 men and 86 women, mean age 52.7 and 48.7 years, respectively) were included. All of them were classified during the period from1998 to 2008 and had a median follow-up of 4.9 years (maximum 12 years). Treatment was provided according to the best knowledge. 59 patients underwent surgery, 85 were regarded as inoperable. All patients received benzimidazoles, and if necessary, interventional measures. Cure, stable disease, and progressive disease or relapse was assessed by imaging techniques including PET/CT scans, and by applying biochemical markers including serology. Of 144 patients 19 were grouped in stage I, 20 in II, 31 in IIIa, 36 in IIIb and 38 in IV. Thus, nearly half of the patients (stages IIIb and IV) had hepatic as well as extrahepatic disease requiring continuous treatment with benzimidazoles. Since surgery in combination with benzimidazoles offers cure, subgroups of patients in the different WHO stages were further analysed. Evaluable were 11 "surgical" cases in stage I, 14 in stage II, 12 in stage IIIa, 10 in stage IIIb, and 12 in stage IV, respectively. Kaplan-Meier plots clearly show a positive outcome for patients allocated in stages I (100%) and II (85%). In contrary, 70% of patients diagnosed in stages IIIb and IV relapsed or showed progression when taken off benzimidazoles after surgery. The subgroup of patients in stage IIIa followed an intermediate course. Thus, prospective classification into the WHO-PNM system clearly separates cases with favourable prognosis when radical surgery was applied, and thus, offers an appropriate basis for treatment decisions of alveolar echinococcosis.

1041

ECHINOCOCCUS MULTILOCULARIS PHOSPHOGLUCOSE ISOMERASE (EMPGI): A GLYCOLYTIC ENZYME INVOLVED IN METACESTODE GROWTH AND PARASITE-HOST CELL INTERACTIONS

Britta Stadelmann, Joachim Müller, Tatiana Küster, **Andrew Hemphill**

University of Berne, Berne, Switzerland

In *Echinococcus multilocularis* metacestodes, the surface-associated and highly glycosylated laminated layer and molecules associated with this structure are believed to be involved in modulating the host-parasite interface. We report on the molecular and functional characterization of *E. multilocularis* phosphoglucose isomerase (EmPGI), which is a component of this laminated layer. The EmPGI amino acid sequence is virtually identical to its homologue in *E. granulosus*, and shares 64% identities and 86% similarities with human PGI. Mammalian PGI is a multifunctional protein that, besides its glycolytic function, can also act as a cytokine, growth factor and inducer of angiogenesis, and plays a major role in tumor growth, development and metastasis formation. EmPGI and multifunctional mammalian PGI share a typical motif that is absent in species without any extracellular function of PGI. Recombinant EmPGI (recEmPGI) is also functionally active as a glycolytic enzyme and was found to be present, besides the laminated layer, in vesicle fluid and in germinal layer cell extracts. EmPGI is released from metacestodes and induces a humoral immune response in experimentally infected mice, and vaccination of mice with recEmPGI renders these mice more resistant towards secondary challenge infection, indicating that EmPGI plays an important role in parasite development and/or in modulating the host-parasite relationship. We show that recEmPGI stimulates the growth of isolated E. multilocularis germinal layer cells *in vitro*, and selectively stimulates the proliferation of bovine adrenal cortex endothelial cells, but not of human fibroblasts and rat hepatocytes. Thus, besides its role in glycolysis, EmPGI could also act as a factor that stimulates parasite growth and potentially induces the formation of novel blood vessels around the developing metacestode *in vivo*.

1042

CLINICAL CONTRIBUTIONS TO A NATURAL HISTORY OF ECHINOCOCCAL CYSTS

Enrico Brunetti[1], Luca Piccoli[2], Francesca Tamarozzi[3], Elisabetta Above[2], Sam Goblirsch[4], Carlo Filice[1]

[1]University of Pavia - IRCCS S.Matteo, Pavia, Italy, [2]IRCCS S.Matteo, Pavia, Italy, [3]University of Pavia, Pavia, Italy, [4]University of Minnesota, Minneapolis, MN, United States

The natural history of cystic echinococcosis (CE) of the liver is still incompletely understood.

Although the standardized ultrasound classification of CE introduced by the WHO Informal Working Group on Echinococcosis improved matters in this respect, the chronic nature of the disease makes it difficult to understand the exact sequence of changes unless short time intervals are used in follow-up. Clinical observations suggests that the sequence might be: CE1 --> CE3a --> CE4 in case of inactivation and CE1-->CE3a --> CE2 and CE4-->CE3b in case of chronicization (both CE2 and CE3b respond poorly to medical treatment or percutaneous drainage). We report two cases in which a CE3a cyst was seen "regressing" to a CE2 stage (transitional to active) and two patients in which a CE4 cyst changed into a CE3b cyst (inactive to transitional). This was possible thanks to continuous sonographic follow-up at short intervals, and both changes were seen shortly after albendazole treatment was discontinued.

The clinical implications of these findings will be discussed.

1043

DIFFERENTIATION OF *DIPHYLLOBOTHRIUM LATUM* AND *D. PACIFICUM* BASED ON ITS2 SYBR GREEN REAL-TIME PCR

Marcos de Almeida[1], Hector Garcia[2], Juan Jimenez[3], Maria Bernadete de Paula Eduardo[4], Blaine Mathison[1], Henry Bishop[1], Alexandre J. da Silva[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Universidad Peruana Cayetano Heredia, Atlanta, Peru, [4]São Paulo State Health Department, Sao Paulo, Brazil

Human diphyllobothriasis is a fishborne zoonosis associated with 14 different cestode species belonging to the genus, *Diphyllobothrium*. Recent data indicate that 20 million people are infected worldwide, despite the decline of cases observed in several countries, particularly in North America. This is probably due to the re-emerging pattern in different areas of the world including European, Asian and South American countries that did not have autochthonous diphyllobothriasis cases in years. *Diphyllobothrium lancelatum* and *D. latum* are the most prevalent species in North America while *D. latum* and *D. pacificum* occur in South America. Morphologic differentiation among *Diphyllobothrium* species requires examination of proglottids or scoleces, since eggs are morphologically identical. Nevertheless, differentiation of among species can be obtained on clinical samples with molecular methods. We developed and evaluated a SYBR Green real-time PCR assay using primers designed on the *Diphyllobothrium* ITS2 region. We evaluated this method on 15 clinical specimens containing *D. latum* (n= 8) and *D. pacificum* (n= 7) from North and South America, including four *D. latum* specimens from the 2004-2005 diphyllobothriasis outbreak that took place in Sao Paulo, Brazil, which is considered not to be endemic for diphyllobothriasis. Species differentiation was achieved with a dissociation curve analysis after the amplification. The differential melting temperature (Tm) of the *D. latum* using this method ranged from 81.5 oC to 82,0oC whereas for *D. pacificum* it ranged from 84.0 oC to 84.5 oC. By using this technique, results can be obtained 3 hours after the DNA is available with an average cost of $ 0.80 per sample. Ova-and-parasite (O&P) examinations can be reliably used in clinical diagnosis of diphyllobothriasis, but not for identification of the parasite at the species level, which is important in studies aimed at tracking the source of infections. For this purpose reliable and inexpensive molecular tools seems to be the best choice.

1044

EVIDENCE OF ANTIBODY-MEDIATED IMMUNITY AGAINST NEUROCYSTICERCOSIS IN INDIANS

T. Jayaraman[1], V. Prabhakaran[1], P. Babu[1], Raghava M. Venkata[1], V. Rajshekhar[1], P. Dorny[2], J. Muliyil[1], A. Oommen[1]

[1]Christian Medical College, Vellore, India, [2]Institute of Tropical Medicine, Antwerp, Belgium

Endemicity of an infectious disease associated with chronic exposure to the infective stage of the organism is linked to the development of acquired immunity that protects against infection. Taenia solium infections are endemic to India but the role of acquired immunity to the prevalence of disease has not been explored. In a study from south India, the prevalence of neurocysticercosis in the total population was found to be 0.13%. In a sample of the population who were free of seizures, taeniasis was detected in 0.8% of the population, Taenia cyst circulating antigens in 4.5%, IgG antibodies to Taenia ova antigens in 40.9% and IgG antibodies to infection specific T. solium cyst antigens in 15.9% of the population. In 93% of the seropositive population cysticercus antibodies were directed against low molecular weight cyst glycoproteins. These results show high exposure of the population to the parasite and a relatively high prevalence of active infections but a low prevalence of clinical neurocysticercosis. The findings may indicate that through constant exposure to low levels of infective Taenia ova the population acquires protective, antibody-mediated immunity to neurocysticercosis. The discussion will argue for a role of IgG antibodies to oncosphere proteins and low molecular weight cyst glycoproteins in protecting the population from neurocysticercosis.

1045

THE MULTIPLE ANTIGEN BLOT ASSAY USING *TAENIA CRASSICEPS* PEPTIDES FROM 14 KDA GLYCOPROTEIN FOR NEUROCYSTICERCOSIS DIAGNOSIS

Regina Helena H. Peralta[1], João Alvaro D'Ippolito[1], Patrícia Mayer Lima[1], Marzia Puccioni Sohler[2], Hercules Moura[3], José Mauro Peralta[2]

[1]Universidade Federal Fluminense, Niterói, Brazil, [2]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

Neurocysticercosis (NC) is caused by the presence of the larval form of *Taenia solium* on the central nervous system and represents the most severe form of the disease. The use of ELISA and EITB for detecting antibodies in cerebrospinal fluid and serum has been proposed for the laboratory diagnosis of NC. *Taenia crassiceps* represents an important experimental model and can be used for antigen preparation. The objective of this study was to standardize and evaluate the ELISA and LIA methods using synthetic peptides obtained from the *T. crassiceps* cysticerci GP14 glycoprotein to detect in patient's serum suspected of NC. It was prepared two biotinylated peptides from the GP14 sequence that was determined by our group using MALDI-TOF/TOF and MS/MS analysis. The results obtained from the ELISA and LIA-Pepbiot in patient's serum having NC were compared to the EITB and ELISA using antigens derived from *T. solium* and *T. crassiceps*. Three groups of patients were studied: NCA - 24 patients with NC confirmed by CT and MRI exams (7 active and 17 inactive NC) ; NG - 10 health individuals with no detectable parasitic disease and the OP - 43 patients with other parasites (OP). Considering the tests reactivity among the patients of group NCA it was observed that the EITB-Tso reacted with six of the 24 serum samples, the EITB-Tcra reacted with five, the ELISA-Tcra reacted with ten, the ELISA-Pepbiot with seven and LIA-Pepbiot with twenty. LIA-Pepbiot showed better results than the other tests. In the active NC six serum samples were positive, while five samples were ELISA-Pepbiot positive, only four samples were EITB-Tso and ELISA-Tcra positive, and three serum were EITB-Tcra positive. In addition, all patients with active NC were reactive at least with one test. The ELISA-Pepbiot and LIA-Pepbiot were negative in all serum from healthy individuals and patients with other parasitoses. The use of multiples synthetic peptides derived from *T. solium* and *T. crassiceps* could be an important tool for laboratorial diagnostic of NC.

1046

IN VITRO MORPHOLOGICAL AND BIOCHEMICAL EFFECTS OF PRAZIQUANTEL AND ALBENDAZOLE ON *TAENIA SOLIUM* CYSTS

Siddhartha Mahanty[1], Adriana Paredes[2], Miguel Marzal[2], Pierre Dorny[3], Silvia Rodriguez[2], Emico Gonzalez[4], Cristina Guerra[2], Hugo Garcia[2], Theodore Nash[5], Cysticercosis Working Group in Peru Cysticercosis Working Group in Peru[6]

[1]National Institutes of Health, Rockville, MD, United States, [2]Laboratory of Experimental Immunopathology, Universidad Paruana Cayetano Heredia, Lima, Peru, [3]Institute of Tropical Medicine, Antwerp, Belgium, [4]School of Veterinary Medicine, Universidad San Marcos, Lima, Peru, [5]National Institutes of Health, Bethesda, MD, United States, [6]Cysticercosis Working Group in Peru, Lima, Peru

Neurocysticercosis (NCC) resulting from *Taenia solium* (Ts) infections is a major cause of adult acquired seizures worldwide. Disease is caused by larval cysts, and treatment consists of anthelmintics drugs, albendazole (ABZ) or praziquantel (PZQ). There are no standard methods to assess drug activity to Ts cysts *in vitro*. Morphological, functional and biochemical changes that might reflect damaging (inhibiting, cytotoxic) drug effects

were analyzed after exposure of cysts to albendazole sulfoxide (ABZ-SO; the major active metabolite of the drug *in vivo*), PZQ, or combinations of both. PZQ exposure led to a decrease in cyst size and inhibition of evagination while ABZ exposure resulted in minimal changes. Alkaline phosphatase is normally secreted by cysts and both drugs inhibited AP secretion at concentrations of 5ng/ml and 50 ng/ml for PZQ and ABZ, respectively. Some combination of both drugs resulted in additive and/or synergistic activities. Parasite specific antigen, that can be detected in the CSF and blood of infected patients, is also normally secreted by Ts cysts *in vitro*. Antigen secretion was inhibited by ABZ and PZQ and a combination of both drugs in a manner similar to AP secretion, suggesting that inhibition of secretion is a common downstream consequence of the activities of both drugs. These studies establish quantitative methods to measure *in vitro* anthelmintic activity and suggest combination therapy with ABZ and PZQ may have clinical benefit.

1047

SEROLOGICAL EVIDENCE OF ARBOVIRAL INFECTIONS AMONG HUMANS IN KENYA

Laura J. Sutherland[1], Ayla A. Cash[1], Rosemary C. Sang[2], Charles H. King[1], A. Desiree LaBeaud[3]

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

Outbreaks of arthropod-borne viral infections occur periodically across Kenya; however, limited surveillance takes place during interepidemic periods. Serosurveys triggered by outbreaks fail to detect potentially high levels of continuing exposure to these pathogens. Using sera from asymptomatic subjects collected across Kenya in 2000-2004, we assessed (via indirect immunofluorescence assay) prevalence of IgG antibodies to yellow fever (YFV), West Nile (WNV), tick-borne encephalitis (TBEV), dengue serotypes 1 - 4 (DENV1-4), and chikungunya (CHIKV) viruses. Seroprevalence estimates were YFV = 34%, WNV = 24%, TBEV = 14%, DENV1 = 51%, DENV2 = 53%, DENV3 = 44%, DENV4 = 36%, and CHIKV = 33%. Older individuals on the Indian Ocean were more likely to be seropositive than inland children. Among inland samples, lowland children were more likely to be seropositive for CHIKV (42% vs. 0%) than highland children. In Kenya, transmission of arboviral infection continues between known epidemics, remaining common across the country.

1048

MOLECULAR EPIDEMIOLOGY OF THE SAINT LOUIS ENCEPHALITIS VIRUS IN THE BRAZILIAN AMAZON: GENETIC DIVERGENCE AND DISPERSAL

Sueli G. Rodrigues[1], Márcio R. Nunes[1], Samir M. Casseb[1], Assis S. Prazeres[1], Daniele S. Rodrigues[1], Mayra Silva[1], Ana C. Cruz[1], José Tavares-Neto[2], **Pedro F. Vasconcelos**[1]

[1]Instituto Evandro Chagas, Ananindeua, Brazil, [2]Universidade Federal da Bahia, Salvador, Brazil

Saint Louis encephalitis Virus (SLEV), a member of the genus Flavivirus (*Flaviviridae*,) is an encephalitogenic arbovirus broadly distributed in the Americas. Phylogenetic analysis based on the full-length E gene sequences obtained for 30 Brazilian SLEV strains was performed using different methods including Bayesian and relaxed molecular clock approaches. A new genetic lineage was described, hereafter named genotype VIII, which co-circulates with the previously described genotype V in the Brazilian Amazon region. Genotypes II and III were restricted to São Paulo state (Southeast Atlantic rainforest ecosystem). The analysis also suggested the emergence of the SLEV common ancestor between 91-189 years ago [Highest Posterior Density -HPD 95% 1875-1973], giving rise to two major genetic groups: genotype II, more prevalent in the North America, and a second group including the other genotypes (I, and III to VIII), broadly dispersed throughout the Americas, suggesting that SLEV initially emerged in South America and spread to North America. In conclusion, the current study demonstrated the high genetic variability of SLEV and its geographic dispersal in Brazil and in other New World countries.

1049

ASTROCYTE ACTIVATION IN JAPANESE ENCEPHALITIS

Khin S. Myint[1], **Robert V. Gibbons**[1], Anja Kipar[2], Guey C. Perng[3], Duangrat Mongkolsirichaikul[1], Yvonne Van Gessel[1], Tom Solomon[2]

[1]Armed Forces Research Institute for Medical Sciences, Bangkok, Thailand, [2]University of Liverpool, Liverpool, United Kingdom, [3]Emory University, School of Medicine, Atlanta, GA, United States

Japanese encephalitis (JE) is a major cause of childhood mortality and morbidity in Asia and the Western Pacific. Mortality rates can be as high as 30% with an additional one-third of survivors suffering severe permanent neuro-psychiatric disability. The neuropathic effects of JE virus (JEV) are still unclear; studies that address the mechanisms that produce inflammation and subsequent neuronal death are needed. While a number of studies have documented the importance of microglia in JEV infection, little is known regarding the role of astrocytes in brain inflammation. The objective of this study was to profile the role of astrocytes and their relationship to neuronal cell death in JEV infection. In a macaque model of JE, we used an immunohistochemical approach to characterize the role of astrocytes by testing for inflammatory markers including tumor necrosis factor (TNF)-alpha, interferon (IFN)-alpha, inducible nitric oxide (NO) synthase (iNOS), and matrix metalloproteinase (MMP)-2 and -9, as well as apoptosis pathways. In our study, we found that astrocytes undergo activation resulting in astrogliosis, produce TNF-alpha, IFN-alpha and MMP-2, and undergo apoptosis through the caspase-dependent intrinsic pathway. Our study confirmed for the first time *in vivo* that astrocytes play a crucial role in JEV infection by producing inflammatory mediators triggering bystander killing of neurons. More research is required to determine the precise role of astroglial activation and its implication on the neuropathogenesis of JE.

1050

MODELING THE LONG-TERM NEUTRALIZING ANTIBODY PERSISTENCE IN ADULTS AFTER ONE DOSE OF LIVE ATTENUATED JAPANESE ENCEPHALITIS CHIMERIC VIRUS VACCINE

Kamal Desai, Laurent Coudeville, Fabrice Bailleux

Sanofi Pasteur SA, Lyon, France

Japanese encephalitis (JE) is the major cause of vaccine-preventable encephalitis in south-east Asia and the western Pacific. A live attenuated JE chimeric virus vaccine (sanofi pasteur, Lyon, France) has been shown to provide 87% seroprotection in 105 JE-naive adults 5 years post one-dose. Because long-term seroprotection data is essential for decision-making on need and timing of boosters, we applied linear and nonlinear statistical models to this data to predict neutralizing antibody titres (Abt) and seroprotection to 10 years post-vaccination. Data on subjects' Abt post one-dose were collected using plaque reduction neutralization test against homologous JE-CV at 0, 14, 28, 56 days, 6 months and then annually for 5 years and used to construct mixed effects statistical models. To avoid assumptions on the functional form of Abt decline, we constructed bi-exponential, linear, piecewise linear and exponential-type models from day 0 or day 28. The adequacy of model fit was based on statistical and heuristic criteria. Individual seroprotection was based on the accepted threshold of 1:10 /dilution units (Abt > 10). Observed Abt were found to rise rapidly by 28 days reaching geometric mean titres (GMT) of 247 (35.7-2136; 95% confidence interval) corresponding to 98.0% (93.1-100) seroprotection. GMT at 6 months declined rapidly to 128 (17.8-1313) corresponding to 95.0% (87.9-100) seroprotection before assuming a much slower rate of decline. The piecewise linear mixed model provided best fit amongst all models implying that long-term decline in Abt from 6 months remains linear. Predicted Abt at 10 years were 46.5 (23.8-91.2)

corresponding to 83.0% (71.6-94.4) seroprotection and average duration of protection of 29.7 years. Other model estimates for seroprotection at 10 years ranged between 66-93%. In conclusion, JE-CV seroprotection post 1 dose in adults is predicted to remain high for at least 10 years. A 5 year follow-up study is ongoing in children.

1051

ASSOCIATION OF THE PROGRESSION OF LIVER FIBROSIS AND RESPONSE TO ANTIVIRAL THERAPY WITH FUNCTIONAL SINGLE NUCLEOTIDE POLYMORPHISMS (*TGF-B1, IFN-G, IL-6, IL-10* AND *TNF-A*) IN PATIENTS INFECTED WITH HEPATITIS C

Fernanda A. Pereira[1], Luciano A. Pereira[1], Maria A. Zarife[1], Theomira M. Carmo[1], Luiz A. Freitas[1], Raymundo Paraná[2], **Mitermayer G. Reis**[1]

[1]Oswaldo Cruz Foundation, Salvador, Brazil, [2]Federal University of Bahia, Salvador, Brazil

Cytokines play a key role in the regulation of immune responses. In HCV infection, the production of abnormal cytokine levels appears to contribute to the progression of disease, viral persistence, and affects response to therapy. Cytokine genes are polymorphic in specific sites, and certain polymorphisms have been shown to affect the overall expression and secretion of cytokines. The aim of the present study was to identify potential markers of cytokines genes associated with the progression of liver fibrosis and response to antiviral therapy.147 patients were enrolled (66 responders and 81 non-responders to antiviral therapy). 120 patients were stratified according to the stage of hepatic fibrosis (METAVIR index). Genotyping was carried out by PCR-SSP. The distributions of the following polymorphisms were compared in these groups: TNF-a(-308G/A [rs1800629]), TGF-b1 (codon 10 T/C [rs1982073], codon 25 G/C [rs1800471]), IL-10 (-1082 A/G [rs 1800896]; -819T/C [rs1800871]; -592A/C [rs 1800872]), IL-6 (-174G/C [rs1800795]), and IFN-g (+874T/A [rs2430561]). This study demonstrated a predominance of *IL-6* high producer phenotype in responders to antiviral treatment compared to the non-responders. No statistically significant difference was observed in allelic, genotypic and phenotypic frequencies of the *TNF*-a, *IFN*-g, *IL-10* and *TGF*-b*1* between these groups. When patients were stratified according to the METAVIR index, no statistically significant difference could be observed in these cytokine genes SNPs. These findings suggest an association between *IL-6* polymorphism in determining the therapeutic response. Further studies are required to determine the role of these polymorphisms on the progression of fibrosis in patients infected with HCV.

1052

LACK OF CORRELATION BETWEEN SERUM AND SALIVA HEPATITIS C VIRAL LOADS

Gisele B. Menezes[1], Fernanda A. Pereira[1], César A. Duarte[2], Theomira M. Carmo[1], Hermes P. Silva Filho[1], Maria A. Zarife[3], Marco A. Krieger[4], Eliana A. Reis[1], **Mitermayer G. Reis**[1]

[1]Laboratório de Patologia e Biologia Molecular, Fundação Oswaldo Cruz, Salvador, Brazil, [2]Instituto de Biologia Molecular do Paraná, Paraná, Brazil, [3]Laboratório Central de Saúde Pública Prof. Gonçalo Moniz (LACEN-BA), Salvador, Bahia, Brazil, [4]Instituto de Biologia Molecular do Paraná, Salvador, Brazil

Hepatitis C virus (HCV) can be detected in blood and other bodily fluids, such as saliva, semen and gastric juices. The latter are potentially alternate routes of transmission. Both qualitative and quantitative assays are important in the diagnosis of Hepatitis C and in monitoring a patient's response to therapy. The aim of the present study was to verify if there is correlation between HCV viral load in saliva and serum of infected patients. Mean viral RNA levels were 3.44 $\log_{10}$ in the saliva and 5.87 $\log_{10}$ in the serum samples. It was observed that saliva HCV viral load was significantly lower than serum. Also, there was no significant correlation between the HCV viral load this may indicate low transmission of HCV by saliva. However, this study demonstrates the importance of epidemiological studies to understand the significance of transmission of HCV and the need to evaluate use of saliva on the diagnostic and transmission of HCV.

1053

SEROLOGICAL EVIDENCE OF CO-CIRCULATING DENGUE VIRUS SEROTYPES, AN UNDERREPORTED ARBOVIRAL DISEASE IN GUINEA

Jaime M. de Araujo Lobo[1], Daniel G. Bausch[2], Christopher N. Mores[1]

[1]Louisiana State University, Baton Rouge, LA, United States, [2]Tulane University, New Orleans, LA, United States

Arboviruses have been studied in arthropods, bats, birds, and non-human primates in Guinea, but only rarely in humans, with the exception of Yellow Fever (YF), which has caused outbreaks roughly bi-annually since 2000. In order to determine the frequency of human dengue disease in the Guinean towns of N'Zerekore and Faranah, we utilized a plaque reduction neutralization test (PRNT) for testing human serum samples. The serum samples were taken from patients that presented to hospital with acute febrile illness, who were ruled not to have malaria or lassa infections. The samples were tested for dengue serotype-specific neutralization antibodies in order to ascertain the occurrence of acute or recent infections. In the case of early infections, the dengue PRNT test presents significant serotype cross-reactivity and therefore it is not always possible to identify the primary virus serotype after a secondary dengue infection. Dengue specific antibodies are present early in the infection and can generate lifelong immunity to the infecting serotype, but only a few months of cross protection to the other serotypes. We found that among the 151 individuals tested, 19% serum samples had greater than 80% neutralization by one specific serotype, in which 33.3% sera samples reacted positively with dengue 1 virus, 33.3% samples reacted with dengue 2, 10% samples reacted positively with dengue 3, and 23.3% of samples with dengue 4. Furthermore, 21% samples had no detectable neutralization for any of the dengue serotypes, 3% bound with greater than 80% neutralization on all serotypes and the remaining 57% of samples cross reacted in diverse combinations of dengue virus 1, 2, 3, and 4. Endpoint titrations and cross neutralizations against YF, Zika, Usutu, and Koutango viruses are underway for these serum samples. These results strongly suggest that dengue, at least, and perhaps other various arboviruses, are circulating in and likely the cause of human disease in Guinea.

1054

THE THREAT OF WEST NILE VIRUS TO THE GALAPAGOS ISLANDS, ECUADOR

Gillian Eastwood

Institute of Zoology, Zoological Society London, and the University of Leeds - United Kingdom, London, United Kingdom

West Nile Virus (WNV) has impacted the health of humans, domestic animals and wildlife across North America since it arrived to the Americas in 1999 to New York City(1-3). Knowledge of the distribution and impact of WNV in South America has been limited to serological assays from Venezuela and Columbia(4, 5), with virus isolation achieved in Argentina in 2006(6). An imminent threat exists to the Ecuadorian islands of Galapagos located 600 miles west of continental South America. I am investigating this risk as part of my PhD of which I am now in the 3rd final year. WNV is maintained in an enzootic 'mosquito vector - avian host' cycle. My studies examine the serological status of Galapagos birds, and the vector ecology of further 2 species of mosquitoes - *Aedes taeniorhynchus* and *Culex quinquefasciatus*, in pertinence to the threat of WNV impacting Galápagos. *A. taeniorhynchus* of Galápagos established across the archipelago before human habitation and are genetically distinct from other strains of this species found elsewhere in the world(7).

C. *quinquefasciatus* alternatively is a relatively recent introduction and a disease vector concerns(8). Abundance and distribution of both mosquito species in Galápagos and their ecological characteristics including feeding preferences are being researched. Historical isolation of Galapagos has produced a range of endemic birds, mammals and reptiles. This exceptional island fauna is likely 'immunologically naïve' having been sheltered from disease exposures prior to introduction of invasive species including pathogens(9). Galapagos is economically important for Ecuador due to the growing number of overseas and national visitors attracted to the archipelago for nature tourism. The islands themselves have developed and now have over 30,000 human inhabitants - populations that have been impacted by another flavivirus, Dengue, with the arrival of a third mosquitoes species to Galapagos, anthrophillic *Aedes aegypti*. In 2009 and during 2010 I examined the capacity of both *A. taeniorhynchus* and C. *quinquefasciatus* to become infected with, and transmit WNV. This Vector Competency work takes place in the USA using field mosquitoes collected in Galápagos. Seasonality in Galápagos is represented by conducting experiments at two temperatures. The capacity of Galapagos mosquitoes to transmit WNV and subsequent consequences for this tropical region will be discussed.

1055

CULEX FLAVIVIRUS ENHANCES WEST NILE VIRUS MOSQUITO INFECTION, CHICAGO USA

Christina Newman[1], Francesco Cerutti[2], Tavis Anderson[1], Gabriel Hamer[3], Edward Walker[3], Uriel Kitron[4], Marilyn Ruiz[5], Jeffrey Brawn[5], Tony Goldberg[1]

[1]University of Wisconsin, Madison, WI, United States, [2]University of Torino, Torino, Italy, [3]Michigan State University, East Lansing, MI, United States, [4]Emory University, Atlanta, GA, United States, [5]University of Illinois, Urbana, IL, United States

Culex flavivirus (CxFV) is an "insect-specific" Flavivirus globally distributed in mosquitoes of the genus Culex. CxFV was positively associated with West Nile virus (WNV) infection in a case-control study of 268 mosquito pools from a focus of WNV transmission in Chicago, USA. CxFV infection rates were high (approximately 100 infected mosquitoes per 1,000 tested), and WNV-positive pools were 4 times more likely to be infected with CxFV than spatiotemporally matched WNV-negative pools. Among WNV-positive individual mosquitoes, 6/15 (40%) were also CxFV-positive, demonstrating that these two flaviviruses co-infect mosquitoes in nature. These results challenge the hypothesis of "super-infection exclusion" in Flavivirus infection and, by contrast, demonstrate an unexpected positive association between CxFV and WNV. Additional analyses suggest that CxFV may be heterogeneously distributed across urban land cover types within the study area, perhaps reflecting variation in mosquito community dynamics. This study provides evidence that CxFV may enhance WNV infection in mosquitoes that are epidemic "bridge vectors" of WNV to humans. Insect-specific flaviviruses such as CxFV may therefore indirectly influence human disease risk.

1056

STABILITY OF REAGENTS ASSOCIATED WITH MICROSPHERE IMMUNOASSAYS OVER TIME USING DIFFERENT STORAGE SOLUTIONS

Alison J. Basile

Centers for Disease Control and Prevention, Fort Collins, CO, United States

The DVBD/CDC in Fort Collins, CO, previously developed microsphere immunoassays to detect IgM to West Nile virus (WNV), St. Louis encephalitis virus (SLEV) and eastern equine encephalitis virus (EEEV). Related assays are being designed with some changes in methodology including the buffering system. As these assays are shared with State health departments, questions regarding the stability and storage requirements for the associated reagents arise. Currently, stock solutions of antigen/antibody-coupled microspheres, and control sera, are prepared and stored at 4ºC for up to 1 month. Working dilutions are prepared immediately prior to use. This time limit was derived from experiments performed during assay development. Storage times greater than this, or alternate storage buffers, were not previously investigated. The convenience factor would be significant if stock or working dilutions could be made in larger volumes to be used over longer periods without deterioration. In addition, less frequent stock preparation could reduce run-to-run error. Using the new methodology, 4 separate series of experiments were performed over the course of 9 months, using buffers manufactured by Candor Bioscience Gmbh, designed specifically to improve stability and test performances. These 4 experiments included: 1) WNV and EEEV antigens were used to illustrate the stabilities of recombinant and suckling mouse brain antigen preparations, respectively, in 4 buffer systems. 2) Positive serum controls to WNV and EEEV were used to illustrate the stabilities of anti-flavivirus and anti-alphavirus sera, respectively, in 3 buffer systems. 3) The effects of lyophilization of the microspheres after reacting antigens with antibody-coupled bead sets were investigated. and 4) The use of an alternate blocking solution in the coupling method was investigated. The results of these experiments are presented, and will be used to finalize methodology for a new generation of arboviral serologic tests. In addition the information may prove useful in knowing how best to provide for long-term storage and shipment of these reagents.

1057

WEST NILE VIRUS RISK ASSESSMENT FOR FOUR MIDWESTERN URBAN AREAS

Susan M. Paskewitz, Patrick Irwin

University of Wisconsin Madison, Madison, WI, United States

Comprehensive knowledge of mosquito populations in urban areas is critical to determining which species pose the greatest risk of West Nile virus transmission to humans. Because mosquito species vary considerably in behavior and ecology, this information is key to directing surveillance and abatement resources for maximum efficacy. Using a previously established WNv risk model and measures of relative mosquito abundance, we determined that Aedes vexans may pose the greatest risk of WNv transmission in the Madison, Wi area. This species accounts for more than 80% of the risk while *Culex* pipiens, usually thought of as the major vector for human WNv transmission in the Midwest, accounts for less than 10% of the risk. Application of the model with local mosquito abundance data for Minneapolis Minnesota also implicates A. vexans as a potential key vector for this area, while the highest risks for Des Plaines, Illinois and for Milwaukee, Wisconsin, come from *Culex* pipiens. The model accurately predicted human WNv infection rates for three of the four urban areas in the study.

1058

TRANSMISSION, AMPLIFICATION, AND EVOLUTION OF WEST NILE VIRUS IN CHICAGO, USA

Tony L. Goldberg[1], Uriel D. Kitron[2], Marilyn O. Ruiz[3], Edward D. Walker[4], Jeffrey A. Brawn[3], Gabriel L. Hamer[4], Tavis K. Anderson[1], Luis Chaves[2], Luigi Bertolotti[5], Giusi Amore[6], Francesco Cerutti[5], Bethany Krebs[3], Christina Newman[1]

[1]University of Wisconsin-Madison, Madison, WI, United States, [2]Emory University, Atlanta, GA, United States, [3]University of Illinois, Urbana, IL, United States, [4]Michigan State University, East Lansing, MI, United States, [5]University of Torino, Torino, Italy, [6]European Food Safety Authority, Parma, Italy

The west suburbs of Chicago, USA, have been a persistent focus of West Nile virus (WNV) transmission since 2002. In this area, WNV undergoes predictable seasonal amplification, characterized by peaks of mosquito infection and human cases in late summer. This multi-year study examines how fine-scale ecological processes within the urban landscape mediate patterns of viral transmission and amplification, and how these outcomes

influence viral evolution locally. Birds and mosquitoes were trapped within an approximately 100 km2 study are each year between 2005 and 2009 and tested for WNV using serological and molecular methods. Viral genome sequence data were used to examine patterns of viral evolution within the study area. Results indicate that WNV amplification in suburban Chicago is disproportionately influenced by a few relatively common bird species, such as the American robin. The mosquito *Culex pipiens* functions as both epizootic and epidemic vector, owing to its selection of both birds and mammals as blood hosts, which in turn reflects the species' complex populations structure. Preliminary field data on avian distributions, coupled with spatially explicit models of WNV transmission, indicate the potential importance of aggregated host distributions (especially nighttime roosts) to localized WNV transmission and persistence within the study area. Microclimate, features of the built environment, and co-circulating pathogens also appear to influence WNV transmission, perhaps explaining the heterogeneous distribution of WNV within the study area. Finally, molecular phylogenetic and phylodynamic analyses provide evidence of distance-limited viral transmission, viral genetic diversification, and fine-scale variation in viral genetic variability across suburban land cover types. Together, these lines of evidence suggest that WNV transmission is spatiotemporally heterogeneous within suburban Chicago. "Hot spots" of arboviral transmission in urban settings may actually represent the coarse-scale aggregation of highly localized ecological interactions.

1059

ALTERED EXPRESSION OF PRO-INFLAMMATORY CYTOKINES: MECHANISMS UNDERLYING MINOCYCLINE-MEDIATED PROTECTION AGAINST WEST NILE VIRUS (WNV)-ASSOCIATED ENCEPHALITIS (WNVE) IN MICE

Moti L. Chapagain[1], Nelson I. Lazaga[2], Mukesh Kumar[1], Esther Volper[1], Saguna Verma[1], Vivek R. Nerurkar[1]

[1]John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI, United States, [2]College of Tropical Agrriculture and Human Resources, University of Hawaii at Manoa, Honolulu, HI, United States

West Nile Virus, WNV, an emerging viral pathogen in the United States causes potentially fatal encephalitis. However, no therapeutic drugs or vaccines are available to prevent or treat WNV infection or its neurological sequelae in human. A recent study demonstrated that minocycline, a broad-spectrum antibiotic, protected mice from Japanese Encephalitis Virus (JEV)-associated encephalitis. However, the role of minocycline in WNVE is unclear. Age and gender matched, 8- to 12-weeks old C57BL/6 mice were inoculated with 1,000 plaque-forming unit (PFU) of WNV (lineage I, NY99), and were either untreated or treated with minocycline and monitored for viremia and survival for up to 3 weeks after infection. Viremia was quantitated by plaque assay, mice survival was analyzed using Log-rank (Mantel-Cox) tests and serum cytokines were quantitated by Luminex assay. Almost 90% mice succumbed to death by day 10 after WNV infection. Viremia peaked on day 3 after infection and clinical symptoms including hind-limb paralysis and hunchback were observed starting at day 7. Interestingly, 30% of WNV-infected mice treated intraperitoneally with minocycline, 40 mg/kg/day, starting on day 3, survived. The survival curve was significantly different between WNV-infected -untreated and -treated mice (Chi-square 6.142, df 1, $p < 0.0132$). There was no difference in the viremia between the mice untreated or treated with minocycline and viremia had no direct correlation with CNS viral load or mortality. Minocycline up-regulated the expression of IL-1β, IL-6, TNF-α and MCP-1, down-regulated IL-12p70 expression, whereas the expression of IL-10 was not altered. In conclusion, minocycline promoted significant survival of mice without altering the viremia. While the precise mechanism of minocycline-associated protection is unclear, minocycline alters the expression of several pro-inflammatory cytokines and it is the intricate balance in the expression of these cytokines that determines ultimate outcome from WNV-associated encephalitis.

1060

WEST NILE VIRUS BINDS TO RED BLOOD CELLS OF SEVERAL VERTEBRATE SPECIES

Caren Chancey[1], Agnes Lee-Stroka[2], Maria Rios[1]

[1]Food and Drug Administration - CBER, Bethesda, MD, United States, [2]National Institutes of Health, Bethesda, MD, United States

West Nile virus (WNV), a mosquito-borne flavivirus, is the most common cause of viral encephalitis among humans in the US. WNV can be transmitted by blood transfusion, and we have reported that WNV binds to red blood cells (RBC) and remains infectious to Vero cells. Since WNV infects a broad range of vertebrates, we have investigated the ability of WNV to bind to RBC from various vertebrate specimens using classical hemagglutination assays (HA) and detection of bound virus by TaqMan RT-PCR amplification. RBC from 18 different vertebrates including human, mouse (BALB/c and C57Bl/6), rat (7 species), rabbit, sheep, ox, calf, sheep, pig, horse, and chicken. HA was performed on a series of WNV dilutions over a pH range of 5.75 to 7.2. To quantify WNV binding at pH 6.2 and 7.2 we performed TaqMan on human, ox, calf, and sheep RBC. For most samples, the optimal pH for HA varied between 6.2 and 6.4. Mouse RBC, from both strains, agglutinated most strongly at pH 5.75. HA in Sprague-Dawley rats was noticeably weaker than HA in cotton rats, occurring over a narrower range of pH and WNV concentrations with no wells showing complete agglutination. Calf RBC agglutinated weakly, and no HA was observed with ox RBC at any pH or viral concentration. Taqman assays performed on calf and ox RBC detected WNV associated with RBC at viral concentrations for which no HA was observed.

The degree to which RBC can be agglutinated by WNV varies between the different animals tested. In addition, WNV HA is dependent on pH, and the optimal pH range of HA also varies by species. RBC from bovine species show significant variation in surface sialic acid content, and the poor performance of bovine RBC in HA and binding assays is consistent with our earlier observations regarding inhibition of WNV-induced HA of human RBCs by sialic acid. Continued comparisons of RBC biochemistry and surface antigens, especially between RBC from oxen, cotton rats, and Sprague-Dawley rats, may yield insights into the nature of the molecular species that mediate WNV-RBC attachment.

1061

EXPERIMENTAL EVOLUTION OF WEST NILE VIRUS EVALUATED *IN VIVO*

Eleanor R. Deardorff[1], Kelly A. Fitzpatrick[1], Pei-Yong Shi[2], Gregory D. Ebel[1]

[1]University of New Mexico, Albuquerque, NM, United States, [2]Novartis Institute for Tropical Diseases, Singapore, Singapore

Arboviruses perpetuate through alternating replication in both vertebrate and invertebrate hosts. The trade-off hypothesis suggests that these viruses maintain adequate levels of replication in two hosts in exchange for superior replication in one host. Releasing the virus from the constraints of a two-host cycle should thus facilitate adaptation to a single host. This theory has been addressed in a variety of systems, but remains poorly understood. We therefore sought to determine the fitness implications of alternating host replication by serially passing WNV twenty times (a) exclusively in mosquitoes (b) exclusively in chicks or (c) back and forth between mosquitoes and chicks. Passed viruses were then competed *in vivo* in fitness assays against a marked reference virus in both mosquitoes and chicks. The ratio of experimental WNV to reference WNV in total virus output was detected by RT-PCR followed by quantitative sequencing and the 'winner' was deemed to have a replicative fitness advantage. Exclusive serial passage in mosquitoes resulted in improved replication in mosquitoes and decreased replication in chicks compared to the co-infected reference virus. Control competitions using viruses passed alternately between chicks and mosquitoes showed no significant difference in ratio of experimental WNV to reference WNV in total virus

output from either host compared to inocula. Consensus sequences for single-host specialized WNV will be examined for sequence elements that can be correlated to either improved or reduced replicative fitness. Additionally, we are conducting fitness restoration studies to evaluate whether fitness in the bypassed host can be regained through alternate passage regimens. Concurrent studies in our lab competed populations of WNV having various levels of genetic diversity against the reference WNV in mosquitoes and chicks. These studies demonstrated that higher levels of genetic diversity were associated with increased replication fitness in mosquitoes but not chickens. Collectively, these results emphasize the importance of virus replication in mosquitoes to WNV adaptation and evolution in North America.

1062

THE IMPACT OF MORTALITY IN BIRDS ON INCIDENCE OF WEST NILE VIRUS HUMAN NEUROINVASIVE DISEASE

Ivo M. Foppa

Tulane University, New Orleans, LA, United States

West Nile virus (WNV) is associated with high mortality in some North American birds, such as corvids. Survival of WNV infection usually leads to sterilizing immunity. The threshold theorem (Kermack&McKendrick, 1927) states that transmission is only supported if the density of immune hosts in the population does not exceed a critical level. WNV-associated avian mortality might thus counteract the dampening effect of increasing avian population immunity on WNV transmission. I present results of an analysis of the relationship between WNV Human neuroinvasive disease (WNNID) annual incidence in ten US states (CA, CO, FL, IL, LA, MA, MD, MN, SC, TN) and indicators of WNV transmission and bird mortality in the previous year. This analysis is based on data from the North American Breeding Bird Survey (annual bird abundance) and annual WNNID incidence data, reported by the CDC. Results from this analysis will contribute to improved prediction of WNNID incidence.

1063

INTEGRATED CONTROL OF MALARIA AND HELMINTHS THROUGH UGANDA'S HEALTH SYSTEM

Ruth Ashton[1], Narcis Kabatereine[2], Simon Brooker[3], Jan Kolaczinski[4]

[1]Malaria Consortium Uganda, Kampala, Uganda, [2]Vector Control Division, Ministry of Health, Kampala, Uganda, [3]Kenya Medical Research Institute (KEMRI)-Wellcome Trust Research Programme, Nairobi, Kenya, [4]Malaria Consortium Africa, Kampala, Uganda

Intestinal worms and schistosomiasis cannot be eliminated unless there is universal access to safe water and sanitation, however, such improvements are likely to take several decades in many African countries. In the interim, the mainstay of control is regular anthelmintic treatment. In recent years the Ugandan Ministry of Health has implemented mass treatment through community and school-based programmes. Concomitantly, donors have increased their support to malaria control including strengthening health systems to ensure that rapid diagnosis, effective treatment and appropriate prevention for malaria are routinely delivered at the facility level. Here there is an obvious opportunity to build on these investments to also integrate worm control into the existing health system, but evidence in practice is limited. To sustain the impact of past and present investment into deworming campaigns in Uganda, delivery of anthelmintics needs to become an integral part of routine health care delivery. A pilot intervention to support health facility based malaria, STH and schistosomiasis control is being implemented in Bulisa and Kibaale districts in western Uganda. We report results of an evaluation of this programme. Results from a baseline needs assessment of health facilities show that very few health workers had ever received training or supervision on STH or schistosomiasis case management. Most had no access to guidelines for diagnosis or treatment, resulting in inconsistency of deworming strategies between facilities. Pregnant women rarely received deworming treatment due to concerns about safety of anthelmintics in pregnancy. There was minimal interaction between mass treatment campaigns and facility-based health workers, with many facility staff believing responsibility for deworming lies with campaigns only. Subsequently, a package of interventions to improve health worker performance in 40 health facilities is described, including job aids, training and initiation of an external supervisory process. Results of the follow-up assessments are also described.

1064

A SURVEY OF SOIL-TRANSMITTED HELMINTHS AND LYMPHATIC FILARIASIS IN SIX PROVINCES OF PAPUA NEW GUINEA

Wayne Melrose, Holly Menzies, Melanie Boer, David Reeve, Jane Connell, Jeffrey Warner, Sonia Harmen

James Cook University, Townsville, Australia

During the setting up of sentinel sites for future monitoring of a national lymphatic filariasis elimination and school-age deworming program, 300- 500 blood and faecal samples are being collected at two sites in each province of Papua New Guinea. This poster provides the results obtained in Bougainville, East and West New Britain, Gulf, New Ireland, and Oro provinces. Faecal samples were examined by microscopy for soil-transmitted helminths, and night blood samples were tested for filarial antigen by the ICT test (and in some instances by Og4C3 ELISA) and microfilaria on a 60ul slide. Hookworm was found at all 1 sites with the prevalence varying from 86-21%. Ascaris lumbricoides was found at 6 sites with the prevalence varying from 39 to 0.3%. Trichuris trichiura was also found at 6 sites with a prevalence between 24 and 0.3%. Filaria antigen positives were found at all sites with a prevalence between 57 and 6%, all but two sites also contained microfilaraemics with a prevalence of between 24 and 11%. These results confirm historical data that Papua New Guinea has a high prevalence of soil-transmitted helminths and lymphatic filariasis and requires a nation-wide integrated campaign to combat these parasites.

1065

THE TRIPLE CO-ADMINISTRATION OF ALBENDAZOLE, IVERMECTIN AND AZITHROMYCIN IS SAFE IN A LYMPHATIC FILARIASIS AND TRACHOMA CO-ENDEMIC AREA OF SIKASSO, MALI

Yaya I. Coulibaly[1], Moussa H. Sankaré[2], Mahamadou M. Keita[2], Drissa Doumbia[2], Moussa Doumbia[2], Modibo Keita[2], Adama Daou[2], Siaka Y. Coulibaly[1], Ousmane Dembele[2], Fadima C. Haïdara[2], David Molyneux[3], Samba O. Sow[2]

[1]Malaria Research and Training Center, Point G, Bamako, Mali, [2]Center for Vaccine Development/CNAM, Bamako, Mali, [3]Centre for Neglected Tropical Diseases, Liverpool, United Kingdom

Neglected tropical diseases (NTDs) are coendemic in many areas of the world, including subsaharan Africa. Consequently, financial and logistical benefit can be gained from integration of preventive chemotherapy programs (PCPs) in such areas. To assess the safety of this approach for the co administration of azithromycin, albendazole and ivermectin, 4 villages in two lymphatic filariasis (LF) and trachoma coendemic districts of Sikasso, Mali, were randomly assigned to triple therapy or standard therapy (albendazole plus ivermectin (A/I) followed 1 week later by azithromycin). These villages had previously undergone 4 consecutive yearly mass drug administration campaigns with A/I and 2 with azithromycin. One village was randomly assigned to each treatment arm in each district. After a baseline assessment, study drugs were administered under direct observation in the clinic. Subjects were encouraged to return to the clinic at any time during the 14 day treatment period to report adverse events and were seen in the clinic on days 7 and 14.The total population of the two villages was 9109, of which 7515 were eligible for treatment (age >5 years). A total of 3016 subjects participated in the study (40.1% of

the eligible population). No serious adverse events occurred during this pilot community trial, and all observed AEs were mild in intensity (mainly diarrhea, headache, abdominal pain, nausea, vomiting). The number of subjects that reported at least one AE was significantly higher in the triple co-administration group (15.75%; 238/1511) as compared to the standard treatment group (18.98%; 286/1507) (OR= 1.26; 95% CI (1.04-1.53); p=0.018). Of note, the overall frequency of AEs in the triple therapy group, 18.98% (286/1507), was comparable or lower than published frequencies of AEs for A/I alone. These data suggest that coadministration of A/I and azithromycin is safe. Additional analyses, including stratification by gender and age group, are currently underway.

1066

ANEMIA, IMPAIRED GROWTH AND EXERCISE INTOLERANCE IN KENYAN CHILDREN: THE ROLE OF SCHISTOSOMIASIS AND POLYPARASITISM

Amaya L. Bustinduy[1], Isabel M. Parraga[2], Charles Thomas[3], Peter L. Mungai[1], Francis Mutuku[4], Eric M. Muchiri[5], Uriel Kitron[4], Charles H. King[1]

[1]Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH, United States, [2]Department of Nutrition ,Case Western Reserve University, Cleveland, OH, United States, [3]Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH, United States, [4]Emory University, Atlanta, GA, United States, [5]Division of Vector Borne and Neglected Tropical Diseases, Ministry of Public Health and Sanitation, Nairobi, Kenya

To quantify burden of disease among children living in areas endemic for multiple parasites, we conducted surveys of affected villages in coastal Kenya. The objectives of the study are to measure the co-prevalence of *S. haematobium*, filariasis, malaria, hookworm, and other geohelminths among residents 5-18 yrs old, and to determine the relationship between parasite load and co-infections with the morbidity outcomes of anemia, reduced fitness, and undernutrition. Cross-sectional data were obtained from three villages during the months of April, August, and November 2009, respectively. After diagnosis (based on urine, stool and blood testing) participating children underwent standardized anthropometric measurement and performed a 20 m shuttle-run fitness test. Results to date from 1382 children reveal some significant heterogeneity among villages. Schistosomiasis prevalence was high in Nganja and Milalani (62 %) and lower in Vuga (25 %). Malaria (by ICT) was variable according to season, ranging from 8%-18%.Filariasis was most prevalent in Vuga (16 %). However, anemia was highly prevalent (50-54%) in all villages, and had a strong association with heavy-intensity schistosomiasis in both high and low prevalence villages. Low-intensity schistosomiasis was also a significant correlate of anemia in Vuga, suggesting a role for infection-related 'anemia of inflammation'.Synergy between infections was observed for hookworm-malaria in anemia, schisto-filaria and schisto-malaria for stunting in Milalani and schisto-filaria and hookworm-*Trichuris* for severe malnutrition in Nganja. Fitness correlated with hemoglobin level and older males were more stunted and wasted in 2/3 villages. We conclude that regardless of location-specific differences between villages (season, slope and nutrition), polyparasitism represents a collective threat to children's health and integrated control approaches appear warranted. Ongoing studies, involving antibody testing for past exposures,malaria PCR and cytokine testing for parasite-mediated inflammation, will refine prevalence estimates and immune response pathway associations.

1067

EVIDENCE TO SUPPORT THE INCLUSION OF VECTOR CONTROL STRATEGIES IN THE GLOBAL EFFORT TO ELIMINATE LYMPHATIC FILARIASIS

Edward Thomsen[1], Lisa Reimer[1], August Kapa[1], Henry Dagoro[1], Manasseh Baea[1], Daniel J. Tisch[2], Peter A. Zimmerman[2], Peter Siba[3], James W. Kazura[2]

[1]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [2]Case Western Reserve University, Cleveland, OH, United States, [3]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea

The Global Programme to Eliminate Lymphatic Filariasis (GPELF) advocates for 4-6 rounds of annual mass drug administration (MDA) to interrupt transmission. Although vector control is not currently a part of the strategy, implementation of vector management plans stands to greatly reduce lymphatic filariasis (LF) transmission and could shorten the time frame for elimination in a given area. Here, we present evidence that the use of long-lasting insecticide treated nets (LLINs), distributed as part of the Global Fund to Fight AIDS, Tuberculosis, and Malaria (GFATM), greatly reduced transmission of lymphatic filariasis in five villages in the East Sepik Province of Papua New Guinea (PNG). Anopheline mosquitoes were collected by human landing catch for four nights/month for one year prior to and one year following LLIN distribution (n=6,898). Half of the samples were dissected and larvae of *Wuchereria bancrofti* were identified under the microscope. The other samples were used for DNA extraction and identification of *W. bancrofti* by PCR. A survey conducted shortly after bednet distribution indicated that 83% of people slept under a bednet the previous night (n=2,459). The man biting rate was significantly reduced ($p<0.001$) post LLIN distribution. In addition, the proportion of mosquitoes identified as infected with *W. bancrofti* by PCR dropped from 15.3% to 4.9% (p=0.02). While 0.6% of mosquitoes were identified as infective pre-LLIN (n=3,935), zero mosquitoes have been found to be infective post-LLIN (n=236). In PNG, where anopheline mosquitoes are the primary vectors, LLINs have proven quite effective in preventing transmission of *W. bancrofti* from human to vector. With the difficulties faced by many LF endemic countries in supporting an MDA program for an extended period of time, integrated vector management may improve durability of programs to eliminate the disease.

1068

EVALUATING PRIMARY HEALTH CARE DELIVERY SYSTEMS FOR NTDS IN WESTERN KENYA

Pauline N. Mwinzi[1], Jane Alaii[1], John M. Ayisi[1], Lorraine Ogange[1], Gladys Odhiambo[1], Erick Muok[1], Susan P. Montgomery[2], W. Evan Secor[2], Diana M.S. Karanja[1]

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

Neglected tropical diseases affect over one billion people in the tropics. Even though effective drugs exist for most of these diseases, they do not effectively reach majority of the persons who need them. Part of the challenge is how to get such existing interventions to affected communities. There is, therefore, an urgent need for better evaluation of the effectiveness of different delivery strategies in achieving and sustaining high population coverage at adequate quality levels. The ongoing study intends to test if the Community Directed Intervention (CDI) approach, that has almost eradicated onchocerciasis in endemic countries in Africa, could be used to strengthen the Primary Health Care (PHC) system in rural Kenya. In the first phase of the study, we have analyzed the prevailing PHC practices in western Kenya, including the Community Strategy (CS) and its ongoing implementation. Study districts in western Kenya were chosen on the basis of PHC delivery and general development levels. These included Rarieda, Kisumu West, Rachuonyo and Homa Bay Districts. Kisumu West and Bondo districts are in the relatively more developed Central Nyanza where PHC delivery is considered average for Kenya, while

Rachuonyo and Homa Bay have lower PHC implementation levels. Views from community members and PHC implementers on priority NTDs were gathered using key informant interviews and focus group discussions. The status of prevailing PHC delivery services were assessed by use of checklists and policy document reviews. Differences in socio-cultural dynamics as related to health care delivery uptake in the study districts are discussed. Data from phase I will be used to design CDI strategies for the four study districts in Phase II for identified NTDs. The outcomes of this study can be used as lessons for implementation of practical integrated health interventions in other regions.

1069

OPERATIONAL RESEARCH ON INTEGRATED NEGLECTED TROPICAL DISEASE (NTD) MAPPING: RESULTS FROM MALI AND SENEGAL

Sonia Pelletreau[1], Massitan D. Soumare[2], Sanoussi Bamani[2], Robert Dembele[2], Rachael Ross[3], Stephanie Lee[1], Sory I. Bamba[2], Els Mathieu[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Ministry of Health of Mali, Bamako, Mali, [3]Emory University, Atlanta, GA, United States

There is increasing interest and funding for integrated approached to NTD programs with preventive chemoprophylaxis. To effectively implement these programs, integrated tools are currently being developed. CDC developed an integrated mapping protocol to help Ministries of Health decide where public health interventions for lymphatic filariasis (LF), trachoma, schistosomiasis and soil-transmitted helminths (STH) are needed. To validate the protocol, the integrated methodology and the current WHO methodologies were implemented in 1 district each in Mali and Senegal. The outcomes evaluated are the prevalence results and its public health intervention implications, survey costs and feasibility.

Both methodologies assessed the WHO-recommended indicators, with the specified age groups, but the sampling frame for the integrated methodology was adapted to make mapping more resource-efficient. The integrated methodology surveyed 2 villages in each sub-district: 1 village was selected randomly; the second was selected based on high suspected schistosomiasis prevalence. In Mali, 1,898 persons, including 900 children (1-9 years), in 18 villages were surveyed for the integrated methodology and 4,479 persons, including 2,738 children (1-9 years) for the WHO methodology, a total of 6,377 persons. Both methodologies indicated no need for mass treatment (MDA) in the surveyed district for trachoma (2.1%, 4.7% TF, respectively) and STH (0%, 0%) and a need for MDA with ivermectin for LF (7%). The integrated methodology indicated 4 sub-districts in need of MDA for schistosomiasis for school-aged children and 5 sub-districts in need of MDA for the whole population. The WHO methodology indicated MDA for school-aged children in the entire area. Using the integrated methodology resulted in a 29% overall cost savings ($7,468 vs $10,539). The integrated methodology used resources more efficiently in the areas of transport, survey time, and teams. Data in Senegal will be collected in June and also will be presented. The new integrated mapping tool could facilitate the beginning and scaling-up of programs by reducing the human and financial resources needed to gather evidence for deciding if a public health intervention is warranted.

1070

THE DEVELOPMENT OF A SPECIMEN SPARING MULTI-CHANNELED BEADED ASSAY TO DETECT IGG4 ANTIBODIES TO *SCHISTOSOMA HAEMATOBIUM*, HOOKWORM AND FILARIAL INFECTIONS IN A POPULATION ON THE COAST OF KENYA

Jessica K. Fairley[1], Adam S. Duvall[1], Rhonda Kimmel[1], Indu Malhotra[1], Christopher L. King[1], Uriel Kitron[2], Charles H. King[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Emory University, Atlanta, GA, United States

To study the combined negative health impact of polyparasitism we developed a novel, diagnostic multi-channeled fluorescent antibody detection assay that simultaneously detects multiple parasitic infections. This assay was developed as part of a study of polyparasitism in coastal Kenya. As there is low cross-reactivity with IgG4, IgG4 responses to *Brugia malayi* antigen (BMA), *S. haematobium* soluble worm antigenic preparation (SWAP), and hookworm excretory/secretory proteins (ESP) were used to detect infections with *Wuchecheria bancrofti*, *Schistosoma haematobium*, and *Necator americanus*, respectively. These responses were assessed using a high throughput bead-based platform (Bioplex, Bio-rad, Hercules, CA). The antigens were coupled to beads, each with a unique dye allowing for automated discrimination of fluorescence. Pooled serum from areas endemic for these infections were used for optimization. With each well containing beads for all three helminthes, diluted serum was incubated with the beads and the RPE conjugated anti-human IgG4 (Southern Biotech) and the plates analyzed using the Bioplex. Due to the low output seen with ESP, beads will be linked to biotin and then coupled to ESP. Cutoff values were set using sera negative for the infections. Standard curves to determine the optimal dilutions were created using a serial dilution of the serum pool. Unlike the SWAP and BMA beads, the ESP beads did not fluoresce significantly with anti-IgG4 secondary antibody despite the detection of anti-ESP IgG4 in the serum with ELISA. Conjugating biotin to the beads will allow for the retention of the native conformation of the ESP and the appropriate binding to serum IgG4. We believe that this type of serologic testing will increase sensitivity of parasite diagnosis with better community participation, providing a better survey of the prevalence of co-infection in this area while offering a specimen sparing method of detection. Given the benefits of this assay and increasing interest in polyparasitism, this could provide a model for serological surveys in future studies.

1071

NATIONWIDE INTEGRATED MAPPING OF THREE NEGLECTED TROPICAL DISEASES IN TOGO

Meyo Dorkenoo[1], Rachel N. Bronzan[2], Didier Ayena[3], Edmond Sognikin[1], Abdou Amza[4], Akpenyo Anthony[5], Yao Sodahlon[6], Els Mathieu[7]

[1]Ministry of Health, Lomé, Togo, [2]HDI, Fair Haven, NJ, United States, [3]Service de Santé des Armées, Lomé, Togo, [4]Ministry of Health, Niamey, Niger, [5]HDI-Togo, Lomé, Togo, [6]Mectizan Donation Program, Atlanta, GA, United States, [7]Centers for Disease Control and Prevention, Atlanta, GA, United States

The Ministry of Health of Togo (MoH) has been working toward integrated control of neglected tropical diseases (NTDs) since 2003. In preparation for nationwide integrated MDA for NTDs in Togo, the MoH and partners conducted an integrated prevalence survey for schistosomiasis, soil transmitted helminthiasis (STH) and trachoma in all districts outside Lomé. Sampling was based on a new, integrated approach developed by the Centers for Disease Control and Prevention. Two villages in each of the 549 peripheral health units (PHU) in 29 of Togo's 35 districts (1096 villages total) were selected based on proximity to water and anticipated high prevalence of schistosomiasis. Trachoma was included for 14 districts. In each village 15 school children age 6 to 9 years were recruited; an

additional 35 children age 1 to 5 years were surveyed for trachoma. Informed consent was obtained. An MoH team collected stool and urine from the children and conducted an eye examination. Urine was tested for blood using a dipstick. Stool was tested for *S. mansoni* and STH using the Kato-Katz method. Over 6 weeks starting in October 2009, 16,440 children were tested for schistosomiasis and STH; 25,000 children were examined for trachoma. At the PHU level, the level planned for MDA implementation for schistosomiasis, the prevalence of *S. haematobium and S. mansoni* ranged from 0 to 100% and 0 to 93%, respectively (national averages: 20% and 3%). The prevalence of STH at the district level ranged from 5% to 70% (national average 32%). Hookworm accounted for 99% of STH detected. The prevalence of trachomatous follicular inflammation, the indicator of active trachoma, ranged from 0.5 to 11.3%. In conclusion, this national, integrated prevalence survey for three NTDs in Togo proved practical and efficient. Integrated MDA in each PHU will target the diseases and population as indicated by the local prevalence of each infection and WHO guidelines. Conducting prevalence mapping at the PHU level allows for focal distribution of preventive chemotherapy, reducing the likelihood of both over- and under-treatment of targeted populations compared to district level MDA implementation, and is more efficient and cost-saving than village level implementation.

1072

THE AMAZONIC NEMATODE *STRONGYLOIDES* AND THE ANDEAN FLUKE *FASCIOLA*: THE NEED FOR TAILORING CONTROL PROGRAMS

Luis A. Marcos[1], Jorge Machicado[2], Angelica Terashima[2], Marco Canales[2], Eduardo Gotuzzo[2]

[1]Infectious Diseases Division, Washington University, St. Louis, MO, United States, [2]Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru

Strongyloides stercoralis infection is a threat in HTLV-I co-infected people (highly endemic in Peru) or in people receiving mainly chemotherapy or steroids. *Fasciola hepatica* infection is able to cause significant hepatic morbidity in people from endemic areas, vegetarians or frequent travelers. Objectives of this study were to update current prevalence rates and reported cases of *S. stercoralis* and *F. hepatica* infections in Peru and to describe the most sensitive diagnostic tests performed in these studies. The inclusion criteria included studies originated in Peru from January 2000 to April 2010 and published in the following databases: MEDLINE, LILACS, SCIELO Peru and LIPECS (Peruvian Literature in Health Sciences). For searching criteria, the following keywords were used: Strongyloides, Strongyloidiasis, Fasciola, Fascioliasis and Peru. A total of 1362 subjects from 52 studies were reported with *S. stercoralis* infection (prevalence rates: 0.3-39%) whereas 1136 subjects from 33 studies were reported with *F. hepatica* infection (0.2-27%). The Lumbreras' Rapid Sedimentation Technique (LRST) was the most effective coprological test for detection of Fasciola eggs; whereas the agar plate, Dancescu culture and the Modified Baermann's Method (MBM) had the highest sensitivity for detection of *Strongyloides* larvae. Strongyloides geographic distribution is mostly in the Amazonic region whereas Fasciola is largely present in the Andean Region. Control programs should pay attention on the distribution of specific hepatointestinal parasites. *Strongyloides* is mostly present in the Amazonic region, requires agar plate for prompt diagnosis and ivermectin is the treatment of choice. Fascioliasis is present throughout the Andean Region, LRST is the coprological test of choice and the most effective treatment is triclabendazole. This is an example of how difficult it can be to approach a national program control of gastrointestinal parasitic infections since two common prevalent parasites have opposite geographic distribution, require especial diagnostic tests and different treatments.

1073

NEGLECTED GEOHELMINTHIASIS OF PERU IN THE NEW MILLENNIUM

Jorge Machicado[1], Luis A. Marcos[2], Angelica Terashima[1], Marco Canales[1], Eduardo Gotuzzo[1]

[1]Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Infectious Diseases Division, Washington University, St. Louis, MO, United States

Infections by intestinal parasites cause significant morbidity including malnutrition, growth retardation and cognitive impairment. Helminthiasis is the fourth cause of outpatient visits in Peru; approximately 1 million cases were seen in 2008 according to the Peruvian Ministry of Health. The objective of this study was to update the current prevalence rates of intestinal parasites in Peru. The inclusion criteria included the studies originated in Peru from January 2000 to April 2010 and published in data bases such as MEDLINE, LILACS (Latin American Literature), SCIELO Peru and LIPECS (Peruvian Literature in Health Sciences). For searching criteria, the following keywords in Spanish and English were used alone or in combination: parasites, intestinal parasites, helminths, and Peru. A total of 101 studies met the inclusion criteria. Out of the 43,803 subjects who were examined in these studies, 55% of them were infected with at least one parasite (n=24,266). The most common helminths found were: *Ascaris lumbricoides* 15% (n=4894; range of prevalence 0.6-82%), *Trichuris trichiura* 14% (n=3018; 0.2-82%) and hookworm 13% (n=1686; 0.1-73%). Fifty-five percent of the studies used at least one sedimentation technique for stool examination. The most common protozoa was *Giardia lamblia* 24% (n=6936; 3-44%). The age group mostly affected was young people below 20 years old and the highest prevalence rates were present in the poorest and most forgotten communities. The highest prevalence rates in Peru in the last years are present in regions with poor health access and on severe poverty. Further studies are warranted to measure the impact of economic, simple and highly sensitive sedimentation techniques in combination with targeted massive population treatments. This is an example of how little we know about a very common public health problem in a developing country which has been forgotten even at the beginning of this new Millennium.

1074

TRYPANOSOMA CRUZI I GENOTYPES IN DIFFERENT GEOGRAPHIC REGIONS AND TRANSMISSION CYCLES BASED ON A MICROSATELLITE MOTIF OF THE INTERGENIC SPACER OF SPLICED LEADER GENES

Carolina I. Cura[1], Ana M. Mejía-Jaramillo[2], Tomás Duffy[1], Juan M. Burgos[1], Marcela Rodriguero[3], Marta V. Cardinal[4], Sonia Kjos[5], Rodrigo Gurgel-Gonçalves[6], Denis Blanchet[7], Luis M. De Pablos[8], Nicolás Tomasini[9], Alex Da Silva[5], Graciela Russomando[10], Cesar A. Cuba Cuba[6], Christine Aznar[7], Teresa Abate[11], Mariano J. Levin[1], Antonio Osuna[8], Ricardo E. Gürtler[4], Patricio Diosque[9], Aldo Solari[12], Omar Triana-Chávez[2], Alejandro G. Schijman[1]

[1]Laboratorio de Biología Molecular de la Enfermedad de Chagas, Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina, [2]Grupo Biología y Control de Enfermedades Infecciosas, Universidad de Antioquía, Medellín, Colombia, [3]Laboratorio de Genética Evolutiva, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina, [4]Laboratorio de Eco-Epidemiología, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina, [5]Department of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States, [6]Parasitologia Médica e Biologia de Vetores, Área de Patologia, Faculdade de Medicina, Universidade de Brasilia, Brasilia DF, Brazil, [7]Laboratoire Hospitalier et Universitaire de Parasitologie Mycologie, Faculté de Médecine, Université des Antilles et de la Guyane, Cayenne, French Guiana, [8]Institute of Biotechnology, Molecular Parasitology Group, University of Granada, Granada, Spain, [9]Laboratorio de Patología Experimental, Universidad Nacional de Salta, Salta, Argentina, [10]Departamento de Biología Molecular, Instituto de Investigaciones en Ciencias de la Salud, Universidad Nacional de Asunción, Asunción, Paraguay, [11]Sección de Biología Molecular, Instituto de Medicina Tropical, Universidad Central de Venezuela, Caracas, Bolivarian Republic of Venezuela, [12]Programa de Biología Celular y Molecular, Facultad de Medicina, Universidad de Chile, Santiago, Chile

The intergenic region of spliced-leader (SL-IR) genes from 105 *Trypanosoma cruzi* I infected biological samples, culture isolates and stocks from 11 endemic countries, from Argentina to USA, were characterised, allowing identification of 76 genotypes with 54 polymorphic sites from 123 aligned sequences. On the basis of the microsatellite motif proposed by Herrera et al. (2007) to define four haplotypes in Colombia, we could classify these genotypes into four distinct Tc I SL-IR groups, three corresponding to the former haplotypes Ia (11 genotypes), Ib (11 genotypes) and Id (35 genotypes); and one novel group, Ie (19 genotypes). Tc Ia was associated with domestic cycles in Southern and Northern South America and sylvatic cycles in Central and North America. Tc Ib was found in all transmission cycles from Colombia. Tc Id was identified in all transmission cycles from Argentina and Colombia, including Chagas cardiomyopathy patients, sylvatic Brazilian samples and human cases from French Guiana, Panama and Venezuela. Tc Ie gathered five samples from domestic *Triatoma infestans* from Northern Argentina, nine samples from wild *Mepraia spinolai* and *Mepraia gajardoi* and two chagasic patients from Chile and one from a Bolivian patient with chagasic reactivation. Mixed infections by Tc Ia + Tc Id, Tc Ia + Tc Ie and Tc Id + Tc Ie were detected in vector faeces and isolates from human and vector samples. In addition, Tc Ia and Tc Id were identified at different tissues from a heart transplanted Chagas cardiomyopathy patient with reactivation, denoting histotropism. *T. cruzi* I SL-IR genotypes from parasites infecting *Triatoma gerstaeckeri* and *Didelphis virginiana* from USA, *T. infestans* from Paraguay, *Rhodnius nasutus* and *Rhodnius neglectus* from Brazil and *M. spinolai* and *M. gajardoi* from Chile are described for the first time.

1075

BOVINE ANTIBODY RESPONSE DIRECTED AGAINST *GLOSSINA* SALIVA: AN EPIDEMIOLOGIC MARKER OF CATTLE EXPOSURE TO TSETSE BITES

Martin B. Somda

CIRDES, Bobo-Dioulasso, Burkina Faso

Our objective is to develop a sero-epidemiological tool to measure the exposure of cattle to tsetse bites based on the host antibody response directed against *Glossina* total salivary antigens. The anti-saliva (IgG) response against *Glossina palpalis gambiensis* saliva was assessed by ELISA-indirect on 102 bovine sera from Burkina Faso: 48 were sedentary bovine from a tsetse free area (North) and 54 were from a tsetse infested area (South-West). High anti-saliva responses were detected in cows from the tsetse infested area. In these animals, the anti-saliva response was significantly higher during the hot dry season (p=0.0004) when animals are the most exposed to *Glossina* bites when watering at gallery forests along permanent streams. Furthermore, there was a strong positive association between the anti-saliva response and the risk of being infected by trypanosomes (p=0.004). These results show that the anti-saliva response may be an interesting marker of exposure of cattle to tsetse bites. However, some animals from the tsetse free area had also elevated anti-saliva responses, suggesting the existence of immune cross-reactivity with salivary proteins from other hematophagous arthropods. We have carried out experimental exposure of cows to the bite by different hematophagous vectors in order to further evaluate the use of *Glossina* total salivary antigens as marker of exposure. Good dynamic of apparition and disappearance of anti-saliva antibodies were observed in animals exposed to the different *Glossina* species whereas some cross reaction with the saliva of horse-flies are suspected. In perspectives, we want to identify and to synthesize tsetse saliva specific antigens in order to develop a more specific and standardized tool. This tool could be used in african trypanomosis endemic areas to (i) identify highly exposed herds toward which vector control should be directed to and to (ii) assess the efficiency of entomological control measures.

1076

PREVALENCE OF HAEMO AND ECTOPASRASITES IN CAMELS SLAUGHTERED AT MAIDUGURI METROPOLITAN ABATTOIR, BORNO STATE, NIGERIA

Lynda U. Odofin[1], A. Kalu[2]

[1]Yale University School of Public Health, New Haven, CT, United States, [2]University of Maiduguri, Borno State, Nigeria

Trypanosoma evansi is the main cause of trypanosomiasis (Surra) in camels. Camels (*Camelus dromedarius*) are a popular source of protein next to beef in Northern Nigeria. It has also been known to be less infected with parasites. To determine the prevalence of hemo and ectoparasites in camels, two hundred camels (*C. dromedarius*) were sampled at the Maiduguri metropolitan abattoir in northern Nigeria, West Africa. Six of these were infected with *T. evansi,* and one was infected with *Babesia.* Also Eighty-three percent of these animals were infested by ectoparasites including ticks. Even though humans are not typically infected with *T. evansi,* there have been case reports of such infections in farmers. This preliminary report suggests a need for further research in this area. Furthermore trypanosomiasis in camels results in huge economic loss for farmers, and like their cattle counterparts camel farmers need to be educated about proper husbandry and disease prevention.

1077

SMALL MAMMAL DIVERSITY AND *LEISHMANIA* INFECTION ACROSS A FOREST COVER GRADIENT IN SOUTHERN COSTA RICA

Mario Baldi[1], Manuel Spinola[1], Bernal Rodriguez[2], Jose Castro[1], Uriel Kitron[3], Luis F. Chaves[3]

[1]Univerisidad Nacional de Costa Rica, Heredia, Costa Rica, [2]Univerisidad de Costa Rica, San Pedro, San Jose, Costa Rica, [3]Emory University, Atlanta, GA, United States

Anthropogenic activities have transformed the landscape leading to gradients of forest cover worldwide. These fragmented and heterogeneous landscape patterns have been associated with the emergence and transmission of Cutaneous Leishmaniasis in Southern Costa Rica. One possible mechanism behind the emergence of this neglected tropical disease is the change in the biodiversity and abundance of mammal species, that can serve as reservoirs of Leishmania parasites, and that is mediated by concurrent changes in the landscape. Here, we present results of six months of small mammal species sampling using the advanced distance sampling, a new method that optimizes the sampling effort to estimate demographic parameters from wildlife populations employed along several plots across a forest cover gradient in southern Costa Rica. We think the use of this new sampling methodology can improve our understanding of the transmission dynamics of this disease by providing demographic information necessary to understand the eco-epidemiology of this pathogen in the reservoirs.

1078

EPIDEMIOLOGY OF AMERICAN TEGUMENTAR LEISHMANIASIS IN THE XAKRIABÁ INDIGENOUS COMMUNITY, STATE OF MINAS GERAIS, SOUTHEAST BRAZIL: A CROSS SECTIONAL STUDY

Elizabeth C. Moreno[1], Célia M. Gontijo[2], Patricia F. Quaresma[2], Janaina Freire[2], Eloiza G. Antonio[2], Raquel Carvalho[2], Renata R. Carvalho[1], Maria N. Melo[1]

[1]Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [2]Laboratorio de Leishmanioses do Centro de Pesquisas Rene Rachou, Belo Horizonte, Brazil

American Tegumentar Leishmaniasis (ATL) is a serious health problem in the Xakriabá indigenous community, in the northeast of Minas Gerais, Brazil. Objectives: to estimate the prevalence and identify possible risk factors of clinical and asymptomatic ATL within the Xakriabá. A survey was carried out in two villages with a higher number of recent human cases of ATL. A sample of the population was interviewed using pre-coded questionnaires investigating the demographic characteristics, socio-economic status, personal and cultural habits, characteristics of domiciles and neighborhoods. Presences of reservoirs and phlebotomies species were also investigated. The diagnoses were carried out using clinical evaluations, skin tests, parasitological (direct examination and culture) and molecular techniques (PCR-RFLP). Immunological profiles are also being constructed. A sample of 164 individuals was studied. The mean age was 22.5 ±16.6 years and females constituted 59% of the sample. Within this low socio-economic community, 65% had only elementary school education levels. Most participants (85%) reported daily contact with the forest. The majority usually burn manure in their houses to protect them against insect bites. Among the participants, 46% reported that a household member has had skin lesions. Only 16 (9.8%) of interviewees reported knowledge of the sandfly, with 15 mentioning either the typical bite with small red points, or the style of flying in short jumps. When photos were shown to the interviewees, 75.3% reported that they had previously seen the disease. Eleven clinical cases were identified: 9 were confirmed by parasitological examinations as *Leishmania braziliensis* and 2 were classified as clinical-epidemiological cases and were cured after treatment. The prevalence was estimated at 6.7% (IC 95% 3.6-11.4). Among the 11 cases, 8 (72.7%) presented scars (probably relapses or reinfections). The prevalence of asymptomatic infection was 18.5% (IC 95% 12.9-25.3). The ages of asymptomatic individuals were significantly higher (P<0.001) than negative individuals. There was no sex difference among asymptomatic, clinical cases, and negative individuals. Other characteristics and adjustments are being investigated. Conclusion: this study confirms the high prevalence of ATL and asymptomatic infection and the high occurrence of relapses and reinfection in the Xakriabá community

1079

INDUCTION OF ATHEROMATOUS PLAQUES IN WISTAR RATS CHRONICALLY INFECTED WITH *TRYPANOSOMA CRUZI* AND FED AD LIBITUM WITH DIET RICH IN FATS

Elio A. Moreno, Jose L. Paredes, Gloria Premoli, Maritza E. Alarcon, Ana Lugo de Yarbuh, Juana Villarreal

Universidad de Los Andes, Merida, Bolivarian Republic of Venezuela

This work is focused on the induction of atheromatous plaques in male albino rats Wistar (*Rattus norvegicus*), chronically infected with *Trypanosoma cruzi* and fed *ad libitum* with diet rich in fats of vegetal origin, during three months. The chronic infection detected by serological and parasitological assays, revealed the presence of antibodies IgG anti-*T. cruzi* and the absence of parasitemias. The diet rich in fats produced in the group of infected rats (A) and the group of healthy rats (C) a significant increase in the weight (P<0.05), in comparison with the control group of infected rats (B) and the group of healthy rats (D), fed with a normal diet. The histopathological study of sections of aorta artery of rats of the group A (infected/diet fats), showed abundants lipid deposits, inflammatory processes (vasculitis) and atheromatous plaques in development. The sections of the heart and skeletal muscle showed pictures of a myocarditis and myositis with features of chronic tissue without parasitism. The immunoistochemestry assay applied to the cuts of artery, heart and skeletal muscle of infected rats A (diet/fast) and B (normal/diet), showed abundants antigen deposits. In conclusion, the rat chronically infected with *T. cruzi* and fed with a diet rich in fats, have a main propensity to develop atheromatous plaques. The results showed that a hyperlipidic diet is a risk factor for the development of atheromatous disease in individuals with Chagas´disease.

1080

CHAGAS' DISEASE AMONG CHILDREN AND MOTHERS IN MAYAN RURAL COMMUNITIES: UTILIZING PDA/GPS UNITS FOR HOUSEHOLD SURVEYS

Rubi Gamboa-León[1], Claudia González-Ramírez[1], Freddy Pacheco-Tucuch[1], Matthew O'Shea[2], Christopher Swalm[2], Jeffrey Shaffer[2], Frances J. Mather[2], Kathryn Rosecrans[2], Julia Pippitt[2], Eric Dumonteil[1], Pierre Buekens[2]

[1]Centro de Investigaciones Regionales, Universidad Autónoma de Yucatán, Mérida, Mexico, [2]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States

The protozoan parasite *Trypanozoma cruzi* is the causal agent of Chagas' disease, a major vector-borne disease in Latin America. We determined the prevalence of *T. cruzi* infection in children (0-12 years old) and their mothers in two communities (Sudzal and Teya) in Yucatán, Mexico by conducting a household survey of mothers and their children. We utilized a suite of mobile technologies (PDA/GPS) to geo-reference 371 households and to collect the survey response data. Within the households, 685 children and 390 mothers were surveyed, which represented a 94% acceptance and participation rate. The ages of the children surveyed were from 0-12 years old; the ages of the mothers surveyed were from 15-59 years old. *T. cruzi* seropositivity was determined with a rapid test (Stat-Pack) and an ELISA assay (Wiener recombinant ELISA) and confirmed by the Institute of Reference and Diagnostic Epidemiology in Mexico, which used two additional diagnostic tests. Participants were considered seropositive when a sample was seroreactive to two or more tests. The

prevalence rates among children were 0.7% (2/193) in Sudzal and 0.3% (1/392) in Teya; the seroprevalence rates among their mothers were 4.4% (7/160) in Sudzal and 0.9% (2/230) in Teya. The higher seroprevalence of *T. cruzi* infection in Sudzal may be due to differences in sociodemographic, economic, or entomologic factors between these communities. These results draw attention to the need for Chagas' disease prevention programs in the region.

1081

IDENTIFICATION OF TRYPANOSOMES ISOLATED FROM HUMANS AND ANIMAL RESERVOIRS IN THE *TRYPANOSOMA BRUCEI GAMBIENSE-T.B. RHODESIENSE* INTERFACE DISTRICTS OF NORTHERN UGANDA

Enock Matovu, Anne J. Kazibwe, Doreen Asiimwe, Alex Boobo

Makerere University, Kampala, Uganda

Human African Trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense* (chronic) or *T. b. rhodesiense* (acute) is a major public health problem in rural Uganda. Continued northward spread of *T. b. rhodesiense* from the Southeast towards *T. b. gambiense* endemic districts (Northwest) is feared to complicate diagnosis and treatment if the 2 diseases co-exist. In order to enable the National HAT programme institute informed control measures, we aimed to characterize trypanosomes from patients and domestic animal reservoirs in the interface districts of Northern Uganda. We carried out surveys in Lira, Dokolo, and Amuru districts. Of 43 persons found positive by the Card Agglutination Test for Trypanosomiasis (CATT) in Lira, 1 was confirmed parasite positive after Haematocrit Centrifugation technique (HCT). Analysis on the 43 CATT positive blood spots on FTA cards by PCR with TBR, SRA and TgsGP primers revealed *T. brucei* signals in 23, *T. b. rhodesiense* in 2; all were *T. b. gambiense* negative. Blood from 20 suspects (*T. brucei* signals only) was inoculated in mice. None became positive, indicating probable exposure to *T. brucei brucei* (non-human infective). We also tested samples passively collected from Lwala hospital that serves Dokolo and Lira, so far all are *T. b. rhodesiense*. From Amuru district in the north (about 100Km from the Lira sites), 1 person was found positive with *T. b. gambiense*. Of 1,782 domestic animals (1,713 cattle, 22 pigs, 19 goats, 18 dogs and 10 sheep) screened in Dokolo, 99 (96 cattle, 2 sheep and 1 pig) were confirmed parasite positive by HCT. Amplification of the rDNA Internal Transcribed Spacer (ITS) in 40 samples from cattle demonstrated *T. brucei* (a complex including the human infective subspecies) in 18, *T. congolense* in 11 and *T. vivax* in 6 animals. This preliminary observation that *T. brucei* (the least pathogenic among animal trypanosomes) is the most prevalent implies that farmers may not readily seek intervention, leading to an unchecked animal reservoir. Indeed 16 of the *T. brucei* were found to be *T. b. rhodesiense* by SRA-PCR.

1082

TRYPANOSOMA BRUCEI PARASITES FAVOR THEIR TRANSMISSION BY MODIFYING THE TSETSE FLY SALIVARY COMPOSITION

Guy Caljon[1], Karin De Ridder[1], Patrick De Baetselier[2], Marc Coosemans[1], **Jan Van Den Abbeele**[1]

[1]Institute of Tropical Medicine Antwerp, Antwerp, Belgium, [2]Free University of Brussels, Brussels, Belgium

Tsetse flies are the notorious transmitters of African trypanosomiasis, a disease caused by the *Trypanosoma* parasite that affects humans and livestock on the African continent. Here, the infected fly/host contact frequency is a key determinant of the transmission dynamics. As an obligate blood feeder, tsetse flies rely on their complex salivary potion to inhibit host haemostatic reactions ensuring an efficient feeding. The results of this experimental study suggest that the parasite might promote its transmission through manipulation of the tsetse feeding behavior by modifying the saliva composition. Indeed, salivary gland *Trypanosoma brucei*-infected flies display a significantly prolonged feeding time, thereby enhancing the likelihood of infecting multiple hosts during the process of a single blood meal cycle. Comparison of the two major anti-haemostatic activities i.e. anti-platelet aggregation and anti-coagulation activity in these flies versus non-infected tsetse flies demonstrates a significant suppression of these activities as a result of the trypanosome-infection status. This effect was mainly related to the parasite-induced reduction in salivary gland gene transcription, resulting in a strong decrease in protein content and related biological activities. Additionally, the anti-thrombin activity and inhibition of thrombin-induced coagulation was even more severely hampered as a result of the trypanosome infection. Indeed, while naive tsetse saliva strongly inhibited human thrombin activity and thrombin-induced blood coagulation, saliva from *T. brucei*-infected flies showed a significantly enhanced thrombinase activity resulting in a far less potent anti-coagulation activity. These data clearly provide evidence for a trypanosome-mediated modification of the tsetse salivary composition that results in a drastically reduced anti-haemostatic potential and a hampered feeding performance which could lead to an increase of the vector/host contact and parasite transmission in field conditions.

1083

A HIGH-THROUGHPUT *IN VITRO* SCREEN TO IDENTIFY INHIBITORS OF THE *TRYPANOSOMA BRUCEI* TRYPANANTHIONE SYNTHETHASE ACTIVITY

Martin Kramer[1], Leah Torrie[2], Michael Booker[1], Daniel Spinks[2], Robert H. Barker, Jr.[1], Sandra Oza[2], Meghan Harris[1], Julie Frearson[2], Iain Collie[2], Alan Fairlamb[2]

[1]Genzyme Corporation, Waltham, MA, United States, [2]Division of Biological Chemistry and Drug Discovery, Wellcome Trust Centre, College of Life Sciences, University of Dundee, Dundee, United Kingdom

The protozoan parasite, *Trypanasoma brucei* is the primary causative agent for HAT (Human African Trypanasomiasis) which results in >50,000 deaths on a yearly basis in sub-Saharan Africa. Current therapies require lengthy hospitalization at a prohibitive cost and are very limited in scope due to toxic side effects and acquired drug resistance. The thiol-redox pathway plays an important role in the defense against chemical and oxidative stress and has been determined to be essential for the survival of *T. brucei*. Due to the absence of this particular biochemical pathway in man, the enzymes within this pathway, including trypanathione synthetase (TryS), represent excellent novel targets for HAT drug discovery efforts. A TryS activity assay was developed at the University of Dundee and a high-throughput screen of 215,000 compounds at 10 uM was undertaken at Genzyme Corporation in an effort to identify inhibitors of the *Tb*TryS. Following hit confirmation, several compounds were chosen to undergo IC50 potency determinations, and three novel series of active compounds were identified around which a medicinal chemistry program could be initiated. The activity of these compounds in a cultured *T. brucei* parasite assay will be determined in an effort to establish a correlation between *in -vitro* activity and *in- vivo* potency.

1084

TWO HIGHLY CONSERVED SECRETORY NUCLEASES FACILITATE PURINE SALVAGE IN *LEISHMANIA MEXICANA*

Yunuen Hernandez, Andrew Y. Kelada, Manju B. Joshi, Dennis M. Dwyer

National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States

All *Leishmania spp* are purine auxotrophs, i.e. they must salvage these molecules from both their insect/mammalian hosts to fulfill their metabolic needs. Since purines are critical to leishmanial survival, we deemed it important to elucidate the mechanisms used for such acquisition. In that regard, we identified two 35kDa secretory nucleases *Lmex*NUCs-1 and -2 in *Leishmania mexicana* which are differentially transcribed and translated through the parasite's life cycle (Am>>Prom). The two 951bp nuclease ORFs share 95% nucleotide homology and possess the five

structural motifs characteristic of the P1/S1 family of fungal nucleases. To understand the biological implications of these two nucleases, we over-expressed *Lmex*NUCs-1 and -2 in *L.mexicana*. Using molecular and biochemical techniques, we demonstrated that *Lmex*NUCs-1 and -2 chimeric enzymes were expressed and secreted/released constitutively by both *L.mexicana* promastigote and amastigote developmental forms. Further, we found that the chimeric and wild type enzymes were functionally active in hydrolyzing a variety of natural and synthetic substrates (i.e. Poly-A, -U, -I, RNA, ssDNA and dsDNA). Such nuclease activity required a metal co-factor and was inhibited by DTT reduction. In addition, we showed that *Lmex*NUCs-1 and -2 were actively synthesized by amastigotes within infected mouse macrophages. Our cumulative results suggest that *Lmex*NUCs-1 and -2 play important role(s) in facilitating the growth, development and survival of this human pathogen.

1085

FUNCTIONAL, MORPHOLOGICAL AND METABOLIC EVALUATION OF MICE ACUTELY INFECTED WITH THREE DIFFERENT STRAINS OF *TRYPANOSOMA CRUZI*

Mara R. Celes[1], Cibele M. Prado[1], Lygia M. Malvestio[1], Erica C. Campos[1], Linda A. Jelicks[2], Herbert B. Tanowitz[3], Marcos A. Rossi[1]

[1]Department of Pathology, University of São Paulo, Ribeirão Preto, Brazil, [2]Department Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY, United States, [3]Department of Pathology, Albert Einstein College of Medicine, Bronx, NY, United States

Strains of *Trypanosoma cruzi* are multiclonal populations that can be classified in groups or genotypes, differing in pathogenicity, virulence, and histotropism. In this study, functional, morphological and metabolic evaluation of mice infected with three strains of *T. cruzi* was performed in the peak of mortality of the animals. CD1 mice infected with 5x104 of the Brazil strain or C57Bl/6 mice infected with 1x103 of the Y strain or C57Bl/6 mice infected with 1x103 of the Tulahuen strain were used. Parasitemia, histopathology, echocardiography (Echo), magnetic resonance imaging (MRI), microPET and body composition (BC) were evaluated. 60% of CD1 mice infected with the Brazil strain died 28-32 dpi, when the parasitemia was decreasing. They presented generalized edema characterized by increased amount of water and fluids and decreased fat at BC analysis. The myocarditis was more evident in the right ventricle (RV) than in the left ventricle (LV). The RV was dilated and the cardiac frequency was decreased in the Echo. The cardiac metabolism was changed from fatty acid to carbohydrate oxidation. 100% of C57Bl/6 mice infected with the Tulahuen strain died 18-24 dpi when the parasitemia was still increasing. They presented increased amount of water and fluids and decreased fat at BC analysis. The myocarditis was present in both ventricles. The RV was dilated and there was no alteration in the Echo. The cardiac metabolism was changed from fatty acid to carbohydrate oxidation. 100% of C57Bl/6 mice infected with the Y strain died 16-23 dpi when the parasitemia was decreasing. They presented increased amount of water and decreased fat at BC analysis. The myocarditis was present in both ventricles. The RV was dilated and the wall thickness of RV and LV was increased. There was no alteration in the Echo. The cardiac metabolism was changed from fatty acid to carbohydrate oxidation. The results corroborate that the genetic differences between the *T. cruzi* strains correlate with their tissue tropism and can help to explain the different findings.

1086

IDENTIFICATION OF PROTEINS DIFFERENTIALLY EXPRESSED IN *LEISHMANIA (VIANNIA) BRAZILIENSIS* AND *L.(V.) PERUVIANA* BY TWO DIMENSIONAL GEL ELECTROPHORESIS

Patricia I. Reyes-Uribe[1], Jorge Arévalo[1], Patricia Cuervo[2]

[1]Instituto de Medicina Tropical "Alexander von Humboldt" - Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Departamento de Imunologia, Instituto Oswaldo Cruz – FIOCRUZ, Rio de Janeiro, Brazil

The cellular and molecular basis that supports the progress from cutaneous to mucous Leishmaniasis (metastasis) is not well understood. *Leishmania (V.) peruviana* and *L.(V.) braziliensis* are two species so closely related that are almost indistinguishable when analysed at the DNA level. However, despite their proximity, *L.(V.) peruviana* only causes cutaneous Leishmaniasis, whereas *L.(V.) braziliensis* can as well disseminate to distant tissues ending in mucous leishmaniasis. For studying this event, a proteomic study was performed to identify proteins differentially expressed by these two species as candidate metastasis markers. Promastigotes of LC2043 (*L.(V.)braziliensis*) and HB86 (*L.(V.)peruviana*) strains were lysed by freeze and thaw cycles and 500ug of soluble proteins were applied in 18cm, non-linear strips for isoelectrofocusing. After, they were separated according their molecular weight by 2DE electrophoresis and gels were analyzed with ImageMaster™ 2D Platinum v7.0. Landmark proteins were detected and gels images calibrated according to their isoelectric point (*pI*) and molecular weight (*Mr)*. While using strips with pI from 3-10, results showed that similar to *L.(V.)braziliensis (1)*, most of the spots from *L.(V.) peruviana* were in the 4-7 *pI* range. From 2 independent experiments, approximately 396 spots were detected in the *L.(V) peruviana* samples, with proteins predominantly from 22 to 80 kD. Comparison of the two proteomes revealed one protein specifically associated with LC2043 mucosal strain. Interestingly, these spot was not present in the HB86 cutaneous strain proteome. On the contrary, other spot (*pI* 7.6) was exclusively expressed in the *L.(V.) peruviana* proteome. In this study, the first *L.(V.) peruviana* protein map was obtained. Furthermore, we have found 2 spots differentially expressed in the *L.(V.) braziliensis* and *L.(V.) peruviana* proteomes that might be markers associated to metastasis. Therefore, it is of pivotal importance to characterize these proteins by mass spectrometry (MALDITOF) and test them on their potential of being metastasis markers.

1087

ALGORITHM PERFORMANCE ASSESSMENT OF PCR AND PCR-RFLP ASSAYS FOR *LEISHMANIA VIANNIA* SPECIES IDENTIFICATION

Nicolas Veland[1], Cristian Valencia[1], Andrea K. Boggild[2], Gert van der Auwera[3], Jean Claude Dujardin[3], Jorge Arevalo[1]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Tropical Disease Unit, Division of Infectious Diseases, Toronto General Hospital, Toronto, ON, Canada, [3]Department of Parasitology, Institute of Tropical Medicine Prince Leopold, Antwerp, Belgium

Leishmania species identification is currently performed based on PCR assays because their high sensibility made them useful on clinical samples. Different targets are used to identify *Viannia* species but there is no consensus methodology described. We present an algorithm based on a three target PCR strategy that can discriminate specifically between *L. peruviana, L. braziliensis* and *L. guyanensis*. The algorithm integrates three different targets previously reported for *Viannia* species identification: mannose phosphate isomerase (MPI) PCR that can identify specifically *L. (V.) peruviana*, cystein proteinase B (CPB) PCR-RFLP that can identify *L. (V.) braziliensis* and heat shock protein 70 kDA (HSP70) PCR-RFLP that differentiates specifically *L. (V.) guyanensis*. We analyze field isolates and clinical samples from patients that yield intense bands in kDNA diagnostic PCR. The samples analyzed include aspirates, scrapings and filter paper. Concerning to field isolates, we successfully identified *Leishmania* species

in 79 out of 80. The species founded were mainly *L. peruviana* (23)*, L. braziliensis* (41) and *L. guyanensis* (14). Only one isolate revealed evidence that suggest that it correspond to a *L. braziliensis/L. peruviana* hybrid. Sensibility of the targets demonstrates that MPI PCR has the highest while lower values were seen in HSP70 PCR. Additionally, when clinical samples from 12 patients were analyzed,analyzed; we successfully identify species in all of them. However, results vary based on different sensibility raised with different types of clinical samples. In general, filter paper reaches higher sensibility values. In conclusion, our algorithm is specific for *Leishmania Viannia* species identification. It can be used to estimate cost of species identification according known species prevalence in different endemic areas, with consequent improvement of hich can be useful to improve leishmaniasis healthcare decision makingpolicies. A consensus strategy as our algorithm is potentially useful for clinicians for decision-making in terms of treatment outcome and patient management.

1088

ASSESSING GENE STATUS AND EXPRESSION PROFILE OF AQUAGLYCEROPORIN, ABC TRANSPORTER MRPA, ORNITHINE DECARBOXYLAE AND Γ-GLUTAMYLCYSTEINE SYNTHETASE IN ANTIMONY-SUSCEPTIBLE AND RESISTANT CLINICAL ISOLATES OF *LEISHMANIA DONOVANI* FROM INDIA

Swati Mandal, Rentala Madhubala

School of Life Sciences, New Delhi, India

Clinical resistance to pentavalent antimonials is interplay between uptake, efflux and sequestration in Leishmania but resistance to this drug in the field isolates is not clearly understood. To address this question, in the present study, we have characterized clinical isolates from India and report that diverse mechanisms of resistance are operative in these isolates. In the present study, we examined the role of Aquaglyceroporin (AQP1), ABC transporter (MRPA), ornithine decarboxylase (ODC) and γ-glutamylcysteine synthetase (γ-GCS) genes as possible biomarkers for monitoring antimonial resistance in Indian leishmaniasis. Aquaglyceroporins (AQPs) have been shown to facilitate uptake of trivalent metalloids. The ABC tranporter gene MRPA confers resistance by sequestration of metal-thiol conjugate. Ornithine decarboxylase (a rate limiting enzyme in polyamine biosynthesis), and γ--glutamylcysteine synthetase (a rate limiting enzyme in glutathione biosynthesis) are two building blocks of the main cellular thiol trypanothione. Susceptibility to trivalent antimony as determined *in vitro* with intracellular amastigotes from both visceral (VL) and post-kala-azar dermal leishmaniasis (PKDL) patients correlated well with the clinical response. Reduced accumulation of SbIII correlated, with a few exceptions, to downregulation of AQP1 RNA as determined by real-time PCR in resistant isolates. Transfection of AQP1 gene in a SAG-resistant field isolate conferred sensitivity to the resistant isolate. However, increased expression of MRPA by real-time PCR was observed in resistant isolates. Transfection of MRPA in a sensitive isolate indeed conferred resistance to SAG in intracellular parasites. Cysteine and glutathione levels were increased in the SAG resistant isolates. Ornithine decarboxylase and γ--glutamylcysteine synthetase were found upregulated in all of the resistant isolates. Transfection of γ-GCS in a sensitive isolate led to 3 fold resistances to SAG in intracellular amastigotes, thereby confirming the role of γ-GCS as one of the factors involved in SAG resistance. We also found that the ODC overexpressors exhibited significant resistance to Pentostam compared to the wild type cells (>8.8 fold). A variety of resistance mechanisms to SAG, most of them consistent with a model based on the study of resistance *in vitro*, were present in clinical isolates from the same geographical region.

1089

DISCOVERY OF 2,4-DIAMINOPYRIMIDINES AS POTENT INHIBITORS OF *TRYPANOSOMA BRUCEI* AND IDENTIFICATION OF MOLECULAR TARGETS BY A CHEMICAL PROTEOMICS APPROACH

Tana Bowling[1], Luke Mercer[1], Joe Perales[1], Jennifer Freeman[1], Tien Nguyen[1], Cyrus Bacchi[2], Nigel Yarlett[2], Robert Don[3], Robert Jacobs[1], Bakela Nare[1]

[1]SCYNEXIS, Inc., Research Triangle Park, NC, United States, [2]Haskins Laboratories, Pace University, New York City, NY, United States, [3]Drugs for Neglected Diseases initiative, Geneva, Switzerland

Human African Trypanosomiasis (HAT) is a fatal disease caused by *Trypanosoma brucei spp*. There is a need for new treatments for HAT because current drugs are costly, difficult to administer and frequently toxic. We have identified 2,4-diaminopyrimidines that demonstrate potent *in vitro* activity against *T. brucei brucei* and the related trypanosomatids *Leishmania spp*. *In vitro* studies performed to characterize the relationship between killing of *T. brucei* and compound exposure demonstrate an early (9-12 hrs) onset of trypanocidal activity as shown by the inability of the parasites to generate ATP. Parasite commitment to death *in vitro* occurs with similar kinetics, even when compound is washed out following a short exposure. A representative 2,4-diaminopyrimidine cured an acute trypanosomiasis infection in mice when administered orally at 20 mg/kg twice daily for 4 days. To identify the molecular target(s) responsible for the mechanism of action of 2,4-diaminopyrimidines against trypanosomatids a representative analogue was immobilized on a solid matrix (sepharose) and used to isolate target proteins from parasite extracts. Mitogen-activated protein kinases (MAPKs) and cdc2-related kinases (CRKs) were identified as the major proteins that were specifically bound to the immobilized compound, suggesting their potential participation in the pharmacological effects of 2,4-diaminopyrimidines against trypanosomatid protozoan parasites. Because they exhibit a good preliminary *in vitro* and *in vivo* pharmacological profile, and they target essential kinases, 2,4-diaminopyrimidines represent a potential lead class of small molecules for development of novel treatments for HAT.

1090

INFLUENCE OF MACROPHAGE IMMUNE RESPONSE TO *LEISHMANIA BRAZILIENSIS* IN THE PATHOGENESIS OF TEGUMENTARY LEISHMANIASIS

Maria Olívia A. Bacellar[1], Angela Giudice[1], Celia Vendrame[2], Lucas Carvalho[1], Thaís Delavechia[1], Edgar Carvalho[1]

[1]Federal University of Bahia, Salvador, Brazil, [2]Federal University of São Paulo, São Paulo, Brazil

Macrophages are preferentially infected by *Leishmania* and serve both as a site of parasite multiplication and also are involved in *Leishmania* killing. In contrast to the wide knowledge of T cell in human leishmaniasis, very little is known about macrophage behavior in human *Leishmania* infection. The current study describes the immune response of macrophages in cutaneous leishmaniasis (CL) and mucosal leishmaniasis (ML) patients, evaluating the chemokines/cytokines produced after *L. braziliensis* infection. We also evaluated the frequency of inflammatory monocytes (CD14+CD16++) in the peripheral blood and TNF-α production by these cells. PBMC-derived macrophages from 21 CL patients 11 ML patients and from14 healthy subjects (HS) were infected with *L. braziliensis* and the levels of CCL2, CCL3, CXCL8, CXCL9 and TNF-α production were measured in supernatants of cultures by ELISA. Expression of CD14, CD16 and TNF-α production in peripheral blood monocytes were analyzed by flow cytometry. CCL2 production was higher in ML than in HS, p=0.0005. The levels of CXCL9 were significantly higher in CL patients and in ML patients when compared with HS, p=0.0001. The levels of TNF-α produced by macrophages from ML and CL patients were also higher than those produced by macrophages from HS, p=0.002 and p= 0.017

respectively. The frequency of CD14+CD16++ monocytes was higher in patients with CL and ML than HS,p=0.01 and p=0.03 respectively. The TNF-α production by cells from CL patients stimulated with soluble leishmania antigen was higher in CD14+CD16++ than in CD14CD16- cells, p< 0.005.The observation that macrophages from tegumentary leishmaniasis patients produce high levels of inflammatory chemokines/cytokines and show a higher expression of CD16++ cells suggest that these cells may participate in the pathogenesis of this disease.

1091

DETECTION AND PERSISTENCE OF HOST DNA IN BLOOD MEALS FROM *TRIATOMA INFESTANS* USING A NOVEL MOLECULAR METHODOLOGY

Luis Gomez-Puerta[1], Dawn M. Roellig[1], Daniel G. Mead[2], Jesus A. Pinto-Caballero[3], Maritza M. Calderon[3], Jenny Ancca-Juarez[3], Victor Quispe-Machaca[3], Michael Z. Levy[4], Robert H. Gilman[5], **Vitaliano A. Cama**[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]University of Georgia, Athens, GA, United States, [3]Universidad Peruana Cayetano Heredia, Lima, Peru, [4]University of Pennsylvania, Philadelphia, PA, United States, [5]Johns Hopkins University, Baltimore, MD, United States

Trypanosoma cruzi, the etiologic agent of Chagas disease, is transmitted by hematophagous reduviid bugs within the subfamily Triatominae. These vectors use a wide range of hosts as blood meal sources, and determining the feeding behaviors of such bugs is useful for ecological and epidemiological studies of *Trypanosoma cruzi*. A number of different approaches for blood meal analysis have been described previously, including serologic and molecular detection methodologies. In the current investigation, the temporal pattern of blood meal detection in *Triatoma infestans* was investigated using a novel molecular approach. Third and fourth instar, lab-reared *T. infestans* nymphs (n=20) were allowed to feed on the blood of one of eight animal species (*Canis familiaris*, *Cavia porcellus*, *Rattus norvegicus*, *Gallus gallus, Mus musculus domesticus*, *Sus scrofa*, *Felis catus*, and *Homo sapiens*). At 7, 14, 21, and 28 days post-feeding, the guts of five bugs were individually collected. The molecular technique used for host identification from vector blood meals was a heminested PCR using novel general mammalian and avian primers. Resulting amplicons were sequenced to determine the species of the experimental blood meal source. Findings indicated that host DNA could be detected up to 28 days post-feeding, and the species of the blood meal source could be differentiated at the same time point. The implications of this study are two-fold. First, unlike other molecular approaches that utilize species-specific primers, this method can be used in an area where the animal species and feeding behavior of the triatomines are unknown. Secondly, this method was able to detect host DNA after an extended period of time in the *T. infestans* gut, indicating the detection capabilities of this assay when used in a field ecological or epidemiological study.

1092

DEVELOPMENT OF LUMINESCENCE-BASED *LEISHMANIA* INTRACELLULAR AMASTIGOTE ASSAY FOR DRUG SCREENING

Susan E. Leed, Dayadevi Jirage, Michael T. O'Neil, Mark R. Hickman, Max Grogl, Jacob D. Johnson

Walter Reed Army Institute of Research, Silver Spring, MD, United States

The requirement for discovering new anti-leishmanial therapeutics remains due to the less than optimal treatments currently available. Since amastigote screening assays most closely reflect the pathological life cycle of the form found in mammalian hosts, many researchers consider axenic or intracellular amastigotes to be the most relevant *in vitro* assay cell types for leishmanial drug discovery. In order to establish a high-throughput *Leishmania* intracellular amastigote assay for use in our cutaneous leishmaniasis drug discovery program, we created *L.braziliensis, L. major, L. panamensis,* and *L. tropica* luciferase-expressin transfectants. Basic assay requirements were established with a manual 96-well assay using *L. major*. The IC50s of a limited panel of known anti-leishmanial compounds were determined to test the validity of the assay. To optimize assay conditions, starting macrophage and promastigote numbers, various macrophage cell lines, assay duration, plate centrifugation, luciferin-d concentration, and time of luciferin addition were examined. Currently, the assay has been automated to 96- and 384-well assay formats with continued IC50 testing of an expanded drug panel. The luminescence-based intracellular *Leishmania* amastigote assay has demonstrated the assay robustness, throughput, and reproducibility lacking from previously described amastigote assays.

1093

EFFECT OF HEAT SHOCK STRESS ON THE EXPRESSION OF THE VIRULENCE FACTOR GP63 IN *LEISHMANIA MAJOR*

Carlos Castrillon, Jorge Arévalo

Instituto de Medicina Tropical Alexander Von Humboldt, Lima, Peru

Leishmania parasites must survive and adapt to different environments, from the vector to the final host. Their gene expression is regulated at the post-transcriptional level, where the 3' UTR regions play a fundamental role. Heat stress affects mRNA stability in parasite heat shock proteins, nevertheless, this heat stress has not been assayed in virulence factors. *In vitro* drug testing is usually made at random, not understanding what processes are being disturbed by promising compounds until long lasting investigations. Then, standardized models for evaluation of drugs that possibly modify gene expression in parasites could be stablished. The objective of this study was to evaluate the effect of temperature in the gene expression of the surface protein GP63 in *Leishmania major*. According to GeneDB, there are 4 copies of gp63 in the *Leishmania major* genome, with main differences at the 3' UTR among them. Primers have been designed to discriminate between them at mRNA level. Promastigote cultures, both in logarithmic and stationary phase, have been exposed to 24, 34 and 37 ºC for 3 hours prior to RNA extraction. Reverse transcription was performed with OligodT to ensure post-transcriptional information, subsequently polymerase chain reaction (PCR) was performed. The PCR products were observed by agarose gel using ethidium bromide. No difference was noticed in expression of gp63 genes 1, 2 and 4 at different temperatures in both phases. However, the expression of gene number 3 shows trends of variation between logarithmic and stationary phases. Effect of temperature remains unclear in those phases for expression of gene number 3. Further investigations on factors modifying gp63 number 3 expression need to be pursued. An *in vitro* model for testing drugs and compounds that impair natural gene expression in Leishmania could be developed in this context.

1094

TRYPANOSOMA CRUZI TRUNCATES APOLIPOPROTEIN-A1 IN HUMAN HDL AND POTENTIALLY RESULTS IN A HYPERFUNCTIONAL HDL PHENOTYPE

Qianqian (Doreen) Miao, Dana Bailey, Cynthia Santamaria, Brian J. Ward, Momar Ndao

McGill University, Montreal, QC, Canada

Chagas disease (CD) is caused by the protozoan parasite, *Trypanosoma cruzi*. Endemic in Central and South America where ~17 million persons are infected, latent infections can persist for decades, causing terminal, cardiomyopathy in ~30% of subjects. Current diagnostic tests often cannot discriminate between disease stages, and as a result, patients are treated with chemotherapy regardless of the disease status. Side effects of chemotherapy include granulocytopenia, rash and peripheral neuropathy. New diagnostic strategies are urgently needed as are tools that permit clinicians to target therapy more effectively. According to previous observations, CD patients, even those who die from cardiac complications have a lower incidence rate of atherosclerosis. However, levels of HDL and Apo-A1 in CD patients are reported to be normal. Recently, our laboratory

discovered several novel biomarkers for CD using SELDI-TOF, as reported previously. This set of biomarkers may indicate the specific disease stages of individual patients and the risk of further disease progression. Most importantly, we have identified intact Apo-A1 as a negative biomarker for CD and several truncated forms of Apo-A1 as positive biomarkers for CD. Apo-A1 is the principle protein found in high density lipoprotein (HDL). We have demonstrated that the principal cystein protease of *T. cruzi* cleaves Apo-A1 in HDL as predicted by mass spectrometry data. We have also shown that HDL is collected onto the surface of the blood-stage form and is internalized into the target host cell by the parasite by immunofluorescent staining. We also confirmed that mice chronically infected with *T. cruzi* show similar patterns of Apo-A1 cleavage. As well, preliminary data suggest that HDL from CD infected mice is 20% better at cholesterol efflux than native HDL. We are currently working with a mice model with HDL depletion to investigate further the interaction between *T. cruzi* and host HDL and the impact of *T. cruzi* infection on host lipid homeostasis. Our unanticipated observations give unique insights into the potential protection against atherosclerosis in CD individuals.

1095

LEISHMANIA AETHIOPICA; THE UNUSUAL ETIOLOGIC AGENT OF CUTANEOUS LEISHMANIASIS IN HO DISTRICT OF THE VOLTA REGION OF GHANA

Godwin Kwakye-Nuako[1], Patrick F. Ayeh-Kumi[1], Manal Jamjoom[2], Israel Mensah-Attipoe[1], Richard H. Asmah[3], Godwin Afebe[4], Paul A. Bates[5]

[1]University of Ghana Medical School, Accra, Ghana, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]School of Allied Health Sciences, College of Health Sciences, Korle-Bu, Accra, Ghana, [4]Disease Control Unit, Ministry of Health, Ho, Ghana, [5]Division of Biomedical and Life Sciences, School of Health and Medicine, Lancaster University, Lancaster, United Kingdom

Leishmaniasis is a parasitic disease of significant public health importance. An outbreak of suspected cutaneous leishmaniasis (CL) was first seen in the Volta Region of Eastern Ghana in 1999, and has remained an endemic area ever since. To improve the level of understanding regarding leishmaniasis in West Africa, particularly in Ghana, there is a need to provide information for the management of the disease. The study focused on the identification of species of Leishmania parasites responsible for leishmaniasis infections reported in the Volta Region of Ghana. The Ho district, located in the middle zone of the Volta region in the south-eastern part of Ghana, was the study site. It borders on the east with Togo in the West African sub-region. Forty four samples were taken for the study. Skin scrapings were collected from the sites of active lesion(s). Primers P5 and P6, were used to amplify a fragment of ~1500 bp of the intergenic region between the ribosomal protein genes RPS7A and RPS7B on chromosome 1 and second primers P1 and P2, were used to amplify an internal fragment of ~1350bp in the nested PCR. Products obtained from the nested PCR were digested using MspI enzyme. The bands produced from some samples showed a match to the control sample L. aethiopica. PCR was shown to be a useful diagnostic tool for Ghanaian CL.

1096

WIDESPREAD SEGMENTAL, FOCAL COPY NUMBER VARIATIONS (CNV) IN *TRYPANOSOMA CRUZI* STRAINS REVEALED BY ARRAY COMPARATIVE GENOMIC HYBRIDIZATION

Todd A. Minning, D. Brent Weatherly, Stephane Flibotte, Rick Tarleton

University of Georgia, Athens, GA, United States

Trypanosoma cruzi is a protozoan parasite and the etiologic agent of Chagas disease, an important public health problem in Latin America. *T. cruzi* is diploid, almost exclusively asexual, and displays an extraordinarily diverse population structure both genetically and phenotypically. Yet, to date the genotypic diversity of *T. cruzi* and its relationship, if any, to biological diversity have not been studied at the whole genome level. In this study, we used whole genome oligonucleotide tiling arrays to compare gene content in biologically disparate *T. cruzi* strains by comparative genomic hybridization (CGH). We observed that *T. cruzi* strains display widespread and focal copy number variations (CNV) and a substantially greater level of diversity than can be adequately defined by the current genetic typing methods. CNV were found in both core genes and gene family-rich regions of the genome, although primarily within the latter. Moreover, our results suggest that there is a greater degree of chromosome resorting between *T. cruzi* strains than previously thought.

1097

UNDERRATING MOSQUITOES AGAIN: SURVEILLANCE EVASION BY *PLASMODIUM FALCIPARUM* ANTIFOLATE DRUG RESISTANCE MUTANTS

Sungano Mharakurwa[1], Taida Kumwenda[1], Mtawa Mkulama[1], Mulenga Musapa[1], Sandra Chishimba[1], Clive J. Shiff[2], David Sullivan[2], Philip Thuma[1]

[1]Malaria Institute at Macha, Choma, Zambia, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

By definition, "malaria" was an initial underrating of mosquitoes, ascribing the disease to "bad air". Ironically, present-day surveillance for drug-resistant *Plasmodium falciparum* alleles is based on genotyping microscopy-positive human malaria infections and presumed epidemiologically representative. By examining *P. falciparum* antifolate resistance genotypes in both human and mosquito hosts, here we show evasion of surveillance by *P. falciparum* drug resistance mutants. Using spray catches, 796 predominantly *Anopheles* arabiensis vector mosquitoes were captured from sleeping rooms of a representative sample of 2279 human residents in the vicinity of Macha, Southern Zambia. The cross-sectional composition of *P. falciparum* DHFR polymorphisms in both human and mosquito infections was examined using PCR and allele-specific restriction enzyme digestion, including DNA sequencing confirmations. We found high levels of pyrimethamine resistance mutants in human *P. falciparum* infections, with nearly saturated S108N (92.7%) and considerably prevalent N51I (81.5%) and C59R (58.5%). In contrast, the odds of these mutants were up to 101-fold lower in the mosquito phase (OR [95% CI]: 101.3 [34.34 - 299.03], $p < 0.001$). Instead, mosquito infections exhibited high prevalence of S108T and A16V, associated with resistance to cycloguanil, a drug never used in the area. One mosquito mid-gut infection carried the I164L mutant, while another bore a novel I164R variant hitherto undescribed for this locus. Currently considered absent from African *P. falciparum* infections, both S108T and A16V, were initially not found in humans and only subsequently detected in microscopy-negative (submicroscopic) infections. Only wild type I164 was found in human malaria samples. It was concluded that the composition of detectable *P. falciparum* antifolate resistance alleles differs in human and mosquito hosts, presumably reflecting drug and (or) immune selection bias. During epidemiological tracking of drug resistance mutants, it is more representative to sample both human and vector infections.

1098

DEVELOPMENT OF AN ALLELIC DISCRIMINATION ASSAY FOR GENOTYPING SINGLE-NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH *PLASMODIUM FALCIPARUM* DRUG RESISTANCE

Edwin Kamau[1], LaDonna Tolbert[1], Samantha Jones[1], John Waitumbi[2], Christian F. Ockenhouse[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Walter Reed Project, Kenya Medical Research Institute, Kisumu, Kenya

Global Malaria control programs are being threatened by spread and emergence of old and new drug resistance parasites. To monitor drug resistance, assessment is performed by *in vivo* drug efficacy studies, *in vitro* testing of patient isolates and detection of molecular markers associated with drug resistance. However, *in vivo* drug efficacy studies and *in vitro* testing of patient isolates pose many challenges for a widespread use in resource constraint environment. The use of molecular markers as a tool for assessing *P. falciparum* drug resistance offers an attractive alternative for wide spread profiling of drug resistance that would benefit malaria control. We have developed an Allelic Discrimination TaqMan Assay for genotyping SNPs associated with *P. falciparum* drug resistance performed on an Applied Biosystems PCR platform. This method differs from conventional SNP detection methods in that it can perform parallel analysis of multiple SNPs on an epidemiological scale, it is rapid, inexpensive, sensitive, field deployable and compatible with the current systems found in most laboratories located in malaria endemic areas. To validate our platform, we selected 21 SNPs that are associated with resistance to antimalarials and tested them in nine *P. falciparum* strains that differ in their genetic profile. Of the 189 SNPs tested, 159 have been previously characterized. We obtained three SNPs which were in disagreement with the published information. We confirmed our data was correct by sequencing. Our data show that this assay is capable of discriminating SNP profile of mixed infection and it is highly sensitive. We tested the performance of the assay in the field by analyzing clinical trial samples. Of a total of 735 SNPs evaluated, the assay successfully detected 100% of all the SNPs assayed. We found polymorphism in 14 of the 16 patient samples tested. In conclusion, our assay is robust, reliable, and amenable to throughput.

1099

MALARIA DRUG SENSITIVITY TESTING USING QUANTITATIVE SPECTROSCOPIC ANALYSIS

Yulia Serebrennikova, Wilbur K. Milhous, Janus Patel, Luis H. Garcia-Rubio, Dennis E. Kyle

University of South Florida, Tampa, FL, United States

Schizont maturation assays have long been a cornerstone in basic malariology to assess parasite growth rates to determine drug susceptibility. With malaria drug resistance increasing in prevalence and severity, new technologies are needed to aid and improve the accuracy and clinical relevance of laboratory or field testing for malaria drug resistance. Here we present a method based on simple and reagentless spectroscopic analysis that provides valuable quantitative information on the morphological and biochemical character of the cells and microorganisms. We used the method to investigate W2 strain of *Plasmodium falciparum* treated with dihydroartemisinin and mefloquine. The size, internal structure, nucleotide and hemozoin composition of the parasites as well as morphology (size and shape) and haemoglobin composition of the infected erythrocytes were determined. Reduction in the sizes of the parasites and their structural organelles was observed after dihydroartemisinin treatment of the ring stage cultures. The nucleotide and hemozoin composition of the treated parasites and haemoglobin composition of the host erythrocytes determined from spectroscopic analysis changed negligibly following the treatment. Although mefloquine treated parasites were growing to the same size as those from parallel non-treated cultures, they lacked hemozoin and had decreased internal structure (organelles). Lesser deformation of the cell shape and no haemoglobin depletion were detected for the infected erythrocytes of mefloquine treated cultures. These results indicate sensitivity of the method for recognition of the effects of antimalarial treatment on the structure and composition of the parasites from the spectroscopic monitoring of the infected erythrocytes. These initial findings in one parasite clone suggest that the spectroscopic analysis can have significant potential for research and clinical applications such as evaluating patient specimens for drug action, drug effects or for therapeutic monitoring.

1100

IN VIVO SELECTION OF *PLASMODIUM FALCIPARUM* PFCRT K76 AND PFMDR1 N86 ALLELES BY ARTEMETHER-LUMEFANTRINE IN MALI

Anastasia Grivoyannis[1], Hamma Maiga[2], Hamidou Niangaly[2], Oumar Bila Traore[2], Aly Kodio[2], Karim Traore[2], Youssouf Tolo[2], Ahmadou Dembele[2], Aliou Traore[2], Amadou Bamadio[2], Zoumana Isaac Traore[2], Kassim Sanogo[2], Mody Sissoko[2], Issaka Sagara[2], Abdoulaye Djimde[2], Ogobara Doumbo[2]

[1]Weill Cornell Medical College, New York, NY, United States, [2]Malaria Research and Training Centre, University of Bamako, Bamako, Mali

Artemether-lumefantrine (AL) is a highly effective artemisinin combination therapy (ACT) that was adopted in Mali in 2005 as first-line therapy against uncomplicated *Plasmodium falciparum* malaria. This study, designed to measure parasite genetic markers associated with and selected by AL treatment failure, was conducted within the framework of a classical WHO drug-efficacy study. A 28-day follow-up efficacy trial of AL was conducted with 337 total participants (children aged >6 mos and adults) suffering from uncomplicated *falciparum* malaria in 4 different Malian villages -- Faladié (n=88), Kollé (n=77), Bandiagara (n=100), and Pongonon (n=72) -- during the 2009 malaria transmission season. Clinical outcomes in 326 patients (96.7%) were analyzed and the 28-day uncorrected adequate clinical and parasitological response (ACPR) rate was 73.9% -- Faladié (68.2%), Kollé (61.8%), Bandiagara (72.4%), and Pongonon (91.3%). Total PCR-corrected 28-day ACPR was 97.5%. Reinfections and recrudescences were then grouped as recurrent infections and analyzed together by PCR-restriction fragment length polymorphism (RFLP) to identify candidate markers for AL tolerance in the chloroquine resistance transporter gene *(pfcrt)* and the multi-drug resistance gene 1 *(pfmdr1). Pfcrt* T76 population prevalence decreased from 49.3% at baseline (n=337) to 38.8% in patients with *P. falciparum* recurrence (n=85) and *pfmdr1* Y86 population prevalence decreased from 11.0% at baseline (n=337) to 0% in patients with recurrent infection (n=85; P=0.001). Treatment with AL selected for the *pfcrt* K76-allele (P=0.014) and the *pfmdr1* N86-allele (P=0.0002) among recurrent infections. These findings suggest that the *pfcrt* K76T and *pfmdr1* N86Y mutations are associated with enhanced *P. falciparum* susceptibility to AL. Parasite populations exposed to AL in this study selected toward chloroquine-sensitivity, and reinforce observations in E. Africa of *pfcrt* K76 and *pfmdr1* N86 as markers of AL tolerance.

1101

EFFICACY OF ARTESUNATE-AMODIAQUINE (ASAQ) AND ARTEMETHER-LUMEFANTRINE (AL) FIXED DOSE COMBINATIONS (FDCS) FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* (PF) MALARIA IN NIMBA COUNTY, LIBERIA

Birgit Schramm[1], Parastou S. Valeh[1], Elisabeth Baudin[1], Charles S. Mazinda[1], Richard Smith[1], Loretxu Pinoges[1], Timothy Sundaygar[1], Yah M. Zolia[2], Joel J. Jones[2], Eric Comte[3], Vincent Jullien[4], Gwenaelle Carn[5], Jean-Rene Kiechel[5], Elizabeth A. Ashley[1], Philippe J. Guerin[1]

[1]Epicentre, Paris, France, [2]National Malaria Control Programme, Ministry of Health and Social Welfare, Monrovia, Liberia, [3]Médecins Sans Frontières, Geneva, Switzerland, [4]Saint-Vincent de Paul, Paris, France, [5]Drugs for Neglected Diseases initiative, Geneva, Switzerland

Monitoring therapeutic efficacy of recommended artemisinin-based combination therapies (ACTs) for treatment of uncomplicated Pf malaria is essential. Our objective was to assess the efficacy of ASAQ FDC in Liberia, where Pf malaria is largely perennial, and efficacy data scarce. The national antimalarial drug policy was AS + AQ since 2004, and was changed to ASAQ FDC in December 2009. An open-label, randomized controlled non-inferiority trial compared the genotype-corrected Day42 cure rates of ASAQ FDC (ASAQ Winthrop®) to AL (Coartem®) in children <5 years (6% non-inferiority margin; one-sided α 5%, power 80%). Day7 desethyl-amodiaquine and lumefantrine concentrations were measured. Three-hundred children age 6-59 months with uncomplicated Pf malaria, were randomized to 3 days of observed ASAQ (once a day) or AL (twice a day) prescribed by weight. AL was given with fatty food. Parasitaemia, clinical or laboratory adverse events (AEs) were recorded at weekly visits. Day42 genotyping-corrected cure rates were 97.3% (ASAQ; 95%CI: 91.6-99.1) and 94.2 % (AL; 88.1-97.2) (mITT, Kaplan-Meier analysis), and ASAQ was non-inferior to AL (cure rate difference -3.1%, upper limit 95%CI 1.2%). Similar findings were obtained with PP analyses. Day3 parasite clearance rates were 100% (ASAQ) and 99.3% (AL), respectively. Day42 re-infection rates were 43% (ASAQ) and 30 % (AL). Among most frequently reported AEs were fatigue (ASAQ: 28.9%; AL: 13.3%), anemia (ASAQ: 22.8%; AL: 15.3%), cough (ASAQ: 18.8 %; AL: 14.0%), increased liver-enzymes (ASAQ: 12.1%; AL: 16%), and vomiting (ASAQ: 10.7%; AL: 6.7%). The majority of AEs were mild to moderate. Three serious AEs occurred (ASAQ: severe malaria, pneumonia; AL: repeated vomiting of study drug). Both ASAQ and AL were highly efficacious in the < 5year population in Nimba County, Liberia. Re-infection rates were high in both arms in this highly endemic setting. Both FDCs were safe and overall well tolerated. These findings describe for the first time the performance of ACTs in Liberia.

1102

DEVELOPMENT OF NEUTRAL SINGLE NUCLEOTIDE POLYMORPHISM MARKERS TO STUDY THE *IN VIVO* DYNAMICS OF *PLASMODIUM VIVAX* INFECTION

Pamela M. Pennington, Maria E. Castellanos, Lucía Ortiz, Norma R. Padilla

Universidad del Valle de Guatemala, Guatemala, Guatemala

Plasmodium vivax anti-malarial resistance is currently assessed with *in vivo* studies. In these studies, blood samples are taken at weekly intervals after treatment to determine the persistence of the erythrocytic stage. If parasites are found seven days after treatment together with suppressive levels of chloroquine in blood, the parasite is assumed to be resistant. However, *P. vivax* can reappear after treatment through relapses from hypnozoite reactivation, recrudescence of surviving erythrocytic stages or re-infection, making it difficult to determine if the parasite observed after treatment is the one that started the infection. Genotyping the parasites at each time point is the best way to verify parasite identity and exclude new infections during the in vivo study. Variable regions of the Merozoite Surface Protein genes (msp) have been used for genotyping. However, these variable regions are under natural selection and mutations may be selected during the infection process, leading to genotype variation. For this reason, a method to detect neutral single nucleotide polymorphisms (SNP) was designed. Using bioinformatics tools, neutral SNPs of genes located in different chromosomes of P. vivax were identified: msp-1, msp-3 alpha, β-tubulin and cell division cycle-2. An allele-specific hemi-nested PCR assay was optimized to detect the identified SNPs in blood spots preserved in filter paper. Genotypes combining these four genes are presented for well characterized strains from different geographic regions. The use of these P. vivax genotype profiles is proposed as a new tool to study the in vivo dynamics of infection during drug trials.

1103

AMAZON MALARIA INITIATIVE: PROVIDING STRATEGIC SUPPORT TO PREVENT AND ASSIST IN MALARIA CONTROL IN THE AMAZON REGION

Pablo F. Aguilar[1], Debora B. Freitas[1], Jaime Chang[2]

[1]Links Media LCC, Gaithersburg, MD, United States, [2]United States Agency for International Development, Lima, Peru

More than 90% of malaria cases in the Americas occur in the Amazon Region. From 1995 to 2000, a significant rise in malaria cases and the emergence and spread of resistance to drugs and insecticides made malaria control a challenge. In 2001, the U.S. Agency for International Development launched the Amazon Malaria Initiative (AMI), establishing a health partnership among six international technical partners and seven countries (Bolivia, Brazil, Colombia, Ecuador, Guyana, Peru and Suriname). Based on capabilities assessments by National Malaria Control Programs (NMCPs), joint work plans for the provision of technical support have been implemented to build sustainable and integrated solutions at local, national and regional levels, through north-south and south-south collaboration. AMI results support that a comprehensive, strategic approach is necessary to strengthen health systems and make a difference in the fight against malaria. AMI's major achievements are the establishment of NMCP's network; the use of evidence-based public health practices by the NMCPs; the improvement of country capacities to monitor resistance to antimalarials and insecticides using *in vivo*, *in vitro* and molecular biology tools; the improvement of the quality control and quality assurance of medicines and insecticides; the transition to Artemisinin based combination therapy following World Health Organization recommendations. Current challenges include sustainability of NMCPs and competing priorities in an epidemiological context.

1104

DEVELOPING AND APPLYING GEOSPATIAL TOOLS FOR MALARIA PREVENTION: AN INTERNATIONAL PARTNERSHIP

Michael C. Wimberly[1], Alemayehu Midekisa[1], Gabriel Senay[1], Paulos Semunigus[2], Hiwot Teka[2], Ting-Wu Chuang[1], Geoffrey M. Henebry[1]

[1]South Dakota State University, Brookings, SD, United States, [2]Anti-Malaria Association, Addis Ababa, Ethiopia

Malaria is a major public health problem in Ethiopia, where outbreaks in highland regions can be affected by climatic variability, land use change, and movements of human populations. Applications of geospatial technologies, including geographic information systems (GIS) and satellite remote sensing, have potential for forecasting the spatial and temporal patterns of malaria risk. Multidisciplinary partnerships can foster the implementation of these tools by linking scientists who have knowledge of geospatial data and techniques with public health practitioners who have a detailed understanding of local needs. We have developed such a partnership involving the Geographic Information Science Center of Excellence (GIScCE) at South Dakota State University, and the Anti-Malaria Association (AMA), a non-governmental organization located in Addis Ababa, Ethiopia. In this partnership, the role of the GIScCE is to

develop models for ecological forecasting of malaria risk using satellite remote sensing, and the role of the AMA is to facilitate data collection, model validation, and implementation of the resulting products. Preliminary results have documented relationships between satellite-derived environmental metrics and malaria incidence in the Ethiopian highlands, confirming the feasibility of environmental risk mapping and forecasting. We have also developed other GIS data products related to land use, health facility accessibility, transportation, and population characteristics that may be useful for enhancan be used to enhance malaria prevention efforts. A key technical challenge in Ethiopia has been implementing internet-based mapping technologies in an environment of low connectivity and low bandwidth. Therefore, another important aspect of the partnership is developing effective, low-cost, and easy-to-use methods for providing public health practitioners with access to digital map products. These approaches include low-bandwidth web applications and standalone applications that do not require internet connectivity. The lessons learned and the tools developed through the ongoing collaboration between GIScCE and AMA can help to inform and enhance other global health partnership efforts.

1105

GENETIC DIVERSITY OF POLYMORPHIC VACCINE CANDIDATE ANTIGENS (*AMA-1*, MSP-3 AND *EBA-175*) IN *PLASMODIUM FALCIPARUM* ISOLATES FROM WESTERN AND CENTRAL AFRICA

Soulama Issiaka[1], Jude D. Bigoga[2], Magatte Ndiaye[3], Edith C. Bougouma[1], Josephine Quagraine[4], Prisca N. Casimiro[5], Rajapandi Thavamani[6], Timothy Stedman[6], Sodiomon B. Sirima[7]

[1]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [2]Department of Biochemistry and The Biotechnology Center, University of Yaunde I, Yahoundé, Cameroon, [3]Service de Parasitologie – Mycologie Université Cheikh Anta DIOP, Dakar, Senegal, [4]Department of Parasitology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana, [5]Unité de Pharmacologie Brazzaville (Congo), Brazzaville, Congo, [6]MR4, American Type Culture Collection, Manassas, VA, United States, [7]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Groupe d'Action et de Recherche en Santé, Burkina Faso

This study was designed to assess the pattern of diversity in three polymorphic genes (*eba-175, msp-3* and *ama-1*) from five countries spanning Central and West Africa. Blood samples were collected from approximately 600 *P. falciparum* infected subjects living in Cameroon, Congo Burkina Faso, Ghana and Senegal. Samples were screened for *eba-175* F- and C- alleles; *msp-3_* K1 and 3D7 alleles; and *ama-1* K1, 3D7 and HB3 alleles using PCR-Digest and nested or semi-nested PCR.. Genetic diversity as measured by mean heterozygosity (*He*) did not differ among genes or countries. However, for some genes, the frequency of alleles did differ among geographic regions. For example, while the eba-175 F-allele predominated in Congo, Cameroon, and Burkina Faso, we found no difference in the frequency of the F- and C- alleles from Ghana and Senegal. Likewise, the frequency of the *ama-1*_3D7 allele was lower in Central African compared to West African countries. Nei's Genetic Distance values for *eba-175* and *msp-3* were close to zero, confirming gene flow while some little high values for *ama-1* (between 0.12 and 0.18) were observed suggesting little or moderate *ama-1* gene flow. The mantel test values, as well as for *eba-175*, msp-3 were close to 0, suggesting the absence or a little of relationship between geographic and genetic distance for each of the maker except for *ama-1* between southern Ghana and Congo (value=0.124), Southern Ghana and Burkina (value=0.118), and Southern and northern Ghana (value=0.100) where mantel test values suggested a little or moderate relationship between geographic and genetic distance.

1106

IN VITRO ERYTHROPOIETIC CELL CULTURE FOR STUDIES OF *PLASMODIUM* PARASITES

Tetsuya Furuya, Timothy T. Stedman

MR4, BioServices, American Type Culture Collection, Manassas, VA, United States

Human erythropoietic cells, including reticulocytes, can be produced *in vitro* from hematopoietic stem cells (HSCs) using combinations of cytokines and co-culture with a mouse stromal cell line (MS5). Reticulocytes produced from HSCs have potential for *in vitro* culture of *Plasmodium vivax*. Erythropoietic cells produced from HSCs also have potential for studies of interactions between proteins of *Plasmodium* parasites and their hosts, since erythrocyte proteins can be modified genetically in HSC culture, which is not practical with donor blood. Here, we report use of erythropoietic cells from HSCs for infection of cryopreserved *P. vivax* parasite and for the study of *P. falciparum* host cell receptor. *P. vivax* parasites from infected *Aotus* monkeys were recovered from frozen stock and cultured overnight. Mature parasites were then enriched with centrifugation on 60 % Percoll and mixed with purified reticulocytes from HSCs. After 48 hr culture, newly infected *P. vivax* parasites were observed. Fewer infected RBC were detected after 72 hour incubation. This result suggests that cryopreserved *P. vivax* parasite can invade reticulocytes from HSCs, but culture optimization is required for maturation of infected parasites. To develop a system for study of potential *P. falciparum* erythrocyte surface receptors, HSCs were transfected with siRNA to reduce specific erythrocyte protein levels in maturing RBC. In a pilot study, transfected siRNA specific for Glycophorin C (GPC) resulted in marked reduction of expressed GPC mRNA and protein. Erythrocytes from the transfected HSCs were infected with purified *P. falciparum* parasites. No significant reduction in invasion events was observed with GPC-silenced erythrocytes. Nonetheless, the results suggest that *in vitro* maturation of erythropoietic cells will be useful to study host-blood stage parasite interaction of *Plasmodium* parasites.

1107

A STRUCTURAL AND FUNCTIONAL ANALYSIS OF TWO NOVEL *PLASMODIUM FALCIPARUM* PROTEINS INVOLVED IN THE ERYTHROCYTE INVASION PROCESS

Anthony Hodder, Peter Czabotar, Alex Uboldi, Ping Canon, Oliver B. Clarke, Clara Lin, Julie Healer and Alan F. Cowman

The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

The clinical symptoms and pathology associated with malaria occur during the asexual erythrocytic phase of the *Plasmodium* life cycle. Central to the severe debilitating effects imposed on the host is the ability of the extracellular merozoites to invade human erythrocytes. Invasion is a tightly controlled process involving specific receptor-ligand interactions between host and parasite molecules. Human erythrocytes are highly polymorphic with respect to the expression of surface molecules. *P. falciparum* has successfully adapted to such diversity by utilizing a number of alternative pathways for invasion that involve distinct erythrocyte receptors. A principal determinant of host cell specificity is the irreversible commitment of the merozoite to the selected host cell by the formation of a junction between merozoite and erythrocyte. The Duffy-binding-like (DBL) family of erythrocyte binding proteins (EBPs), are key parasite ligands that interact with host receptors during invasion. The DBL domain(s) of these proteins are responsible for binding to the receptor molecules. The determination of the *Plasmodium falciparum* genome has enabled other potential paralogues of this family to be identified. Pf10_0348 and Pf10_0355 are two related proteins that contain predicted DBL domains within their structures but unlike other DBL-EBP members also possess structural characteristics found uniquely in the MSP3-like family, making them hybrid molecules. We have utilized both parasite-derived and recombinant proteins to characterize some of the biochemical properties and the

function of these unique molecules. We will also discuss the structure of the DBL domain of Pf10_0355 which we have recently determined to a resolution of 2.8A.

1108

MEASURING GENETIC COMPLEXITY OF *PLASMODIUM VIVAX* INFECTIONS BY A HETERODUPLEX TRACKING ASSAY

Jessica T. Lin[1], Jonathan J. Juliano[1], Jetsumon Sattabongkot[2], Steven R. Meshnick[3]

[1]University of North Carolina School of Medicine, Chapel Hill, NC, United States, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]Gillings School of Public Health, University of North Carolina, Chapel Hill, NC, United States

High rates of polyclonal *Plasmodium falciparum* infections in hyperendemic settings are well-characterized, and the ability to measure this genetic complexity affects classification of clinical outcomes in drug efficacy trials. In the same fashion, *P. vivax* infections often contain multiple genetically unique variants even in areas of low transmission intensity such as Thailand. We applied a genotyping strategy that has been successful in measuring genetic complexity of *P. falciparum* infections - the heteroduplex tracking assay (HTA) - to study the complexity of *P. vivax* infections from northwest Thailand. A radiolabeled probe targeting the *P. vivax* merozoite surface protein-1 (PvMSP1) was developed and validated. When annealed with PCR amplified PvMSP1 from different Thai *P. vivax* infections, the HTA detected as many as six variants within a single infection. Comparison of this assay to the more traditional method of RFLP analysis of 40 Thai isolates showed the HTA to consistently reveal more genetic complexity and the ability to uncover minority variants. We plan to use this method to assess the genotypes of relapsing *P. vivax* parasites. This may shed light on the genetic complexity of parasites arising from activation of hypnozoites vs. inoculated parasites in a newly acquired primary infection.

1109

ASSESSMENT OF *PLASMODIUM FALCIPARUM MSP1, MSP2* AND *GLURP* ALLELE DIVERSITY AND FREQUENCY IN SUB-SAHARAN AFRICA

Felista Mwingira[1], Gamba Nkwengulila[1], Sonja Schoepflin[2], Hans Peter Beck[2], Georges Snounou[3], Ingrid Felger[2], Pierre Olliaro[4], Kefas Mugittu[5]

[1]University of Dar Es Salaam, Dar Es Salaam, United Republic of Tanzania, [2]Swiss Tropical and Public Health Institute, Basel, Switzerland, [3]Faculté de Médecine Pitié-Salpêtrière Université Pierre and Marie Curie, Paris, France, [4] UNICEF/UNDP/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases, Geneva, Switzerland, [5]Ifakara Health Institute, Dar Es Salaam, United Republic of Tanzania

Molecular genotyping of highly diverse *Plasmodium falciparum msp1*, *msp2* and *glurp* loci is recommended to distinguish recrudescence from new infections in efficacy studies and clinical trials. However, the parasite's genetic profile in many areas has not been systematically documented. Low heterozygosity would result in over-estimation of recrudescence and consequently unnecessary treatment policy changes. Here we present *msp1 msp2* and *glurp* genetic diversity and allele frequency in areas with different transmission intensities namely Malawi, Tanzania, Uganda, Burkina Faso and Sao Tomé. A total 780 baseline (Day 0) blood samples from children under 7 years, from ACT clinical trials done between 1996 and 2000 were genotyped. DNA was extracted; allelic frequency was investigated by PCR followed by genescan for *msp2* and fragment sizing by a digitalized gel imager for *msp1* and *glurp.* The obtained genotypes were further grouped into 5bp, 3bp and 20bp "bins" for *msp1*, *2 and glurp* respectively. Out of 780 DNA samples 599 (76.8%) were successfully amplified at *msp1*, 679 (87%) at *msp2* and 575 (73.7%) at *glurp* loci. The *msp2* gene showed a slightly higher average MOI (2.24), followed by *msp1* (1.48) and lastly *glurp* (1.4). A total of 67 *msp1* genotypes [30 MAD20, 1 Ro33 and 35 K1-types] were recorded. Out of 116 *msp2* genotypes, 83 and 33 represented the 3D7 and FC27 allelic families, respectively. Overall, 31 *glurp* genotypes were detected. All 5 sites recorded very high HE values (0.95 - 0.99). HE was highest in *msp2* locus in Tanzania (HE=0.99), and lowest for glurp in Sao Tomé and Uganda (HE=0.95) (P<0.0001 In conclusion, *P. falciparum msp1, msp2* and *glurp* markers are highly polymorphic and have low allelic frequencies in Sub-Saharan Africa, hence useful in distinguishing *P. falciparum* populations in respective study sites. With the expanding access to ACTs and current changes in malaria epidemiology, allele frequency/genetic diversity should be monitored regularly to ensure reliability of adjusted treatment outcome.

1110

THE *PLASMODIUM VIVAX* TROPHOZOITE PROTEOME: A COMPARISON WITH GENOME AND TRANSCRIPTOME DATA PROVIDES VALUABLE INSIGHTS

Sheila Akinyi[1], Cindy C. Korir[1], Esmeralda V.S. Meyer[1], John W. Barnwell[2], Mary R. Galinski[1]

[1]Emory University, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States

Plasmodium vivax causes between 130 to 390 million cases of illness annually. Like other *Plasmodium* species, *P. vivax* antigenically and structurally alters its host red blood cell (RBC) in order to survive. In this study, we constructed the *P. vivax* trophozoite stage proteome using a global proteomic approach; liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is a highly sensitive method by which we identified the peptides extracted from solubilized *P. vivax* Salvador I strain trophozoite infected RBCs, acquired from blood-stage infections in *Saimiri boliviensis* monkeys. We utilized the *P. vivax* genome database to annotate our data and determine the number of known transcripts that are translated into proteins at the trophozoite stage. We report the identification of 688 proteins, which include 191 hypothetical proteins, members of known and recently identified multigene families such as Vir proteins, *Plasmodium* helical interspersed subtelomeric (PHIST) proteins, Pv-fam-a, Pv-fam-d, Pv-fam-e and Pv-fam-h proteins. We analyzed the identified proteins for the presence of export characteristics such as signal peptides, *Plasmodium* export element (PEXEL), and transmembrane motifs. The identification of hypothetical proteins with no known homologs in other species may represent a class of *P. vivax* proteins that could be further characterized as targets for species-specific drug and vaccine development, and also investigated for their contribution to this species' unique characteristics. We compared our *P. vivax* proteome data to published genome and transcriptome data to gain insights into how this species regulates transcription and translation at the trophozoite stage to ensure its survival within the human host. Comparisons with evolutionarily closely related species such as *P. cynomolgi* and *P. knowlesi*, and *P. falciparum*, are also revealing the identity of shared and unique orthologs and enhancing our understanding of expressed proteins and their biological functions.

1111

PLASMODIUM KNOWLESI SICAVAR GENE EXPRESSION IN SICA[+] AND SICA[-] CLONES

Stacey Lapp[1], Cindy Korir[1], John Barnwell[2], Mary Galinski[1]

[1]Emory University, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States

The SICAvar variant antigen gene family of *Plasmodium knowlesi* allows for chronic infections in its non-human primate host by the expression of antigenically distinct SICA proteins on the surface of infected erythrocytes, which switch phenotype *in vivo* in the face of immunological challenge. In the rhesus monkey model system, many transcripts representing a host of SICAvar genes can be detected in ring and trophozoite stage infections, however, most are not detected as full length transcripts and they do not go on to be translated into protein and targeted to the

RBC membrane. In this system, the means by which these transcripts are generated and regulated is unknown. Here we show the proteomic and transcriptional profiles of 3 SICA[+] isogenic clones, as well as 2 SICA[-] clones derived from serial subpassages in splenectomized rhesus macaques. Using immunoprecipitation and LC-MS/MS, we identified the major SICA proteins in each of the SICA[+] clones, and confirmed the lack of expressed SICA antigens in the SICA[-] clones. In each SICA[+] clone, there are 2-3 major SICA antigens in terms of abundance, and several others made at lower levels. We also characterized the genes that encode these proteins by quantitative RT-PCR and northern blot, and show that the relative abundance of transcripts matches the proteomic profiles in these clones. By IFA and flow cytometry we attempt to determine whether individual infected RBCs possess multiple SICA antigens. How our findings relate to the orthologous PfEMP1 (var gene) family will be discussed.

1112

ELEVEN *PLASMODIUM VIVAX* MEROZOITE SURFACE PROTEIN-3 (PVMSP3) PROTEINS ARE EXPRESSED AT DIFFERENT LEVELS DURING THE ERYTHROCYTIC SCHIZOGONY

Jianlin Jiang[1], John W. Barnwell[2], Korir Cindy[1], Esmeralda V.S. Meyer[1], Mary R. Galinski[1]

[1]Emory University, Yerkes National Primate Research Center, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention, Division of Parasitic Diseases, Atlanta, GA, United States

Members of the *Plasmodium vivax* merozoite surface protein-3 (PvMSP3) are considered potential vaccine candidates because these abundant merozoite surface antigens are exposed to the host's humoral immune system when merozoites are released from the infected erythrocytes. Three members, PvMSP3-α (PvMSP3.10), PvMSP3-β (PvMSP3.3), and PvMSP3-γ (PvMSP3.1) of this gene family have been identified and characterized previously. Here we report the expression profile of the transcripts and proteins of the PvMSP3 family comprising 11 gene members. The hallmark of the PvMSP3 family of proteins is the secondary structure formed by an alanine-rich, central domain (coiled-coil motif) containing variable heptad repeated sequences. These coiled-coil structures span the majority of the coding region. Importantly, the putative MSP3 proteins in *P. vivax*, *P. falciparum*, *P. knowlesi* and *P. cynomolgi* share a conserved Asp-Leu-Arg-Asp (Gly/Ala) motif located 8-17 amino acids downstream from the putative signal peptide cleavage site. We confirmed that all *Pvmsp3* gene transcripts are detected in full-length during the schizont stage and that only two *Pvmsp*3 transcripts change their level of expression dramatically between stages. With exception of PvMSP3.11, all other ten PvMSP3 proteins are expressed during the schizont stage at different levels as determined by quantitative immunoblot. Indirect Fluorescent Assays (IFA) showed that ten PvMSP3 proteins were expressed at surface of merozoites and in the parasitophorous vacuole of schizonts as well. In conclusion, we present a detailed analysis of the gene arrangement, transcript profile and protein expression of eleven members of the MSP3 gene family in *P. vivax*.

1113

INVESTIGATING TEMPO AND MODE IN THE EVOLUTION OF HUMAN MALARIAL PARASITES

Fabia U. Battistuzzi[1], M. Andreina Pacheco[1], Lisa Jones-Engel[2], Mike Cranfield[3], Sabrina Krief[4], Georges Snounou[5], Anne Charlotte Gruner[6], **Ananias A. Escalante**[1]

*[1]Center for Evolutionary Medicine and Informatics, Arizona State University, Tempe, AZ, United States, [2]Division of International Programs, University of Washington-NPRC, Seattle, WA, United States, [3]Research and Conservation Program, The Maryland Zoo in Baltimore, Baltimore, MD, United States, [4]Eco-Anthropologie et Ethnobiologie, Muséum National d'Histoire Naturelle, Paris, France, [5]Faculté de Médecine Pitié-Salpêtrière, Université Pierre and Marie Curie, Paris, France, [6]Laboratory of Malaria Immunology, Singapore Immunology Network, Agency for Science Technology and Research (A*STAR), Biopolis, Singapore and Institut Cochin, Universite Paris Descartes, CNRS (UMR 8104), Paris, France*

G. G. Simpson, one of the founders of modern evolutionary biology, was interested in variations on the rate of evolution (tempo) and the mechanisms driving those changes (mode). Those are still valid concerns whereas new data from African Apes has raised questions about when and how human malarias originated. In this investigation we are focusing on how can species be defined in the absence of morphological/biological data and how can we time the origin of human malarias? While these may appear to some as "academic questions", such studies provide the basis for comparative genomics. In addition, they allow us to understand host-switches as a mechanism driving the evolution of malarial parasites and to explore the time frames at which novel species could emerge in humans. Whereas it may be difficult to agree upon a " molecular golden standard", mitochondrial genomes (mtDNA) provide reproducible results when used to define species, though the inclusion of nuclear loci is always advisable. Such nuclear loci could be chosen by testing whether their rates of evolution allow for distinction among closely related *Plasmodium* species (e.g. those found in rodents). The use of mtDNA and nuclear loci from parasites found in African apes indicated that two new species closely related to *P. falciparum*, *P. billbrayi* and *P. billcollinsi*, are good phylo-species. However, the dynamics of host-switches among humans and African apes is far more complicated than previously thought and the use of short sequences (e.g. cytochrome b fragments) makes difficult the detection of otherwise well defined phylo-species. In term of timing the origin of human malarias, we explored different methods for estimating the time of origin of *P. falciparum* and *P. vivax* using complete mtDNA. We found that time estimates are seriously affected by the calibration points and violations in the assumptions used by the molecular timing methods. We explore potential calibration points and further discuss the limitations of the mitochondrial genome in estimating the time of origin of malarial parasites.

1114

MOLECULAR BASIS FOR HOST SPECIFICITY IN AVIAN MALARIA

Criseyda Martinez, Claire Loiseau, Chris Smith, Ravinder N. Sehgal

San Francisco State University, San Francisco, CA, United States

Malaria is a disease that infects multiple organisms: humans, chimpanzees, reptiles and birds. Malaria is caused by *Plasmodium* blood parasites and has dramatically affected bird populations. The extinction of several avian populations in the Hawaiian Islands was partially due to a newly introduced *Plasmodium* strain that was able to infect multiple bird species (a generalist parasite). Host-specific parasites (specialists) can infect only one bird species. Previous work has shown that specialists can become generalists in host switching events, making them more virulent. The molecular basis of host specificity in avian *Plasmodium* parasites is unknown, but our project aims at identifying genes responsible for host specificity. Previous data has implicated the erythrocyte binding-like (*ebl*)

genes in human and chimpanzee *Plasmodium* strains as potential host-specific determinants. Our studies will focus on identifying the *ebl* genes in avian *Plasmodium* strains infecting African rainforest birds by using PCR to amplify these genes. Currently we have acquired a genetic region for a putative candidate *ebl* gene in *Plamodium gallinaceum,* an avian strain. We further seek to sequence the genes and analyze their DNA and amino acid sequence to determine whether sequence variability correlates with host specificity. We plan to map the distribution of *ebl* gene alleles of African rainforest birds. We expect to identify the *ebl* family of genes in avian malaria and correlate them to host-specificity. The identification of *ebl* genes in avian parasites characterized in this study will allow us to predict potential emerging diseases in avian populations. Predicting potential host-switching events could allow us to slow down the spread of malaria.

1115

COMPARISON OF TWO PCR METHODS FOR THE DETECTION OF *PLASMODIUM SP*. USING DNA OBTAINED FROM MALARIA RAPID DIAGNOSTIC TEST

Juan Jose Contreras[1], Anne Leenders[2], Dionicia Gamboa[3]

[1]Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Institute for Life Science and Chemistry, Hogeschool Utrecht, Utrecht, The Netherlands, [3]Instituto de Medicina Tropical Alexander von Humboldt, Departamento de Bioquímica, Biología Molecular y Farmacología, Facultad de Ciencias, Universidad Peruana Cayetano Heredia, Lima, Peru

Polymerase Chain Reaction (PCR) is a method that has shown a better sensitivity and specificity than microscopy and malaria rapid diagnostic tests (MRDT). It was previously described that the MRDTs can be used as a source of DNA for molecular epidemiology studies. The key for this approach is to have an appropriate DNA extraction method to ensure the quality of DNA to be used in the PCR. The objective of this study was to compare two PCR methods for the malaria diagnosis using DNA extracted from MRDTs using the phenol chloroform method. 187 MRDTs collected in the Department of Loreto, Peru, between February and September 2006 were used as source of DNA. From these MRDTs, 5 were positive to *P. falciparum*, 38 were positive to *P. vivax* and 144 were negative. The DNA was extracted by phenol chloroform method. We used for detection and identification of *Plasmodium* species two different PCR methods using as target the 18S rRNA gene: a Simplex PCR (S-PCR) and Semi Nested Multiplex PCR (SnM-PCR). S-PCR amplifies products of 250 bp for *P. falciparum* and two bands of 220 and 270 bp for *P. vivax*. In SnM-PCR, the first reaction amplifies a band between 783 to 831 bp and in the second reaction a band of 400 bp for *P. falciparum* and a band of 500 pb for *P. vivax*. S-PCR identified 8 *P. falciparum* (S: 57.1%; Sp: 99.4%) and 31 *P. vivax* (S: 63.1%; Sp: 95.3%), while 148 were negative. SnM-PCR identified 5 *P. falciparum* (S: 80% Sp: 99.4%), only 11 *P. vivax*, (S: 23.6%; Sp: 98.6%) and 171 were negative. In conclusion, S-PCR was more sensitive compared with the SnM-PCR because the S-PCR uses smaller DNA target and have more probability to amplify the DNA extracted from MRDTs. Due to the damage during the DNA extraction from the MRDTs, the use of SnM-PCR would not be appropriate because it amplifies longer DNA target.

1116

DISTRIBUTION OF *PLASMODIUM VIVAX* CIRCUMSPOROZOITE PROTEIN SUBTYPES IN DIFFERENT REGIONS OF PERU

Lorena Tapia, Stella Chenet, Carola Salas, Carmen Lucas, Paul C. Graf

Naval Medical Research Center Detachment, Lima, Peru

Plasmodium vivax is the most prevalent malaria parasite in South America. In Peru most cases are reported from the Amazon basin region. Since this is the most endemic zone of the country, the majority of the genetic diversity studies have been conducted here. However, the rest of the country has largely been neglected for malaria studies. Pvcsp is an important protein on the surface of *P. vivax* sporozoites which is involved in binding to heparin on the surface of hepatocytes and the subsequent invasion of these cells. There are also some published reports that Pvcsp genotype seems to segregate with the ability of the parasite to infect different species of *Anopheles* mosquitoes. Pvcsp has also been used extensively to assess genetic diversity in P vivax populations. There are two subtypes, VK210 and VK247, which differ in the amino acid composition of the central repeat region. In order to identify the *Pvcsp* variants present in different regions of Peru, the *Pvcsp* central repeat region was amplified and sequenced. A high degree of variability was found in 106 samples from Iquitos with 32 alleles of the VK210 subtype found in 98 samples and 3 alleles of the VK247 subtype found in 8 samples. Both subtypes were also found in other zones of the Peruvian jungle, namely, Yurimaguas, southwest of Iquitos, and Madre de Dios, even further to the south. In the central part of the country, from Junin, 10 isolates were genotyped as VK210,with 3 alleles present. Isolates from the North Coast (21 from Piura and 21 from Tumbes) all contained the same allele of the VK247 subtype. The two Pvcsp subtypes are present throughout the jungle of Peru with VK210 more prevalent than VK247. On the North Coast only one allele of the VK247 subtype was detected.

1117

NOVEL MASS SPECTROMETRY APPROACHES FOR THE ANALYSIS OF THE EXPRESSION OF ANTENNAL SOLUBLE OLFACTORY PROTEINS IN *ANOPHELES GAMBIAE*

Francesca Romana Dani[1], Elena Michelucci[1], Guido Mastrobuoni[2], Antonio Felicioli[3], Alberto Niccolini[3], Beniamino Caputo[4], Paolo Pelosi[3], Gloriano Moneti[1], **Alessandra della Torre**[4]

[1]CISM, Mass Spectrometry Center, University of Florence, Florence, Italy, [2]Max-Delbrück Centrum for Molecular Medicine, Berlin, Germany, [3]University of Pisa, Pisa, Italy, [4]University of Rome "Sapienza", Rome, Italy

Olfactory cues are the most important group of external stimuli affecting mosquito behavior (e.g. mating and partner recognition, search for sugar sources and, in females only, host-seeking and oviposition). Perception of volatile semiochemicals in mosquitoes is mediated by chemosensory neurons segregated within specific olfactory sensilla located mainly on the antennae and maxillary palps. Odourant Binding Proteins (OBPs) and Chemo-Sensory Proteins (CSPs) are soluble proteins responsible of the peri-receptor events leading the detection of odour molecules and to the activation of odorant receptors. *Anopheles gambiae* genome contains a remarkable number of genes encoding OBPs (57) and a lower number of genes encoding CSPs (7). So far, the expression pattern of these genes has been almost exclusively investigated by genomic approaches (i.e. RT-PCR and microarrays): some OBPs have been shown to be specific of the olfactory organs, while others appear to be more ubiquitous. Moreover, OBPs have been found to be differential expressed between males and females and between females at different physiological stages. Traditional proteomic approaches have been proved difficult on mosquito antennae, due to low concentration of OBPs and CSPs in these tissues and difficulties in obtaining sufficient material for 2-dimensional gels. We here present the results of OBPs and CSP expression analyses in *An. gambiae* antennae carried out by an innovative shotgun proteomic approach, based on the nano HPLC-ESI LTQ Orbitrap analysis of the peptides obtained from the enzymatic digestion of the whole protein extract. The sensibility of this approach is compared to that of a more time-consuming traditional approach based on (i) separation of proteins on a 2-dimensional gel, (ii) excision and enzymatic digestion of gel spots, followed by (iii) micro or nano HPLC separation of peptides and (iv) online analysis of peptides through MS and MS/MS experiments on a ESI LTQ Orbitrap mass spectrometer. The advantages/disadvantages of the two approaches are discussed in order to provide a novel perspective in the analyses of soluble proteins in mosquito olfactory organs. Differences observed between male and female antennae are described.

1118

MOLECULAR REGULATION OF AUTOGENY IN THE ARBOVIRAL VECTOR, *CULEX TARSALIS*

Katie N. Provost-Javier, Jason L. Rasgon

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

Autogeny, the ability of a female mosquito to produce eggs without blood feeding, is a alternate reproductive strategy with important implications for vector-borne disease transmission. Very little is known about the molecular mechanisms that regulate autogeny and how they compare with blood meal regulated reproduction (anautogeny). In order to compare these reproductive strategies, we selected for and characterized autogenous and anautogenous populations of an important arboviral vector, *Culex tarsalis*. While autogeny is a genetic trait, its expression in *C. tarsalis* is severely compromised by the availability of adequate larval nutrition, similar to the availability of nutrients following a blood meal in anautogeny. We cloned components of two nutrient-sensitive pathways with demonstrated functions in anautogeny in other mosquito species: *C. tarsalis* Target of Rapamycin (CtTOR), the kinase that functions in the TOR signaling pathway, and the *C. tarsalis* Insulin Receptor (CtInR), the receptor for the insulin signaling pathway. We investigated tissue specific expression and used RNAi mediated knockdown to examine the effect of these pathways on vitellogenin gene expression and egg development in our selected *C. tarsalis* populations.

1119

MOLECULAR CLONING OF THE SODIUM-METHIONINE SELECTIVE SYMPORTER FROM THE YELLOW FEVER MOSQUITO

Ella Meleshkevitch[1], Dmitry Voronov[2], Jeffrey Fox[1], Ryan Metzler[1], Lyudmila Popova[3], Dmitri Boudko[1]

[1]Rosalind Franklin University, North Chicago, IL, United States, [2]Moscow State University, Moscow, Russian Federation, [3]A.N. Belozersky Institute, Moscow State University, Moscow, Russian Federation

Nutrient Amino acid Transporters (NATs) comprise a recently identified subfamily of the Neurotransmitter Sodium Symporter Family (NSS or SLC6). In vector mosquitoes NAT-SLC6 population includes a large number of paralogous (species-specific) transporters with unknown physiological properties and biological functions. Here we report the molecular cloning, functional heterologous expression, and in situ hybridization of AeNAT5, a new insect NAT from the yellow fever mosquito, *Aedes aegypti* (NCBI accession # ABZ81822). In contrast to the previously characterized an Aedes broad spectrum AAT1 and the *Anopheles* L-tryptophan- and L-phenylalanine- selective transporters, AgNAT6 and AgNAT8 respectively, AeNAT5 selectively absorbs L-methionine (K 0.5 L-Met = 20+/-9 uM; K0.5 Na+ = 46 +/-17 mM; stoichiometry 1:1) and, with ~20 fold reduced transport efficiency, L- cysteine and Homocysteine. It rejects other canonical amino acids and amine neurotransmitters. AeNAT5 transcript is abundant in the larval alimentary canal. However, substantial differences in expressions of AeNAT5 vs. AeAAT1 were found in the anterior and posterior domains of the larval gut. AeNAT5 is also identified in the mosquito brain and a few other tissues. These findings are consistent with our earlier proposal that the NAT populations evolve and act synergistically as a principal molecular mechanism of absorption and redistribution of essential amino acids. The narrow substrate spectra AeNAT5, AgNAT6 and AgNAT8 comprise a mosquito-specific addition to the NAT population, which balances the acquisition of the most underrepresented essential amino acids vs. more redundant nutrients. Hence, the essential NATs may be especially critical for extensive protein synthesis during mosquito development and egg maturation. The essential linage-specific NATs may provide more specific targets for the management of medically and economically important insects.

1120

CHARACTERIZATION OF DOUBLE STRANDED RNA BINDING PROTEINS INVOLVED IN THE RNA INTERFERENCE PATHWAY OF THE DENGUE MOSQUITO, *AEDES AEGYPTI*

Mary Etna Richter, Kevin M. Myles, Zach N. Adelman

Virginia Tech, Blacksburg, VA, United States

Aedes aegypti mosquitoes are the vectors of several arboviruses of global health significance, including dengue viruses and yellow fever virus. The RNA interference (RNAi) pathway is an important defense mechanism used by invertebrate organisms to protect against viral infection, and we have shown previously that RNAi plays a direct role in vector competence. While the structure and function of many genes involved in the *Drosophila* RNAi pathway have been characterized, the corresponding mosquito orthologs have only been peripherally described or remain unknown. We have characterized the gene structure of *Ae. aegypti* double stranded RNA binding proteins predicted to be important to the RNAi pathway. Two genes, *r2d2* and *r3d1* are orthologs of *Drosophila* genes known to have important roles in the RNAi initiator complex. A third member of the same family, which we refer to as *extra loquacious* (*exloqs*), appears to have no known orthologs outside of the *Aedes* genus. Our characterization of these genes has revealed new exons and alternative splice variants for both *r3d1* and *exloqs*. In addition, we examined single-nucleotide polymorphisms, as well as differential expression of all three genes for specific tissues and developmental stages. This work increases the accuracy of the annotations for all three genes, and provides valuable insight for future mechanistic and biochemical studies involving these gene products and their roles in RNAi.

1121

VALIDATION OF NOVEL PROMOTER SEQUENCES DERIVED FROM TWO ENDOGENOUS UBIQUITIN GENES IN TRANSGENIC *AEDES AEGYPTI*

Azadeh Aryan, Michelle Anderson, Tiffany Gross, Kevin M. Myles, Zach N. Adelman

Virginia Tech, Blacksburg, VA, United States

To date, only a limited number of promoter sequences have been described to drive transgene expression in the disease vector *Aedes aegypti*. We sought to increase this repertoire by characterizing the ability of upstream sequences derived from the *Ae. aegypti* UbL40 and polyubiquitin genes to drive the expression of marker proteins. Both genomic fragments were able drive robust expression of luciferase in cultured mosquito cells. Following Mos1-transformation, the UbL40 promoter drove strong expression of a fluorescent marker in early larvae and in ovaries, while the polyubiquitin promoter drove robust EGFP expression in all stages of development, including constitutive expression throughout the midgut. In addition, both promoters drove robust expression of luciferase within 12 hours after injection into newly laid embryos. These promoter elements will thus be useful for the expression of anti-pathogen effector genes in genetics-based control strategies; hairpin RNAs in gene knockdown experiments; and as drivers of transposase/recombinase expression in new helper systems.

1122

INCREASED INSULIN SIGNALING IN TWO KEY REPRODUCTIVE TISSUES, THE FAT BODY AND OVARIES, INCREASES MOSQUITO FECUNDITY

Anam J. Arik, Kendra M. Quicke, Michael A. Riehle

University of Arizona, Tucson, AZ, United States

Mosquito reproduction is regulated by a complex series of hormonal cues. In the ovaries and fat body the insulin/insulin growth factor 1 signaling (IIS) cascade regulates steroidogenesis and vitellogenesis respectively.

Phosphotase and tensin homolog (PTEN) is a direct antagonist of IIS. Knockdown of AaegPTEN or its specific splice variant AaegPTEN6 by RNAi resulted in a 15-63% increase in egg production and had no effect on the egg viability during the first reproductive cycle. Knockdown of a second splice variant, AaegPTEN3, had no effect on reproduction. However, RNAi has limitations. The dsRNA injections resulted in knockdown of AaegPTEN in both fat body and ovaries making it impossible to elucidate whether the ovaries fat body, or both tissues are responsible for the increased egg production. In addition, the transient nature of RNAi knockdown prevents us from determining whether increased reproduction occurs throughout the life of the mosquito or whether there is a life history change to earlier reproduction. To address these questions we are generating transgenic mosquitoes with either increased or decreased IIS in the fat body and ovary. We have successfully engineered transgenic lines using the vitellogenin promoter linked to an insulin signaling inhibitor, PTEN, or to its activator, Akt. We characterized how changes to IIS specifically in the fat body effects egg production during multiple reproductive cycles and the impact is has on mosquito lifespan. We have also identified a putative ovary specific promoter with expression in the ovarian follicle cells where the IIS cascade regulates steroidogenesis. We have linked this promoter to an activator of the IIS cascade and are generating and characterizing transgenic lines with modified IIS in the ovaries.

1123

TEMPORAL SILENCING OF TWO *CULEX PIPIENS QUINQUEFASCIATUS* (DIPTERA: CULICIDAE) MOSQUITO GENES: METHOD FOR SILENCING POTENTIAL IMMUNE RESPONSE GENES

Chelsea T. Smartt, Sheri L. Anderson, Samantha A. Yost, Stephanie L. Richards

University of Florida, Vero Beach, FL, United States

A mosquito's ability to transmit a virus is influenced in part by how active its immune response is to the invading virus. Components of the mosquito Toll and immune deficiency pathways are known to counter foreign pathogens, including viruses. Preliminary studies on gene expression responses in midgut tissues of *Culex pipiens quinquefasciatus* infected with West Nile virus (WNV) revealed two genes, one encoding a Toll-like protein and the other a gram-negative bacteria binding protein, whose expression was altered after infection. Changes in expression after viral infection could indicate that these genes are members of the immune response pathway. Injection of double-stranded (ds) RNA representing the Toll-like gene, CQ G12A2, into *Cx. p. quinquefasciatus* showed decreased expression in midgut tissue beginning 6 days post-injection (dpi) through 9 dpi. Blood feeding the dsRNA-injected mosquitoes 4 days after injections shifted the expression knockdown in midguts to 8 dpi. Injection of dsRNA representing the gram-negative bacteria binding protein gene from *Cx. p. quinquefasciatus*, CQ G1A1, knocked out the expression in midgut tissue 1 dpi through 9 dpi. When these injected mosquitoes were blood fed 4 days following injection, gene expression levels did not decrease until 7 dpi. Here we present results that indicate the importance of tracking the time required for change in expression for each RNA target. With this information we can pinpoint the time of maximum knockdown to study the involvement of these genes in mosquito-WNV interactions. We also discuss the role of blood ingestion in delaying gene expression knockdown; this issue should be considered in studies that use RNAi technology to assign a gene a function in vector competence.

1124

SPATIAL AND TEMPORAL PATTERNS OF GENE EXPRESSION IN SALIVARY GLANDS OF *AEDES AEGYPTI*

Jennifer Juhn[1], Unsar Naeem-Ullah[2], Judy Coleman[1], Anthony A. James[1], Osvaldo Marinotti[1]

[1]University of California, Irvine, Irvine, CA, United States, [2]University of Agriculture, Faisalabad, Pakistan

Transgenic mosquitoes designed to impact vector competence have been proposed as tools for the control of dengue viral transmission. In addition to the development of anti-dengue effector molecules that block viral replication and dissemination, promoters are needed that target effector gene expression to key mosquito tissues where the viruses and host interact. It has been shown in transgenic mosquitoes that expression of anti-dengue effector molecules in the distal-lateral lobes of *Aedes aegypti* salivary glands reduces prevalence and mean intensities of infection of the virus. We anticipate greater efficacy of viral suppression if we target the effector genes to all lobes of the salivary glands. We report here the hybridization in situ patterns of 19 genes expressed in the salivary glands of adult *Ae. aegypti* females. Distinct spatial expression patterns were identified. Eight genes are expressed exclusively in the proximal-lateral lobes, five genes within the distal-lateral lobes, and two genes in the medial lobe. Four genes are expressed in the distal-lateral and medial lobes. Quantitative real-time RT-PCR was used to measure relative levels of gene expression following blood feeding for seven genes that represent the four major classes of spatial expression patterns. All analyzed genes are expressed constitutively. It is possible that continuous expression of anti-viral effector molecules throughout the salivary glands will completely disrupt dengue transmission. Based on our data, a minimum of two promoters is necessary to drive the expression of one or more anti-dengue genes in all cells of the female salivary gland.

1125

INSULIN SIGNALING IN THE MOSQUITO: UNDERSTANDING AKT PHYSIOLOGY IN THE FAT BODY AND PTEN REGULATION AT THE MOLECULAR LEVEL

Hemant B. Badgandi, Michael A. Riehle

University of Arizona, Tucson, AZ, United States

The insulin/insulin growth factor 1 signaling (IIS) cascade regulates aging, reproduction, and innate immunity in a wide range of organisms. To assess the impact of IIS in mosquitoes we examined two critical IIS molecules, the inhibitor Phosphatase and Tensin homolog (PTEN) and the activator Akt. We generated transgenic *Anopheles* stephensi lines overexpressing an active Akt (myr-AsteAkt) regulated by the vitellogenin promoter to enhance IIS activity in the fat body. We assessed mRNA transcript and protein expression levels, and myr-AsteAkt was expressed only in the fat body in a bloodmeal dependant manner as expected. The impact of increased IIS on lifespan and reproduction is examined. This work assesses the link between changes in lifespan, reproduction, innate immunity and IIS manipulation in the fat body. We are also studying the biochemistry of the key IIS inhibitor in the mosquito, PTEN. Regulation of PTEN is critical for its lipid phosphatase activity, membrane association, and subcellular localization. One of the key regulatory mechanisms of PTEN is the phosphorylation of serine and threonine residues leading to decreased enzymatic activity. Using a proteomics approach, we identified several phosphorylated serine and threonine residues on the C-terminus of the mosquito PTEN6. However, the kinases involved and the signaling context of the phosphorylation is unknown. Sequence analysis predicts several phosphorylation sites on *Aedes aegypti* PTEN6. Four sites on the C-terminal regulatory region are predicted as Casein Kinase I (CK1) and Casein Kinase II (CK2) substrate sites. *In vitro* kinase assays were used to determine if recombinant mosquito PTEN acts as a substrate for CK1 and CK2. Understanding how PTEN is regulated at the molecular level will allow us to utilize this IIS inhibitor in unique mosquito control strategies.

1126

DEFECT OF DSRNA PROCESSING IN *AEDES ALBOPICTUS* C6/36 CELLS

Baojun Yang, Hongwei Li

University of Hawaii at Manoa, Honolulu, HI, United States

Double-stranded RNA (dsRNA) is the trigger of RNA interference (RNAi)-mediated gene regulation. Dicer protein processes dsRNAs into short interfering RNAs (siRNAs), which are incorporated into the effector RNA induced silencing complex and direct degradation of homologous target mRNAs. In plants and insects, RNAi can acts as an antiviral mechanism through generation of viral specific siRNAs from a replicating virus by Dicer. In this study, we analyzed the RNAi machinery in mosquito C6/36 cells, an *Aedes albopictus* cell line commonly used for propagation of dengue virus and some other flaviviruses. Transfection of long dsRNAs (~500bp) did not result in specific knockdown of cognate reporter genes (GFP and Renilla luciferase) in C6/36 cells, and showed no significant difference between the effects caused by specific target dsRNAs and unrelated dsRNAs. However, expression of the GFP gene can be efficiently inhibited by chemically synthesized GFP siRNA, indicating that there may be defects in dsRNA processing in C6/36 cells. To test this possibility, *in vitro* Dicer assays were performed by using crude cell extracts prepared from C6/36, *Aedes aegypti* Aag2, *Anopheles gambiae* 4a-2s4 and Drosophila melanogaster S2 cells. Consistent with the inefficacy of dsRNAs in the cell-based analysis, production of siRNAs was not detected when radiolabeled long dsRNAs were incubated with the C6/36 extract. In contrast, dsRNAs were efficiently processed into siRNAs in extracts from all other cell lines. This result suggests that in C6/36 cells dsRNA processing in RNAi pathway is defective and such a property may provide an advantage to virus replication.

1127

MACROPHAGE NUCLEAR RECEPTOR SIGNALING MODULATES DEFENSES AND SUSCEPTIBILITY TO MYCOBACTERIUM TUBERCULOSIS

H. Kitdorlang Dkhar, Sahil Mahajan, Sandeep Dave, Vemika Chandra, **Pawan Gupta**

Institute of Microbial Technology, Chandigarh, India

We are studying host pharmacological effectors, contributing towards Mycobacterium tuberculosis (*Mtb*) pathogenesis or clearance. The project has a "look beyond NF-kB approach" an overrated pharmacological molecule in macrophages, to other orphan/ nuclear receptor that have recently been reported to find expression in immune cells such as macrophages. Preliminary observations suggest that these orphan/ nuclear receptors may contribute towards susceptibility or resistance of natural host. We have studied differential expression of orphan/ nuclear receptor in macrophages. We have confirmed altered expression of these receptors to H37Ra strain. We have confirmed responding receptors by cell based bug survival assays and using avirulent strains such as *BCG*, *M. smegmatis*, *H37Ra*. We have identified that these receptors have pro-*Mtb*/ anti-*Mtb*/ neut-*Mtb* function largely by promoting/ resisting macrophage foam cell formation. Some of these receptors are 'Lipid Sensing', to mean that while some of them have lipid as their ligands, others have lipids as post translational modification. We are studying host-pathogen interaction at level of *Mtb* lipid repertoire to modulate these LSNRs. All the above studies are being verified to virulent strain H37Rv in cell based and animal based assays. Also clinical samples from human volunteers from interesting family history are being attempted to be looked for differential expression of these factors in monocytes/macrophages derived there from.

1128

A PROSPECTIVE COHORT STUDY TO EVALUATE INCIDENCE OF TUBERCULOSIS IN INFANTS, WESTERN KENYA

Grace K. Kaguthi[1], Videlis N. Nduba[1], Ellen Mitchel[2], Susan Verver[2], Martiens Borgdorff[3], Anthony Hawkridge[4], Kayla Laserson[1], Anja V'ant Hoog[5]

[1]Kenya Medical Research Institute-Centre for Disease Control, Kisumu, Kenya, [2]KNCV Tuberculosis Foundation, Hague, The Netherlands, [3]University of Amsterdam, Hague, The Netherlands, [4]AERAS Global TB Foundation, Capetown, South Africa, [5]University of Amsterdam- Academic Medical Centre, Hague, The Netherlands

Cohort studies which include comprehensive diagnostic methods to provide reliable estimates of TB incidence and other epidemiological parameters in infants are needed to guide the planning of future TB vaccine trials. We set out to determine the incidence of TB, latent TB infection and all cause and TB specific mortality rates in Siaya district, western Kenya. To demonstrate a TB incidence of 0.5%, and make inferences for a phase III trial, ~2900 infants are being enrolled and followed up for a minimum of one year. Through 4-monthly follow up visits and health facility (HF) surveillance, those determined to be TB suspects by history of contact, TB symptoms, hospitalisation history, are admitted to a case verification ward. Specimens are collected for microscopy and culture by induced sputum and gastric aspiration. Chest radiographs, mantoux tests, and HIV testing are performed. Additional morbidity and mortality surveillance is conducted through HF record reviews. From June 2009-February 2010, 1553 BCG vaccinated infants have been enrolled with cumulative follow up of 325 person years. Of 1553 participants, 199(12%) were TB suspects. Of those 3(1%) had a history of contact with a TB case, 34(15%) had TB symptoms, and 139(70%) had protocol defined hospitalisation history. Six incident TB cases(2%) have been identified based on suggestive radiological and clinical features, however, none have been confirmed by bacteriology. Two of these are HIV coinfected, and 4(1%) are eligible for Isoniazid therapy following positive mantoux, negative cultures and symptoms. In conclusion, our preliminary analysis suggests that the majority of the TB suspects and TB cases were generated from the protocol defined hospitalisation history. To intensify case finding in infants in TB vaccine trials, the criteria for TB suspect identification need to be reconsidered, and go beyond contact history and Tb symptoms.

1129

DIFFERENTIAL RELATIONSHIP OF ENDOGENOUSLY ACTIVATED TH1/TH2 CYTOKINE SECRETING CELLS IN PULMONARY TUBERCULOSIS AND HEALTHY COMMUNITY CONTROLS IN A BCG VACCINATED HIGH TB BURDEN COUNTRY

Najeeha Talat[1], Firdaus Shahid[1], Sindhu Muzaffar[1], Ghaffar Dawood[2], Rabia Hussain[1]

[1]Aga Khan University, Karachi, Pakistan, [2]Masoomeen General Hospital, Karachi, Pakistan

Pakistan ranks 8th among 22 high TB burden countries despite wide BCG coverage (>90%). Tuberculin skin test positivity (TST+) in healthy community controls is as high as 50% increasing to 80% in recently exposed contacts. T effector cells are activated in the lymphoid compartment, develop homing receptors (CCR7) and transit to infected tissue sites via the blood compartment. We hypothesized that the relationship of Th1 and Th2 activated cells in transit may be different in tuberculosis patients compared to healthy controls. We therefore, analyzed the relationship of Th1 (IFNγ, IL2, TNF α) and Th2 (IL4 and IL6, IL10) cytokine secreting cells in the peripheral blood compartment of tuberculosis patients with active pulmonary disease (PTB=17) compared to healthy controls with latent infection (EC TST+=18). Whole blood (1:10) was cultured in the absence of exogenous stimulus for 2 days and

supernatants were tested for Th1/Th2 cytokine using the Cytometric Bead Array system. All Th1/Th2 cytokines were significantly elevated (Mann Whitney U test $p < 0.01$) in PTB compared to EC TST+. Spearman Rank analysis was carried out to determine the relationship between Th1 and Th2 cytokines. Differential association between Th1 and Th2 cytokines in the two groups was observed with IFNγ Vs IL6 (PTB, rho= -0.023, p>0.1; EC TST+, rho= 0.665, p=0.003) and between IL2 Vs IL4 (PTB, rho= -0.419, p=0.083; EC TST+, rho=0.668, p=0.002). Differential endogenous activation of Th1/Th2 cytokines in pulmonary tuberculosis and healthy TST+ community controls may have important implications with respect to disease pathogenesis and protection.

1130

THE USE OF MOLECULAR TECHNIQUES FOR THE IDENTIFICATION OF *MYCOBACTERIUM BOVIS*

Adwoa Asante-poku[1], K.G. Aning[2], D. Yeboah-Manu[1], T. Bodmer[3], G. Plushke[4]

[1]Noguchi Memorial Institute for Medical Research, Greater Accra, Ghana, [2]Animal Science Department, College of Agriculture and Consumable Science, University of Ghana, Greater Accra, Ghana, [3]Institute for Infectious Biology, Bern, Switzerland, [4]Swiss Tropical Institute, Basel, Switzerland

Mycobacterium bovis, member of *M. tuberculosis* complex (MTBC) causes tuberculosis (TB) mainly in cattle but has a broad host range and causes disease similar to that caused by *M. tuberculosis* in humans. Identification of *M. bovis* traditionally has been based on phenotypic characteristics and biochemical properties.Several molecular methods have been developed for the identification of *M. bovis* including DNA sequence variations in the direct repeat region of MTBC complex -spoligotyping or single nucleotide polymorphisms (SNPs) in the *oxyR* gene or be differentiated by large sequence polymorphisms or regions of difference (RD). The objective of this study was to determine a molecular method for the Detection of *M. bovis* from cattle. 17 suspected lesions from positive rectors (cattle) from Comparative tuberculin test were cultivated on LJ medium containing pyruvate. Isolates were identified using biochemical assays and PCR using Insertion sequence IS6110, Allele-specific(oxyR) and Spoligotyping Three(16%) of the isolates gave phenotypic properties were characteristic of (MTBC) while the remaining three were identified as non tuberculosis mycobacteria Insertion sequence of isolates gave 50% identification while with *oxyR* 3(50%) were identified as *M. tuberculosis*. Spoligotyping identified *Mycobacterium tuberculosis Ghana*, *Mycobacterium africanum,*while sequencing of the 16rRNA identified two non tuberculosis bacteria - *Mycobacterium. fla*vescens and *Mycobacterium. moriokaense* which have been known to infect animals example cattle presenting histological form similar to those presented in cattle infected with *Mycobacterium bovis.* As far as can be gathered from literature this is the first time *Mycobacterium tuberculosis* in cattle has been identified in Ghana through molecular typing of appropriate isolates.

1131

CAN JOB TITLES BE PREDICTORS FOR RECENT ONSET LATENT TUBERCULOSIS IN HEALTH CARE WORKERS?

Kittisak Sawanyawisuth[1], Naesinee Chaiear[2], Kanlayanee Sawanyawisuth[3], Panita Limpawattana[1], Janpen Bourpoern[4], Vichai Senthong[1], Wipa Reechaipichitkul[1]

[1]Department of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, [2]Department of Community Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, [3]Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, [4]Infectious Control Unit, Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

Latent tuberculosis (LTB) is the stage of *Mycobacterium tuberculosis* that is asymptomatic, dormant and non-contagious. Although health care workers are considered as high risk for LTB, it has been debating if job types are associated with the risk of LTB. In addition, there is limited data of this issue on the recent onset LTB. We determined the association of job types and tuberculin conversion or recent onset latent tuberculosis in healthcare workers in an endemic area of tuberculosis. A case-control study was done at Srinagarind hospital, Thailand. Cases were subjects with tuberculin conversion, while controls were subjects with negative results of tuberculin skin test (TST) in two consecutive years. There were 1,025 subjects completed two consecutive TST during 2001-2009. The incidence rate of tuberculin conversion was 19.8% or 203 subjects. In a multivariate model, the only three significant factors for tuberculin conversion were male gender, having BCG scar, and job types. Only nurses, nurse assistants, and workers were significantly associated with tuberculin conversion with adjusted odds ratio [95% confidence interval] of 2.3 [1.3-4.1], 2.3 [1.3-4.7], and 3.0 [1.8-5.0], respectively. Tuberculosis infection control program should be emphasized in those job types of healthcare workers who are at risk.

1132

COMPARISON OF DIFFERENT MOLECULAR AND CULTURE-BASED STOOL TECHNIQUES IN PULMONARY TUBERCULOSIS DIAGNOSIS

Svenja J. Albrecht[1], Luz Caviedes[2], Patricia Sheen[2], Fanny Arenas[2], Patricia Fuentes[2], Teresa Valencia[2], Vivian Kawai[3], Giselle Soto[3], Laura Martin[2], Diana L. Williams[4], Robert H. Gilman[1], Carlton A. Evans[5]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Laboratorios de Investigación y Desarrollo, Facultad de Ciencias, Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Asociación Benefica Prisma, Lima, Peru, [4]Laboratory Research Branch, Molecular Biology Research, National Hansen's Disease Program LSU-SVM, Baton Rouge, LA, United States, [5]IFHAD: Innovation for Health and Development, London, United Kingdom

The diagnosis of pulmonary tuberculosis (TB) is difficult in patients unable to provide sputum. Most sputum is swallowed and we evaluated molecular and culture-based tests for detecting *M. tuberculosis* from swallowed sputum in stool for the diagnosis of pulmonary TB. Stool samples from adults with suspected and proven pulmonary TB, prior to and during treatment were tested. The diagnostic performance of the following techniques was compared: an IS6110 nested polymerase chain reaction (PCR); sputum smear fluorescence microscopy with centrifuge concentration and Auramine staining; the Microscopic-Observation Drug-Susceptibility (MODS) broth culture technique; culture on antibiotic-enriched selective Middlebrook 7H10 thin-layer agar (TLA); and culture on conventional Lowenstein-Jensen (LJ) solid culture medium. Stool was decontaminated with the NALC-NaOH technique as used for sputum. Of 1,086 stool samples, 129 were culture positive. For these samples, the diagnostic sensitivity of MODS was 92%, higher than LJ (81%, P=0.02), PCR (75%, P<0.01), all of which were more sensitive than TLA 59%, P<0.01) and only 40% were microscopy positive. Considering the 934 samples with results for all tests, PCR was positive for 19% and culture 12%: MODS in 9.2%, LJ in 7.3%, TLA in 6.0% and microscopy in 5.3% (all comparisons P<0.01). Contamination caused test failure for 1.8% of MODS tests, 3.4% of TLA and significantly more for LJ cultures (15.2%, P<0.01). 567 of the PCR were performed after two DNA extraction techniques and positivity was significantly more frequent with commercial spin columns (Qiagen), than the in-house Chelex technique (16% vs 12%, P=0.03). In conclusion, PCR of stool specimens has higher sensitivity than culture for the diagnosis of pulmonary TB. Qiagen columns performed better than Chelex extraction. MODS had the highest sensitivity and lowest contamination rates among the three culture techniques.

1133

GENETIC VARIABILITY IN HUMAN METAPNEUMOVIRUS CIRCULATING IN CENTRAL AND SOUTH AMERICA

Merly Sovero[1], Josefina Garcia[1], Ada Romero[1], Maria E. Gamero[1], Victor A. Laguna-Torres[1], Jorge Gomez[2], Felix Sanchez[3], Ana E. Arango[4], Tadeusz J. Kochel[1], Eric S. Halsey[1]

[1]Naval Medical Research Center Detachment, Lima, Peru, [2]Directorate of Epidemiology, Lima, Peru, [3]Hospital Infantil Manuel de Jesus Rivera, Managua, Nicaragua, [4]Universidad de Antioquia, Medellin, Colombia

The human metapneumovirus (hMPV) is a recently discovered member of the family Paramiyxoviridae responsible for acute respiratory tract infections in young children, elderly patients, and immunocompromised hosts. Based on genomic sequencing and phylogenetic analysis, there are two major hMPV subtypes: A and B. These analyses are based on the sequencing of the N, M, F, G, or L genes and genotype grouping are concordant regardless of which gene is studied. The major differences between the A and B genotypes are nucleotide polymorphisms concentrated mostly on the G and SH proteins. The G gene of hMPV displays significant strain-to-strain variability. In this study we genetically analyzed the circulating hPMV in Central and South America from July 2008 to June 2009 and characterized the strains present in this region and their genetic variability. Samples were collected during an international collaborative febrile surveillance study. All were cultured and analyzed by inmunofluorescence for influenza, hRSV, parainfluenza, enterovirus, adenovirus, herpes simplex virus (1 and 2), and hMPV. Only those hMPV culture-postive samples were confirmed by RT-PCR and sequenced. The primers used were specific to hMPV G and N genes. This study analyzed 32 culture-positive samples. Of these, 50% were male and 50% were female. Nineteen of the samples came from children under 12 years of age, 3 from adolescents age 12-17, 7 from adults age 18-50, and 3 from participants older than 50 years.

Nucleotide comparison of the samples revealed the existence of two major genetic clusters. The phylogenetic analysis for the N gene of samples showed high similarity between the viruses, while the amino acid sequence of G gene clearly showed more diversity in the strains; genotypes A2, B1, and B2 were detected. The partial comparison of amino acid sequences of the G protein of hMPV revealed that all changes were base substitutions, with no deletions or insertions. In conclusion, our results show that two distinct clusters of hMPV circulated in Central and South America during the July 2008- July 2009 period. Similar clusters were reported in Canada in 2002, suggesting that a relative homogeneous population of hMPV is circulating throughout the world. These results differ from the previously described isolated viruses in 2003 in Peru which showed subtype A as the predominant subtype.

1134

CLIMATE AS SEASONAL INFLUENZA PREDICTORS

Radina P. Soebiyanto, Richard K. Kiang

NASA Goddard Space Flight Center, Greenbelt, MD, United States

Seasonal influenza continues to be a significant public health burden. Despite vaccination and the largely mild cases, influenza causes up to 300,000 deaths each year worldwide. Moreover, influenza virus inherently undergoes rapid mutation that has the potential to bring about pandemic at any time. Hence understanding transmission pattern and capabilities to accurately project influenza cases can contribute to reducing the disease burden, as well as facilitating the preparedness effort. Toward this end, our group has examined the role of climatic and environmental factors in influenza seasonality. We have previously shown the dependencies between climate and influenza incidences in two regions with warm temperature. We now extend our analysis to cities with temperate climate, and subsequently compare the dominating climatic indicators between the regions. Remotely-sensed climatic indicators from NASA satellites - such as Land Surface Temperature (LST), precipitation as a measure for rainfall - as well as meteorological measures from the ground stations are used as covariates in our empirical models. In general, the resulting model can identify the timing of influenza peak reasonably well. We further produced influenza forecasts for the following season using the climate-based model. The resulting one-season-ahead prediction provides baseline cases that can be used to estimate vaccine production. The identification of influenza-associated environmental parameters from this work could also guide further biological studies on the transmission mechanism.

1135

ACUTE RESPIRATORY INFECTION, MAIN CAUSE FOR MORBIDITY FOR CHILDREN 0-5 YEARS OF AGE, IN POST-EARTHQUAKE, HAITI, 2010

Ribka Amsalu Tessera

Save the Children, Wahington, DC, United States

An earthquake measuring 7.0 struck Haiti on January 12th, 2010 at 16:53hours local time. It had devastating effect on the capital Port-au-Prince, the towns of Carrefour, Leogane, Grand Goave, and Jacmel in the South East Department. A total of 3million people were affected by the earthquake of whom 2million are estimated to have been displaced from their homes. Save the Children, a humanitarian organization, that has been operational in Haiti for 21 years responded immediately by establishing mobile medical team and providing emergency medical services in Port-au-Prince, Leogane, and Jacmel. Disease surveillance is one of the key tasks of the medical team to prioritize health action and determine the occurrence of disease outbreaks. From January 31st - April 4th, 21 Mobile Medical team of Save the Children composed of medical doctors, midwife and nurses, had 52,761 consultations in Port-au-Prince and Jacmel, out of which 22% were due to Acute Respiratory Infections (ARIs). ARI accounted for 48.4% of consultations among children 0-5yr of age, and 12.5% among those over the age of 5yrs. The total consultation due to ARIs was four fold higher than the total number of consultations due to diarrhea and suspected malaria among children 0-5yr of age. The World Health Organization (WHO), estimates that prior to the earthquake pneumonia accounted for 20% of mortality among children in Haiti, much higher than diarrhea (16%) and malaria (1%). Crowded leaving conditions, low vaccination coverage, and poor nutritional situation have exacerbated the risk for pneumonia among children in post-earthquake Haiti. Efforts to have a strict case definition for pneumonia in the surveillance system is critical to monitor disease trend, prioritize public health action including advocacy to improve shelter, food, and vaccination among the internally displaced persons in Haiti.

1136

EFFICACY OF A RAPID DIAGNOSTIC ASSAY FOR DETECTING PANDEMIC INFLUENZA A: H1N1 IN A RESPIRATORY SURVEILLANCE COHORT IN PERU

Victor E. Gonzaga, Yeny Tinoco, Hugo Razuri, Ernesto Ortiz, Claudia Guezala, Abel Estela, Candice Romero, Patricia Brena, Maria Luisa Morales, Joel M. Montgomery

Naval Medical Research Center Detachment, Lima, Peru

A population-based active surveillance cohort to estimate the burden of influenza and influenza like illness (ILI) was initiated in 4 geographically distinct regions of Peru in June-July 2009, in the early phase of the pandemic. A rapid diagnostic assay, QuickVue Influenza A+B®, was performed in the field as the first step in diagnosis of influenza among ILI cases. In the context of the pandemic, we evaluated the sensitivity, specificity and usefulness of this test as a tool to aid clinical and public health decisions. Nasal/throat swabs were collected from subjects with ILI (fever ≥38°C with cough and/or sore throat). For this study, in order to evaluate the usefulness of this test during periods of higher virus circulation, we selected samples collected during the peak of the pandemic in Lima (A), Tumbes (B), and Puerto Maldonado (C). The fourth site was excluded because a pandemic peak could not be clearly defined.

Epidemic peak periods were selected considering the highest ILI attack rates per site observed in 2009. Samples were tested by QuickVue A+B® within 24 hours of ILI case detection, stored at -70ºC, transported to the lab and tested by real-time PCR for pH1N1. Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) per site were calculated in order to measure the usefulness of the test. A total of 570 samples were collected during selected epidemiological weeks (EW): EW 25-29 for A (n=186), and EW 37-41 for B (n=248) and C (n=136). The specificity was similar in these sites: 96, 97, and 100% for A, C and B, respectively. However, the sensitivity was variable: 38, 48 and 55% in B, C and A, respectively. Similar results were obtained for PPV: 94, 97, 100, and 94% in C, A and B, respectively. The NPV was 47% in A, 63% in C and 70% in B. In conclusion, the sensitivity of the QuickVue Influenza A+B® appears to be low for the specific pH1N1 genotype. As expected, NPV decreased when prevalence of infection was higher in the population, as in Lima. An NPV as low as 47%, under this context, makes the test of limited use as a screening tool during a pandemic. Therefore, final diagnoses should be confirmed with the accepted gold standard (real-time PCR for pH1N1). However, while this particular rapid diagnostic assay suffers from low sensitivity rates, utilization of such assays allows clinicians to make informed decisions and respond in a more rapid fashion.

1137

MYCOBACTERIUM LEPRAE IS SUSCEPTIBLE TO CEM-101

Ramanuj Lahiri[1], Tom Gillis[1], Prabhavathi Fernandes[2]

[1]National Hansen's Disease Program at Lousiana State University, Baton Rouge, LA, United States, [2]Cempra Pharmaceuticals, Chapel Hill, NC, United States

CEM-101, a new macrolide-ketolide in clinical development, has been found to be a minimum of 4-fold more active than other macrolides, mainly clarithromycin and azithromycin and 2-4 fold more active than telithromycin. It is active against a variety of macrolide-resistant pathogenic strains of *S. aureus*, *S. pyogenes*, and *S. pneumoniae*. The efficacy of CEM-101 against *Mycobacterium leprae*, the causative agent for leprosy, was investigated in the present study. The Thai-53 isolate of *M. leprae*, maintained by serial passages in athymic *nu/nu* mice footpads, was used for all experiments. For axenic testing freshly harvested viable *M. leprae* were incubated in medium along with different concentrations of the drugs (CEM-101, clarithromycin and rifampin) for 7 days at 33oC. At the end of this incubation drug-treated *M. leprae* were subjected to radiorespirometry to assess viability based on oxidation of 14C palmitate and staining with viability dyes to assess the extent of membrane damage. For intracellular testing peritoneal macrophages from Swiss mice were infected with freshly harvested viable *M. leprae* at a MOI of 20:1 for 12 hours. At the end of the infection extracellular bacteria were washed and drugs added at different concentrations and incubated for 3 days at 33oC. At the end of 3 days cells were lysed to obtain the intracellular *M. leprae* for viability testing by radiorespirometry and viability staining. CEM-101 at 0.15 µg/ml was able to significantly (P<0.001) reduce the viability of *M. leprae* in both axenic and intracellular cultures when compared to controls. Inhibition by CEM-101 was not statistically different from inhibition obtained with clarithromycin under identical conditions and at the same concentration against the claithromycin-susceptible Thai-53 strain. In conclusion, CEM-101 is effective against *M. leprae* potentially expanding the drugs available to treat leprosy.

1138

CHANGES IN ILI CASE REPORTING PATTERN IN AN ELECTRONIC SURVEILLANCE SYSTEM DUE TO PANDEMIC INFLUENZA A (PH1N1) IN THE PERUVIAN NAVY, 2008-2009

Delphis M. Vera, Ricardo A. Hora, Joan M. Neyra, Jose A. Quispe, Joel M. Montgomery

United States Naval Medical Research Center Detachment Lima Peru, Callao, Peru

During 2008-2009, the Department of Health of the Peruvian Navy closely monitored influenza-like illness (ILI) cases among active duty and retired military personnel and their families using an electronic disease surveillance system. As pandemic influenza A (H1N1) (pH1N1) virus spread globally in 2009, the system detected a steady increase in ILI cases as compared to 2008. We sought to determine and characterize such differences between cases reported during the period from Jan 2008-Dec 2009. Alerta DISAMAR is the Peruvian Navy's electronic disease surveillance system currently implemented in 121 reporting units nationwide. Reporting of ILI cases by age group was carried out weekly via Internet and/or telephone. We retrospectively reviewed ILI cases reported during 2008-2009 and compared cases by year using the Mann- Whitney's U test. P-values <0.05 indicated statistical significance. During the period from 2008-2009 a total of 5469 ILI cases were reported to Alerta. A total of 2282[41.73%] and 3187[58.27%] cases occurred in 2008 and 2009, respectively (p=0.02). Analysis by month between 2008 and 2009 showed differences in May and June [pandemic awareness in Peru] (p<0.01 and p=0.02). The number of cases reported in 2009 (n=1526) in persons 17 years old and under was significantly higher than those reported in 2008[n=856] (p<0.01). The analysis also showed significant differences in Lima [1643 vs. 2443, 2008 vs. 2009] and Iquitos [168 vs. 339] (p=0.032 and p=0.017). Our findings showed a difference between the ILI cases reported in this period, particularly among persons under 17 years of age. We may attribute this difference to pandemic periods distorting both case reporting patterns among stakeholders in surveillance systems, and health care seeking behavior among populations. Public health professionals utilizing surveillance systems should be cognizant that over reporting may occur during similar situations. Therefore, they should aim to confirm "real" cases from those that may be influenced to seek medical care.

1139

DEFINING ELISPOT CUT-OFFS FROM UNREPLICATED TEST AND CONTROL WELLS

Neal Alexander[1], Annette Fox[2], Vu T. Lien[3], Tao Dong[3], Le Q. Mai[3], Peter Horby[2]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]Oxford University Clinical Research Unit, Hanoi, Vietnam, [3]National Institute for Hygiene and Epidemiology, Hanoi, Vietnam

The enzyme-linked immunospot (ELISpot) assay is widely used to detect antigen-specific cytokine-secreting T cells, in particular cell-mediated immune response to vaccines. Various criteria have been used to define positive response, some of them arbitrary. Rigorous methods have been devised for plate layouts with replication, but often this is not present. We present a method for selecting cut-offs which requires negative control wells but not replication. The method uses the proportion of plates in which the number of spot forming units in the test well exceeds that in the control well by a certain amount, rather than the same size difference the other way round. If this proportion exceeds 50% by more than sampling error we can infer that the assay is detecting a signal. Moreover, plotting this proportion, and its confidence interval, against the size of difference suggests the most powerful cutoff to use. We illustrate the method using data from a community-based study of influenza transmission in Vietnam. The complete proteome of H3N2, the haemagglutinin and neuraminidase of H1N1 and the haemagglutinin of H5N1 were represented as peptides of 9-20 amino acids. Preliminary results are available from blood samples of 751 residents aged 5-84

years. Among the peptides with a strong signal was matrix protein: 92 samples had a difference of more than 2 between square-root-test and square-root-control counts, and, of these, 79% had the test well larger than control, rather than the other way round, suggesting a strong signal. By comparison, acidic polymerase, for example, had 47 samples with differences of this magnitude, but about equal proportions of these had test greater than control (47%) and the other way round (53%), suggesting low discriminatory power. The assay has since been refined and we will also present analysis of 1317 paired results based on two different reader settings. This approach should prove useful for those experiments in which testing a greater number of different peptides is preferred to a smaller number of peptides with replication.

1140

BCG STATUS IN NIGERIAN CHILDREN WITH TUBERCULOSIS

Adeola Orogade

Abu Teaching Hospital, Kaduna, Nigeria

Currently there is only one vaccine against tuberculosis available worldwide: Bacille Calmette-Guérin (BCG). This vaccine, used since 1921, can protect children from severe forms of tuberculosis. There are however varying reports in different countries about the efficacy of BCG in preventing pulmonary TB in children. To evaluate the effect of BCG in preventing pulmonary tuberculosis, 191 HIV negative children aged between 8 months and 14 years with clinical and radiological diagnosis of tuberculosis, receiving antituberculous therapy at a government facility were studied. 95 of them had received BCG and 96 had not. Sixty five percent in BCG group and 76% and in non BCG group were aged over 5 years, while 32% and 20% were 1-5 years old respectively. The others in each group were infants. The commonest presentation was Pulmonary TB in 80% in BCG group and 76% in the non BCG groups, 15% and 20% of children had disseminated TB, while cervical adenopathy was seen in others. Tuberculin skin tests were negative in 34% and 60% in these categories respectively. Only about 50% of children in this series received BCG, but there was not much difference in proportions of children that had pulmonary TB or disseminated TB in both groups. Negative tuberculin test in up to one third of children receiving BCG is noted. Due to the upsurge in incidence of pulmonary tuberculosis locally and globally, there is need to further investigate and review current recommendations for BCG vaccination in this region as well as consider other vaccine candidates that would be effective against most forms of childhood Tuberculosis.

1141

ARE PARAGONIMIASIS AND OTHER PARASITIC INFECTIONS MISDIAGNOSED AS TUBERCULOSIS IN RESOURCE LIMITED AREAS?

Bernard Nkrumah[1], Henry Owusu Frempong[2]

[1]Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana, [2]Agogo Presbyterian Hospital, Agogo, Ghana

Paragonimiasis (PG) is a food-borne zoonosis caused by trematode worms. This and other parasitic or fungal agents cause infection that predominantly affects the lungs with clinical symptoms similar to tuberculosis (TB) (Vidamalya et al., 2009). Due to the lack of awareness of local health professionals to parasitic agents, they are little evoked in face of chronic cough simulating pulmonary TB. TB offends PG and other parasitic infections by reason of their flagrant clinicoradiologic similarities, thus they are least considered in the face of a chronic cough simulating pulmonary TB, as reported previously. The objective of this study was to estimate the prevalence of PG and other parasitic or fungal causes of respiratory diseases in suspected TB patients. Sputum samples were collected from all patients reporting to the TB clinic of the hospital for the first time. After patients consent, three sputum samples were collected, pooled and processed within 24 hours of collection by: wet preparation with 3% NaOH concentration and microscopy of sediment for parasites and ZN staining of sediment for Acid fast bacilli (AFB). Ethical approval was obtained for this study. 70 patients were recruited within the study period. 44 (62.9%) were males. The mean age was 44.9 years (95% CI: 40.1-49.5). 16 (22.9%) samples were positive for parasitic agents by wet preparation, 2 (2.9%) by ZN staining and 17 (24.3%) were AFB positive. Sputum collected were grouped into four: blood stained, 4(5.7%); muco-purulent, 6(8.6%); muco-salivery, 57 (81.4%) and salivery, 3 (4.3%). 15 (21.4%) of the study patients were HIV/AIDS positive. Parasites identified in wet preparation were Paragonimus *uterobilateralis* (PU) (2/70), Strongyloides *stercoralis* (SS) (1/70) and fungal elements (13/70) of which 8/13 were HIV/AIDS patients. Cryptosporidium parvum (2/70) were found in the ZN staining. Apart from SS, all other parasites were identified in AFB negative patients. Both PU were identified in blood stained samples. The prevalence of PG, fungal infection, Cryptosporidiosis and Strongyloidiasis were 2.9%, 18.6%, 2.9% and 1.4% respectively.These were lower compared to studies carried out in parts of Nigeria and Cameroon, as reported previously. Our sample size might have contributed to this. Our study shows that parasitic infections of the lung are clearly being misdiagnosed as TB; there is the need for awareness to be created among local health care providers.

1142

MODELING CATCH-UP GROWTH FOLLOWING DIFFERENT DRUG TREATMENT REGIMENS FOR ENDEMIC SCHISTOSOMIASIS

David Gurarie, **Xiaoxia Wang**, Amaya L. Bustinduy, Charles H. King

Case Western Reserve University, Cleveland, OH, United States

Schistosomiasis is a chronic parasitic infection that results in persistent inflammation that can cause growth stunting and nutritional wasting among affected children. We develop a mathematical model of early childhood development (ages 0-20 years) and the effect of chronic helminth infection on some basic developmental indicators (height, weight). The model was calibrated using the available developmental data in the US (CDC/NCHS), along with infection data and developmental statistics collected in Kenya. We utilized our calibrated model to examine and predict long term outcomes of different age-targeted treatment control strategies. Our results demonstrate the need for early treatment and repeated coverage through the primary school years in order to prevent the under-recognized but disabling sequelae of stunting and undernutrition.

1143

PARASITOLOGICAL AND SEROLOGICAL OF SCHISTOSOMIASIS IN TWO COMMUNITIES ALONG THE VOLTA BASIN

Naa Adjeley Tawiah[1], Irene Ayi[2], Patrick Ayeh-Kumi[1], Daniel Boakye[2]

[1]University of Ghana Medical School, Accra, Ghana, [2]Noguchi Memorial Institute for Medical Research, Accra, Ghana

Schistosomiasis is an important health problem in many developing countries including Ghana. Humans living in schistosomiasis endemic areas develop anti-parasitic antibody responses,which may play distinct roles in immunity. Insight regarding humoral responses in the pattern of *Schistosoma haematobium* and *S. mansoni* infections in any endemic locality is relevant in understanding the pathogenesis of schistosomiasis. This study estimated prevalence of Schistosomiasis in five hundred and eighty three participants aged 5-90 years in Klamadaboe and Torgome. Participants were interviewed to gather Schistosomiasis-related information and screened for *S. haematobium* and *S. mansoni* eggs by microscopy through urine filtration and Kato-Katz technique. ELISA technique was used to confirm infection status of 115 microscopy negative and positive participants to detect anti-IgM and IgG against soluble adult worm and egg antigens.Prevalence of *S. mansoni* and *S. haematobium* infections by microscopy was (17.0%, 10.1%) (N= 159) in

Klamadaboe and (2.6%, 4.7%) in Torgome (N = 424) respectively. Out of 583 participants tested, (10.1%, 39.0%) were positive for haematuria and proteinuria in Klamadaboe and (12.3%, 32.1%) in Torgome. Serum anti-IgM and IgG was detected in (60%, 51%) of people negative for *S. mansoni* eggs (N= 105) and (68%, 54%) respectively for *S. haematobium* (N=112). Fifty nine percent (N =115) had mixed infections with antibodies against both infections. A total of 3.94% (N=76) and 52% (N=27) previously treated participants were positive by microscopy in Torgome and Klamadaboe respectively. Serum antibody detection was useful in confirming infected and uninfected individuals.

1144

SUPPRESSION OF IMMUNOPATHOLOGY IN EXPERIMENTAL MURINE SCHISTOSOMIASIS BY INTERLEUKIN-18-TARGETED FUSION TOXIN, DAB_{390}IL-18 I-STUDIES OF *IN VITRO* AND *IN VIVO* EFFICACY

Saleh A. Bahashwan[1], Mohamed A. Ali[1], Khalid A. Shadid[1], Ahmad A. Fayed[1], Naif O. Al-Harbi[2]

[1]College of Health Sciences, Taibah University, Al-Madina Al-Munawara, Saudi Arabia, [2]College of Health Sciences- King Saud University, Riyadh, Saudi Arabia

Schistosomiasis causes pathology in an estimated 200 million individuals. Clinical disease is caused by a complex immunopathologic response to parasite ova, which are deposited in the host tissues. This immunopathologic response is initiated and caused by Antigen Presenting Cells (APC) which express the high affinity IL-18 receptor (IL-18R). DAB390IL-18 is a diphtheria toxin IL-18 fusion toxin protein which functionally inactivates or kills cells which bear the high affinity IL-18R. DAB390IL-18 has been used for the prevention of murine Experimental Auto-immune Encephalitis (EAE). Therefore, we reasoned that DAB390IL-18 might suppress immunopathology in schistosomiasis. In these studies we assessed the *in vitro* and *in vivo* effects of DAB390IL-18 on the development of immunopathology in murine schistosomiasis. DAB390IL-18 suppressed IL-18, lectin mitogen (Con A), and soluble *Schistosoma mansoni* egg antigen -induced lymphocyte proliferation and *in vitro* granuloma formation. In addition, DAB390IL-18 suppressed *in vitro* IL-18R expression. DAB390IL-18 also suppressed the development of granulomas and collagen deposition *in vivo* in the livers of infected animals. Therefore, DAB390IL-18 may have potential for the targeted reduction of immunopathology due to schistosomiasis in man

1145

THE ASSOCIATION BETWEEN SCHISTOSOME INFECTION, ATOPY AND AUTOREACTIVITY IN A ZIMBABWEAN POPULATION

Francisca Mutapi

University of Edinburgh, Edinburgh, United Kingdom

In animal experimental models, parasitic helminth infections can protect the host from atopy and autoimmune diseases. We have conducted the first population-scale human study investigating the relationship between helminth parasitism and autoimmune-reactivity. In addition we have conducted a study of the relationship between atopy and infection intensity in 2 villages of differing schistosome infection intensity. In people naturally exposed to the blood fluke helminth parasite causing schistosomiasis, we found that autoimmune-reactivity and atopy is inversely associated with current infection intensity but is independent of host age, sex and HIV status. Autoimmune-reactivity increases 6 months after anti-helminthic treatment. The implications of these findings are relevant in understanding both the aetiology of autoimmune diseases and in predicting the long-term consequences of large-scale schistosomiasis control programs.

1146

THE APPLICATION OF INTEGRATED MODEL OF S LANDSCAPE PATTERN ANALYSIS AND BAYESIAN MODELS ON SCHISTOSOMIAIS CONTROL

Kun Yang

Jiangsu Institute of Parasitic Diseases, Wuxi, China

With the ecological environment changing, including climate warming and human activities, the prevalence of schistosomiasis japonica will also change. The aim of this study is to establish an integrated model to evaluate and predict the change of the endemic status of schistosomiasis before and after the project of land conversion for restoration in the Dongting Lake region. The data collected was the sampling survey on schistosomiasis from 1995 to 2006 in Hanshou County in Hunan province, P.R China. Normalized difference vegetation index (NDVI) and Wetness, land surface temperature (LST) and landscape factors (land-use/type) were extracted from remote sensing images. Landscape metrics were calculated from land-use/type images. Spatio-temporal Bayesian models based on landscape analysis and the sensitivity/specificity of diagnostic test(s) were established to understand and predict the spatio-temporal pattern of schistosomiasis. The results showed that the change of spatial structure each year during 1996 and 2005 were significant. The negative correlation of the prevalence of *S.japonicum* infection and NDVI were significant as well. The prediction map of 2002 showed the whole prevalence of *S.japonicum* infection was low, and the areas whose prevalence was more than 1% were mostly along bodies of water such as the Muping Lake and Yuanshui River. The average prediction prevalence was about 2.22% in 2005, and these areas were also along bodies of water. The changing map of *S.japonicum* infection prevalence in the southern areas between 1996 and 2005 were not found to be significant. However, the prevalence increased significantly in the northern areas. The impact of the project, land conversion for restoration on the prevalence of *S.japonicum* was significant, with the impact of partial abandonment for restoration being stronger than the complete abandonment part of the project.

1147

CURRENT EFFICACY OF PRAZIQUANTEL AGAINST *SCHISTOSOMA JAPONICUM* INFECTION: A FIELD EVALUATION IN MAIN ENDEMIC FOCI OF CHINA

Wang Wei, Liang Yousheng, Dai Jianrong, Li Hongjun

Jiangsu Institute of Parasitic Diseases, Wuxi, China

Praziquantel is widely used for the treatment of human schistosomiasis. However, in recent years, there is a increasing concern about the resistance of *Schistosoma* species to praziquantel. Under laboratorial conditions it is possible to induce resistance of *S. mansoni* to praziquantel with multiple sub-curative doses, and a decreased sensitivity of *S. mansoni* to praziquantel has been found in many endemic areas. There are also several schistosomiasis cases caused by *S. hematobium* in whom repeated standard failed to clear the infection reported. Since 1992, the World Bank Loan Project for Schistosomiasis Control initiated in China, praziquantel-based chemotherapy has been conducted to control the morbidity and reduce the prevalence and intensity of *S. japonicum* infection. After extensive, long-term repeated praziquantel chemotherapy, whether there is decreased efficacy of praziquantel against *S. japonicum* is paid many attentions. The study we described here was designed to evaluate the efficacy of praziquantel against *S. japonicum* in main endemic foci of China. During the non-transmission period of schistosomiasis, a random sample of 4760 subjects from 11 villages of 5 provinces in China that are endemic for *S. japonicum* were examined using the miracidium-hatching method for detection of the stool samples, and a total of 584 subjects were identified as being infected with *S. japonicum*, with a prevalence rate of 12.27%. Among them, 565 stool-egg-positive subjects were treated with praziquantel in a single oral dose of 40 mg/kg. Six weeks post-treatment, among the 505 villagers re-examined, 480 (95.05%)

had no detectable *S. japonicum* eggs. Twenty-one subjects still excreting eggs after the first treatment were treated with praziquantel for the second time. All stool samples, including those from those participants with second treatment were re-examined six weeks after the second treatment, and no stool-egg-positives were found. The results indicate that the efficacy of praziquantel against *S. japonicum* is still high, which has not changed after more than two decades of repeated, expanded chemotherapy in main endemic areas of China. It is suggested that no evidence of tolerance or resistance of *S. japonicum* to praziquantel is detected in China.

1148

CD23B MRNA EXPRESSION DOMINATES OVER CD23A MRNA EXPRESSION IN B CELLS FROM *SCHISTOSOMA MANSONI*-INFECTED OR UNINFECTED ADULTS IN WESTERN KENYA

Daniel O. Onguru[1], Pauline N. Mwinzi[1], Ayub V. Ofulla[2], Evan W. Secor[3], Diana M. Karanja[1], Daniel G. Colley[4], Lisa M. Ganley-Leal[5], YanMei Liang[6]

[1]Kenya Medical Research Institute, Kisumu, Kenya, [2]Maseno University, Kisumu, Kenya, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]University of Georgia, Athens, GA, United States, [5]Boston University School of Medicine, Boston, MA, United States, [6]Boston University Medical Center, Boston, MA, United States

IgE is associated with resistance to schistosomiasis and exerts its functions through its receptors, the high affinity receptor FcεRI and the low affinity receptor FcεRII (CD23). We previously demonstrated that expression of CD23 by circulating B cells correlates with the development of resistance to schistosome infection in a multiply treated cohort in an endemic area in western Kenya. There are two isoforms of the 45-kD CD23; CD23a and CD23b, which differ only in their cytosolic domains. CD23a is constitutively expressed by B cells and functions in the endocytosis of CD23-IgE antigen complexes, but the role of CD23b, which is IL-4-inducible, in B cells is not known. The objective of this study was to define the role of CD23 in generating immunity to *S. mansoni* re-infection. B cells were isolated from the peripheral blood of occupationally-exposed adult males assessed for expression of CD23a and CD23b and compared to uninfected cohorts. CD23a is the dominate isoform in peripheral blood B cells from uninfected/unexposed subjects. In contrast, CD23b was found to be elevated relative to CD23a in B cells from both *S. mansoni* infected and non-infected endemic controls. Functionally, IL-4-generated CD23b+ B cells appear to display altered B cell differentiation pathways. These results suggest that CD23b has functional significance for B cells and studies are underway to define the role of CD23b+ B cells in schistosomiasis.

1149

HUMAN MONOCYTE EXPRESSION AND ACTIVITY IN *SCHISTOSOMA HAEMATOBIUM* IMMUNE RESPONSES

Laura J. Appleby, Francisca Mutapi

University of Edinburgh, Edinburgh, United Kingdom

Monocytes derived from myeloid bone marrow precursors circulate in the blood before entering tissues and maturing into macrophages with a distinct phenotype dependent on the cytokine signals received. Macrophages and monocytes that are stimulated with Th1 cytokines, IFNg and TNFa, develop a classically activated phenotype, catalysing L-arginine to nitric oxide in response to microorganisms. In contrast, Th2 stimulated monocytes develop into alternatively activated monocytes and macrophages (AAM), characterised by a metabolic pathway in which L-arginine is converted into L-ornithine and urea leading to the suppression of T cell proliferation. AAM are associated with wound healing, controlling inflammatory immune responses and parasitic infections. While classically activated macrophages and monocytes (CAM) behave similarly in murine and human systems, AAMs do not seem to have such an overlap, and indeed the characteristic murine marker genes for AAM are notably absent in the human genome. However, an alternatively activated genotype in human filarial infections, with an upregulation of the arginase encoding gene ARG-1 has been reported previously. Using PBMCs isolated from a cohort of 200 participants in a *Schistosoma haematobium* endemic area in rural Zimbabwe we investigated the influence of infection on the phenotypic and genetic expression patterns in human monocytes, identifying markers for alternative activation. Furthermore the arginase expression patterns were elucidated and associated with liver pathology and infection intensity.

1150

COMPATIBILITY PATTERN OF *BIOMPHALARIA ALEXANDRINA* SNAILS IN WATER COURSES OF ALEXANDRIA GOVERNORATE, EGYPT

Suzan El Nasery, **Iman Abou El Naga**, Sonia Allam, Rasha Mady

Faculty of Medicine, Alexandria University, Alexandria, Egypt

Schistosomiasis is one of the ten tropical diseases specially targeted for control by the World Health Organization. Despite the major advances in the control of the disease, yet the transmission of the disease shows little evidence of slowing down globally and continues to spread to new geographic areas.

Our aim was to determine the compatibility pattern of Biomphalaria alexandrina snails collected from two districts representing eastern (Abees) and western (Alamreya) areas of Alexandria governorate. This will pave the way for further epidemiological and biological control studies of the intermediate host of Schistosoma mansoni. The results denoted that the natural infection rate of the snails from the field of the two areas was 2.3 and 3.3% respectively. At the age of 4-6 weeks, three hundred snails of the first generation of the field snails (laboratory adapted snails) from each group, were exposed to eight miracidia of the local strain of S. mansoni. Infection rate of the laboratory adapted snails of both groups was 15.67 and 18.33% respectively. Total cercarial production/100 exposed snails were 27484.56±8828.47 and 32937.33±10315.83 while the prepatent period was 31.96 and 30.58 days. No statistical significant differences in the results were present between both groups. By using Random Amplified Polymorphic DNA (RAPD-PCR) technique, differentiation between susceptible and resistant snails was achieved. Bands of resistance as well as bands of susceptibility could be detected by five out of eight primers used. This technique was proved to be easy, applicable and does not need previous information of nucleotide sequences.

1151

NATIONAL SEROLOGICAL SURVEY OF *HAEMATOBIUM* SCHISTOSOMIASIS IN MOROCCO: EVIDENCE FOR ELIMINATION

Mohamed Rhajaoui

Institut National D'Hygiene, Rabat, Morocco

The Moroccan Public Health Ministry launched a process of eliminating schistosomiasis in 1994. From 2005 to 2009 the epidemiological situation was marked by interruption of transmission at the national level, with only a few residual cases recorded. Our present study is the first systematic serologic survey to evaluate the transmission status in remaining endemic foci. Two thousand three hundred and eighty two children, born after the date of the last autochthonous cases, were selected from provinces with histories of high schistosomiasis transmission: Errachidia, Tata, El Kelaa Des Sraghna, Chtouka Ait Baha, and Beni Mellal. Specific antibodies directed to *Schistosoma haematobium* adult worm microsomal antigens (HAMA) were targeted using an enzyme-linked immunoelectro-transfer blot (EITB) assay. The results showed an absence of antibodies in all the sera. Consequently, our findings confirm a very low transmission status or a likely interruption of haematobium schistosomiasis transmission within these last endemic hot-spots.

1152

PROFILE OF SPECIFIC HUMORAL RESPONSES AGAINST SCHISTOSOMIASIS IN AN ENDEMIC RURAL AREA OF MINAS GERAIS STATE, BRAZIL: A LONGITUDINAL STUDY

Leonardo F. Matoso[1], Ricardo T. Fujiwara[1], Humberto F. Quites[1], Lorena J. Souza[2], Luciana Lisboa[2], Helmut Kloos[3], Rodrigo C. Oliveira[2], Andrea Gazzinelli[1]

[1]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [2]Fundação Oswaldo Cruz, Belo Horizonte, Brazil, [3]University California, San Francisco, CA, United States

We examined the pattern of specific IgE and IgG4 against *Schistosoma mansoni* antigens (SEA or SWAP) of residents of an endemic area in Brazil. Parasitological and serological analyses were performed in 152 individuals who participated in the entire period of evaluation. The follow up consisted of collection of three serum and fecal samples (time zero, one and three years after treatment), in the period between 2001 and 2006. Before treatment, the prevalence of *S. mansoni* was 57.9% (CI95% 50.06-65.74) and the geometric mean egg counts 64.1 (CI95% 52.67-75.51). After treatment, the prevalence decreased significantly to 15.1% (CI95% 9.41-20.79) and 27.6% (CI95% 20.50-34.70) in 2002 and 2006, respectively. The intensity of infection was significantly reduced to 37.8 (CI95% 35.07-40.55) and 36.4 (CI95% 33.93-38.89) eggs/g after treatment. Analysis of IgE-SEA demonstrated that significant differences in antibody production was only detected after three years of treatment, with infected individuals presenting higher anti-SEA IgE levels when compared to those egg-negative. Although differences in anti-SWAP IgE production were observed among infected and egg-negative individuals, they were limited to the initial evaluation before treatment. Anti-SEA and SWAP IgG4 levels were significantly higher in infected individuals before and after treatment, when compared to egg-negative individuals. Significant association was observed only to SEA-specific antibodies at time zero, showing a correlation between intensity of infection and IgE ($r = -0.266$, $P = 0.012$), and also between geometric mean egg counts and IgG4 ($r = 0.239$, $P = 0.025$). Similar association was observed between parasite burden, IgG4 to SWAP ($r = 0.502$, $P = 0.015$) and parasite burden and IgE to SEA ($r = 0.470$, $P = 0.002$), one and three years after treatment, respectively. Our results may contribute to the evaluation of the use of specific IgE and IgG4 ELISA to predict susceptibility/resistance to human schistosomiasis. Analysis to evaluate the association between antibody production and infection is under investigation.

1153

LONG TERM INFECTIONS; GENE EXPRESSION PROFILES OF SCHISTOSOME/SNAIL COMBINATIONS

S.K. Buddenborg, S.D. Stansbury, J.W. Dragoo, C.M. Adema

University of New Mexico, Albuquerque, NM, United States

Oligo-based microarrays bearing features derived from either *Biomphalaria glabrata* or *Schistosoma mansoni* are probed with transcribed sequences derived from whole bodies of snails with *S. mansoni* infections of varying age. This will enable measurement of response profiles from both host and parasite a) at 3 weeks post-infection (wpi), before cercariae production and release (referred to as "shedding") commences; b) early in the interval of cercarial shedding (about 5 wpi); and c) at a time following more prolonged periods of cercariae shedding (8 wpi). It will be determined if immune or other genes in snails undergo pronounced up- or down-regulation before or after cercariae production, and if parasite components, potentially including factors like innate immune pathway or stress-related proteins that could contribute directly to protection of the infected snail, or that could modulate snail defense responses, are significantly altered in their expression during the same time points. An understanding of which transcripts from snail host or schistosome parasite, separately or combined, prolong the life of cercariae-shedding snails may provide inroads for control aimed at reduction of release of cercariae (infective for humans) by infected Biomphalaria snails.

1154

DIVERSIFICATION OF SNAIL RESPONSE FACTORS TO PARASITE INFECTION, EXCEPTION OR TREND?

S.D. Stansbury, J.W. Dragoo, S.K. Buddenborg, C.M. Adema

University of New Mexico, Albuquerque, NM, United States

Microarrays incorporating sequences expressed by *Biomphalaria glabrata* exposed to various immune challenges (wounding, bacteria, exposure to schistosomes) were used to identify candidate immune response factors from both BB02 (susceptible to S. mansoni) and BS-90 (resistant) *B. glabrata* laboratory strains at 12h, 24h and 120h post exposure to bacteria or schistosome parasites. Known immune factors as well as putative immune factors among novel (unknown) sequences were selected for analysis to expand insights into immunity of B. glabrata, especially transcripts that are held in common among all responses, as well as those that are unique to schistosome challenge. Five groups of candidate defense genes will be characterized by full-length sequencing. In light of indicated antigenic variation by schistosomes of mucins and of somatic diversification of fibrinogen-related proteins (FREPs), a category of snail immune lectins, it becomes highly relevant to investigate whether the occurrence of diverse or diversified immune factors in an invertebrate host that contributes to parasite transmission like the snail *B. glabrata* is more common than previously assumed. Accordingly, results from application of SSCP and from sequence comparsions of multiple cloned cDNA amplicons of putative non-self recognition factors (including FREPs and other lectins) will be presented.

1155

CROSS-SECTIONAL AND LONGITUDINAL STUDY ON SCHISTOSOMIASIS AND ITS RELATIONSHIP WITH SOCIOECONOMIC VARIABLES IN A RURAL AREA IN MINAS GERAIS STATE, BRAZIL

Humberto F. Quites[1], Helmut Kloos[2], Leonardo F. Matoso[1], Dener C. Reis[1], Elivelton S. Fonseca[1], João Paulo A. Haddad[1], Rodrigo C. Oliveira[3], Andrea Gazzinelli[1]

[1]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [2]University California, San Francisco, CA, United States, [3]Fundação Oswaldo Cruz, Belo Horizonte, Brazil

The objective of this study was to identify the socioeonomic determinants of schistosomiasis in a hyperendemic rural area in Minas Gerais State, Brazil, during the period 2001-2009. The study population comprised 528 persons in 2001 and 533 in 2009, including 389 individuals who lived in Virgem das Graças at these two points in time. Socioeconomic and water contact data were collected in household surveys. All participants were examined parasitologically in 2001, 2005 and 2009 and treated with praziquantel. The results showed a reduction in prevalence from 57.7% to 26.5% and in geometric mean egg counts from 57.98 (CI95% 55.84-60.11) in 2001 to 13.45 (CI95% 11.29-15.60) in 2009. *S. mansoni* infection was significantly correlated with ownership of motorized vehicles, electricity, latrines and water storage tanks ($p<0.001$). The number of houses using the streams decreased during those 8 years, largely as a result of increased use of piped water from shallow wells and springs ($p<0.001$). In the multivariate logistic regression model *S. mansoni* infection in 2009 was significantly correlated with age group 10-19 years (OR=1.18 CI95% 1.04-1.34) and well ownership (OR=1.09 CI95% 1.01-1.18) and faucet (OR=1.25 CI95% 1.06-1.47). Self evaluation of family health conditions showed that 30.0% of all households attributed health improvements during the study period to increased income from the government's "Bolsa Familia" social program, which benefits poor families and was implemented in 2004. The finding that improved socioeconomic conditions were associated with increased use of a safer water supply and declines in *S. mansoni* infection warrants further studies in other communities.

1156

INVESTIGATION OF SCHISTOSOMIASIS TREATMENT ROTATION STRATEGIES TO MAKE THE MOST OF EXPENSIVE PRAZIQUANTEL

Lindsay Rakers[1], **Frank O. Richards**[1], Abel Eigege[2], Emmanuel Emukah[3], B. Ibrahim[4], G. Ogah[5], Ndudi Okocha[6], Emmanuel Miri[2]

[1]The Carter Center, Atlanta, GA, United States, [2]The Carter Center, Jos, Nigeria, [3]The Carter Center, Owerri, Nigeria, [4]Plateau State Ministry of Health, Jos, Nigeria, [5]Nasarawa State Ministry of Health, Lafia, Nigeria, [6]Delta State Ministry of Health, Asaba, Nigeria

Schistosomiasis is a parasitic disease affecting 200 million people worldwide, and about 35 million in Nigeria alone. The drug praziquantel (PZQ) effectively combats the disease, and is used in annual mass treatment programs targeting the urinary and intestinal forms of the disease, *S. haematobium* [SH] and *S. mansoni*. When it is not donated, the cost of PZQ (about $0.20 per treatment) is a major constraint to scaling up treatment programs in countries that need it most. To maximize impact, the State Ministries of Health Delta, Nasarawa and Plateau States, assisted by The Carter Center, have assessed the feasibility of PZQ treatment rotations (drug holidays). Mass drug administration (MDA) with PZQ was provided in villages with >20% prevalence of SH (determined through rapid reagent strip test for hematuria in school children). A strategy has been adopted wherein targeted communities in endemic local government areas (LGAs) receive annual PZQ treatment for three to five years followed by a "drug holiday." MDA was rotated to other LGAs while health education and monitoring for recrudescence among school children continued in sentinel villages in LGAs on "holiday." From 2004 - 2007, prevalence data has been collected in communities after three to five years of annual treatment, and during treatment holiday. Communities treated for three years recrudesced in two years to an average of 24.5%, exceeding the 20% threshold for MDA, suggesting that retreatment was necessary. In contrast, those receiving four years of treatment experienced average recrudescence ranging from 3.6% (Delta state) to 13.2% prevalence (Plateau and Nasarawa States) after 2 years. Those receiving five years of treatment recrudesced to 11.6% prevalence after 2 years. We concluded that four to five years of annual treatment prior to holiday is the best rotation strategy in terms of the speed of disease recrudescence and the need for retreatment.

1157

SCHISTOSOMIASIS IN MINIGO, TANZANIA: A STUDY ON PREVENTIVE BEHAVIOR AND ITS CORRELATION TO DISEASE PREVALENCE

Laura N. Marshall, Amanda S. Chan, Eiman Mahmoud

Touro University - California, Vallejo, CA, United States

Schistosomiasis, the second-most prevalent parasitic disease in humans, manifests as a granulomatous inflammatory response to *Schistosoma* eggs, primarily within hepatic and intestinal systems. We evaluated the efficacy of preventive behavior for schistosomiasis within the village of Minigo, Tanzania (pop. 3635). Daily activities with infested waters in this endemic Lake Victoria Region put villagers at risk for contracting the trematodes (*S. mansoni* and *S. haematobium*). A comprehensive on-site screening and preventive education program produced stool and urine samples. Light microscopy was used to verify schistosomes. Test positive subjects were given a physical exam and treated with Praziquantel. 13.1% of participants (n=229, m=136, f=93) were test positive, fishermen composing the largest proportion (5.7%). Approximately half (n=110) of the subjects participated in an oral questionnaire and reported preventative (45.5%) and non-preventative (54.5%) behavior. Of the test positive cases in this subgroup, 33.3% reported preventative behavior and 66.7% reported no preventative behavior, a difference of two fold. Individual prevention was assessed with three criteria: previous testing, previous schisto study participation, preventive behavior. Although the three largest subgroups were students, farmers, then fishermen, results displayed an inverse relationship with prevention. Finally, ROC analysis, used in the cost/benefit analysis of diagnostic decision-making, was applied to subjects with respect to an understanding of schistosomiasis. Subgroups displayed a high sensitivity (0.78-1) for risks and awareness of infection, but overall low specificity (0.04-0.32), which is interpreted as poor understanding of the disease factors and risks. Data show preventative behavior reduces schistosomiasis prevalence. Regular extension of services from point-of-care facilities to communities is a preventive approach, which would encourage compliance to preventive intervention and treatment and may facilitate an increase in ROC specificity and an overall reduction of prevalence.

1158

THE ROLE OF CD11B+ MONONUCLEAR PHAGOCYTES IN SCHISTOSOME DEVELOPMENT

Colleen D. Walls, Stephen J. Davies

Uniformed Services University of the Health Sciences, Bethesda, MD, United States

Worldwide, over 200 millon people are infected with schistosomiasis. Importantly, transmission and pathology of the disease is dependent on worm maturation. Earlier, we demonstrated that naïve CD4+T cells facilitate worm development in the absence of TCR signaling. We further correlated the presence of naïve CD4+T cells with changes in steady-state expression of genes required for monocyte/macrophage maturation. Interestingly, direct stimulation of mononuclear phagocytes in the absence of CD4+T cells restores worm development. Hence, we hypothesize that schistosome development is dependent on innate immune signals, which are facilitated by the presence of naïve CD4+T cells. Recent studies have implicated a role for naïve T cells in priming dendritic cell maturation through the interaction of B7-H1 on resting T cells. Here, we further hypothesize that resting naïve CD4+T cells prime monocyte/macrophage maturation through B7-H1-PD1 signaling, and that this interaction is required for the appropriate innate immune signals that direct worm development. To support this hypothesis, we show that inert OVA-specific CD4+T cells, expressing B7-H1, directly interact with CD11b+ mononuclear cells in OTII-RAG-/- transgenic mice. Moreover, during the course of a schistosome infection, CD11b+ cells segregate into two distinct populations (CD11b+Hi and CD11b+Lo) that differ in their scatter properties and relative expression of Ly6C, CD115, and PD1. Remarkably, the relative ratios of these populations significantly differ between OTII-RAG-/- mice and RAG-/- mice, which lack T cells. Specifically, RAG -/- mice accumulate a larger proportion of CD11b+Lo cells that express less CD115, which is required for monocyte/macrophage maturation, and less PD1, which is a receptor for B7-H1. This evidence suggest that there is inadequate maturation of macrophages in the absence of naïve CD4+T cells, which correlates with the lack of schistosome development, and thus indicates that naïve CD4+T cells indirectly facilitate worm development by modulating monocyte/macrophage function.

1159

OROPOUCHE FEVER VIRUS: MOLECULAR EPIDEMIOLOGY AND EVOLUTION

Helena B. Vasconcelos, Márcio R. Nunes, Valéria L. Carvalho, Eliana V. da Silva, Mayra Silva, Lívia M. Casseb, Samir M. Casseb, **Pedro F. Vasconcelos**

Instituto Evandro Chagas, Ananindeua, Brazil

Oropouche virus (VORO; *Bunyaviridae, Orthobunyavirus*) is one of most important arbovirus which infects humans in the Brazilian Amazon, and is also the causal agent of Oropouche fever. Between 1961 and 2009, dozens of epidemics were registered in several urban centers of the Brazilian states of Acre, Amapá, Amazonas, Maranhão, Pará, Rondônia and Tocantins, and also in Panama, Peru and Trinidad & Tobago. This work aimed to develop a retrospective epidemiologic and molecular study of

VORO emphasizing its distribution, epidemic dynamics in the period, as well as the dispersion of the VORO genotypes in Brazil and other Latin American countries as a contribution to understanding of the molecular epidemiology of it. A total of 66 VORO isolates of the Instituto Evandro Chagas collection were growth into VERO cells and suckling mice; then, RNA was extracted and cDNA prepared by RT-PCR; the amplicons were purified and submitted to nucleotide sequencing to further molecular and evolution analyzes including genetic reassortment, molecular clock and viral dispersion. It was demonstrated the circulation of four different genetic lineages of VORO in the Brazilian Amazon (genotypes I, II, III, and IV); the genotypes I and II were respectively the most distributed VORO genotypes in Occidental and Oriental Amazon areas. These and the genotype III have been continuously under evolution pressure and changing by the mechanism "boom and boost" which result in an emergence of new VORO sub-genotypes that replace the older circulating sub-lineages in an area. The genotype III which was previously recognized in Panama was identified in the Amazon and Southeast regions. The results obtained by the comparative phylogenetic analyses of the SRNA and MRNA topologies suggest that VORO uses the genetic reassortment as mechanism to further generate its viral biodiversity, and the genotype I is the most stable, while the genotype II is the most unstable, and therefore under higher evolutionary pressure; it was recognized a new VORO genotype in this study, the genotype IV. The molecular clock analysis showed that VORO emerged in Pará state approximately 223 years ago, and along of the years did its dispersal and evolution through the Pan-Amazon as well as to the Caribbean and Central America region, and the genotype I was responsible by the emergence of all other VORO genotypes.

1160

ISOLATION OF H5N1 INFLUENZA VIRUSES FROM WILD TERRESTRIAL BIRDS IN KAZAKHSTAN

Zhailaubay Kydyrbayev

Research Institute for Biological Safety Problems, Gvardeiskiy, Kazakhstan

Highly pathogenic avian influenza viruses of subtype H5N1 were identified in Southeast Asia in 1996 and have spread in recent years across broad regions of Eurasia and Africa. These viruses have been shown to be highly lethal in chickens and other poultry species as well as several types of aquatic birds, however, there are only limited reports of H5N1 surveillance in terrestrial birds. In this study, we examined the presence of H5N1 in wild terrestrial birds in Kazakhstan. Wild birds were caught at the ornithological station "Chockpack" in the Zhambylskaya oblast. Cloacal swabs were collected according to accepted FAO standards. Field samples were tested by several methods including RRT-PCR, for H-, and N- genes. During the period 2007-08, cloacal swabs from 993 representatives of wild terrestrial birds in the family's Meropidae, Corvidae, Muscicapidae, Sylvidae, Falconidae, Hirundinidae, Motacillidae, Turdidae, Paridae, Laniidae, Accipitridae, Emberizidae, Upupidae, Strigidae, Sturnidae, Ploceidae, Columbidae, Fringillidae, Accipitridae, Motacillidae, Prunellidae, Caprimulgidae, Cuculidae, Tytonidae, and Fringillidae were collected. Of the 993 samples, 44 (4.43%) positive samples for type A influenza viruses including 28 (2.8%) positive samples from the birds of the Columbidae family (pigeon), 5 (0.5%) positive samples from birds of Corvidae family (jackdaw, rook), 4 (0.4%) positive samples from birds of the Ploceidae family (sparrow), 3 samples (0.3%) from birds of Meropidae family (golden bee-eater), 2 samples (0.2%) from birds of Hirundinidae family (swallow), 1 sample (0,1%) from birds of Sturnidae family(starling) and 1 sample (0.1%) from birds of Turdidae family (blue throated robin). Seven strains of subtype H5N1, including 3 from pigeons (Columbidae family), 2 from golden bee-eater (Meropidae family), 2 from starlings (Sturnidae family) and 1 from a rook (Corvidae family) were detected. The data support that AI viruses of H5N1 subtype circulate among wild terrestrial birds which live close to humans and domestic birds in Kazakhstan.

1161

ACUTE HEPATITIS E IN IMMIGRANTS AND NATIVE PATIENTS IN VICENZA, ITALY

Maria T. Giordani[1], Paolo Fabris[1], Enrico Brunetti[2], Luisa Romanò[3]

[1]Infectious and Tropical Diseases Unit, San Bortolo Hospital, Vicenza, Italy, [2]Division of Infectious and Tropical Diseases, University of Pavia, IRCCS S.Matteo Hospital Foundation, Pavia, Italy, [3]Public Health Microbiology Virology Department, University of Milan, Milan, Italy

Hepatitis E (HEV) virus is the most common cause of acute viral hepatitis in developing countries, but is gaining increasing global attention. Hepatitis E is generally self-limiting but in pregnant women and patients with chronic liver disease death can occur. In non-endemic countries, the infection occurs in sporadic cases: a zoonotic transmission involving pigs and other mammalians has been demonstrated.

We report our experience on HEV acute hepatitis in the Infectious and Tropical Diseases Department of the S.Bortolo Hospital in Vicenza, Italy. From 1995-2010, 20 cases of acute hepatitis E (17 male and 3 female, none pregnant, mean age 30 + 4.9 y) were admitted to our Department. The diagnosis was made by the detection of anti-HEV IgM together with HEV-RNA in blood and in stool. Four patients had recently immigrated to Italy (mean stay 19 days), 14 patients acquired the infection after travelling to their country of origin (15 Bangladesh, 4 India and 1 Pakistan) after several years of residence in Italy (mean 9.13 ± 2.3 years). Only one patient, an immigrant from Bangladesh who had been living in Italy for 9 years, had no history of recent travels. However, he was a household contact of another patient with HEV infection (secondary case). Only in one Italian male no travels and contacts with hyperendemic areas could be found. In this case, genotype 3 of HEV can be assumed, while all the other cases had genotype 1 (Burmese). All patients had a self-limited icteric illness (mean bilirubin level 8,6 ± 6.44 mg/dl, mean ALT level 2837,6 ± 1559 UI/l). None of the patients had pre-existing chronic liver disease. In conclusion, HEV infection is generally an imported disease in our town, but secondary and the odd autochthonous case can occur. The number of cases reported slightly increased since 2004. (1,1 cases/y in 1995-2003 vs 1,8 in 2004-2009). In particular, the infection is more common in immigrants travelling to the country of origin after staying in Italy for several years: a loss of previously acquired immunity can be hypothesized in these cases.

1162

MONOCLONAL AND POLYCLONAL ANTIBODIES FOR DEVELOPMENT OF RAPID IMMUNOASSAYS FOR DETECTION OF SAND FLY FEVER VIRUSES

Raymond Houghton, Stan Morkowski, Yvonne Stevens, Jean Chen, Jennifer Moon, Syamal Raychaudhuri

InBios International Inc., Seattle, WA, United States

Various viruses and Leishmania parasites are carried by the sand fly *Phlebotomus papatasi* and cause sand fly fever, or cutaneous and visceral Leishmaniasis. These diseases are designated high on the list of infectious threats to deployed troops. The development of simple field tests to detect and differentiate the causative agents of these diseases directly in sand flies or in humans would be of great importance. Sand Fly Fever is caused by a group of viruses often termed Sand Fly Fever Viruses (SFFV), or Papatasi viruses, or Phleboviruses and is typically caused by e.g. Toscana, Naples and Sicilian viruses which are closely related to Rift Valley Fever Virus (RVFV). The Toscana virus is confined to Italy and the Mediterranean basin, whereas the Naples and Sicilian viruses are more prevalent and often found together in the Middle East through Pakistan and Afghanistan, and recently in Algeria. The Toscana virus can lead to severe meningitis, whereas the Naples and Sicilian virus lead to short term febrile illness. Recently, other viruses related to the Naples virus (Massilia virus, Southern France and Punique, Tunisia) and Sicilian virus (Cyprus virus, Algeria and Utique, Tunisia) have been identified. Serological responses

to the viral Nucleocapsid (N) protein, in particular, specific IgM and IgG responses as detected in ELISA or western blot, are frequently used in SFFV diagnosis. However, high degree of cross reactivity between different SFFV and low antibody titers can complicate differential diagnosis. We have developed polyclonal and monoclonal antibodies to the different SFFV N proteins and have designed several rapid immunoassays for antigen detection either for use in sand flies or in human sera. In this study we describe the use of these antibodies in development of two rapid antigen tests 1) to differentiate SFFVs from Leishmania parasites; and 2) to differentiate Sicilian virus from Naples and Toscana virus. The potential field use of these tests will be discussed.

1163

DIAGNOSTIC PERFORMANCE OF ROTAVIRUS AND NOROVIRUS TESTING ON RECTAL SWAB SPECIMENS: IMPLICATIONS FOR OUTBREAK INVESTIGATIONS

Wences Arvelo[1], Alejandra Estevez[2], Beatriz Lopez[2], Nicole Gregoricus[1], Jan Vinje[1], Jon Gentsch[1], Umesh Parashar[1], Kim Lindblade[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Universidad del Valle de Guatemala, Guatemala City, Guatemala

Diarrhea outbreaks can result in high morbidity and mortality, particularly in children in developing countries. Identifying etiological agents is critical in controlling outbreaks. We evaluated the diagnostic performance of specimens collected using rectal swabs for the detection of rotavirus and norovirus. Patients meeting a diarrhea case definition (≥3 loose stools in 24 hours during the last seven days) were enrolled through an ongoing population-based surveillance system based at a hospital and an ambulatory clinic in the Department of Santa Rosa, Guatemala. From January through March 2009, we attempted to collect a rectal swab and whole stool sample from patients <5 years old with diarrhea. Rectal swabs were placed in phosphate-buffered saline media. Both specimens were kept at 4°C, and tested for norovirus using real time-reverse transcription-polymerase chain reaction and for rotavirus via enzyme linked immunosorbant assay using monoclonal antibodies to detect group specific antigen present in Group A rotavirus. We calculated sensitivity and specificity assuming testing from whole stool samples as the gold standard. We enrolled 102 cases with paired whole stool and rectal swab samples. The median age of patients was one year (range: 0-4 years); 38 (37%) were ambulatory patients and 64 (63%) were hospitalized. Twenty (22%) were positive for norovirus and 56 (55%) were positive for rotavirus. The overall sensitivity for rotavirus testing on rectal swabs was 90% [95% Confidence Interval (95% CI): 79% - 96%] with a specificity of 92% (95% CI: 81% - 98%). For norovirus overall sensitivity was 57% (95% CI: 33% - 79%) and specificity 91% (95% CI: 83% - 95%). Performance for norovirus was highest among children <1 year old with vomiting, with a sensitivity of 71% (95% CI: 36% - 92%) and specificity 92% (95% CI: 74% - 98%). Sensitivity and specificity did not vary significantly among hospitalized or ambulatory patients. In conclusion, sensitivity and specificity for rotavirus testing from rectal swabs were high, but sensitivity for norovirus was lower. Testing of specimens from rectal swabs is a viable alternative to whole stool for detection of rotavirus, particularly during outbreaks where collection of whole stool may be difficult. Norovirus testing from rectal swabs could also be used to confirm the etiology of the outbreak.

1164

ESTABLISHING THE CAPACITY FOR INFLUENZA SENTINEL SURVEILLANCE IN LIMITED-RESOURCE SETTINGS: A PROGRESS REPORT FROM WEST AFRICA

Ndahwouh Talla Nzussouo[1], Serge Manoncourt[2], Deidra Shuck-Lee[3], Claire Cornelius[3], Karl Kronmann[4], William Ampofo[5], H. Kadjo-Adje[6], Ousmane M. Diop[7], Oyofo Buhari[3], Chris Duggar[8], Richard Davis[8], Michael Deming[8]

[1]Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia/Global Disease Detection and Response Program/United States Naval Medical Research Unit-3/Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Global Disease Detection and Response Program/United States Naval Medical Research Unit 3, Cairo, Egypt, [3]Viral and Zoonotic Diseases Research Program/United States Naval Medical Research Unit 3, Cairo, Egypt, [4]Viral and Zoonotic Diseases Research Program/United States Naval Medical Research Unit 3 and Noguchi Memorial Institute for Medical Research, Accra, Ghana, [5]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [6]Institut Pasteur, Abidjan, Côte D'Ivoire, [7]Institut Pasteur de Dakar, Dakar, Senegal, [8]Influenza Division, Centers for Disease Control and Prevention, Atlanta, GA, United States

The detection of highly-pathogenic avian influenza in poultry in West Africa in 2006 led to recognition of the urgent need for widespread sentinel surveillance for influenza. Only Senegal and Côte d'Ivoire were conducting influenza surveillance at this time. Since then, training and laboratory equipment have been provided by Centers for Disease Control and Prevention, NAMRU-3, the US Navy Global Emerging Infections System, the US Department of State, and the Institut Pasteur with the goal of building influenza surveillance capacity based on real-time PCR testing in several other West African countries, both among outpatients with influenza-like-illness (ILI) and among inpatients with severe acute respiratory infection (SARI). Initial assessment visits were conducted in several countries in 2008 and 2009. To summarize current surveillance capability, these assessments and subsequent trip reports were reviewed, and ministries of health were contacted. Influenza surveillance has been conducted in Senegal since 1996, in Côte d'Ivoire since 2003 and in Ghana and Nigeria since 2007. In 2010, the numbers of ILI and SARI sites in these countries were 14/4, 9/8, 15/3, and 4/4, respectively, and they tested a total of 1550, 1382, 3848 and 1408 specimens, respectively, from April 1, 2009 to March 31, 2010. The initial assessment visits were conducted in Benin, Burkina Faso, Liberia, Mali, Mauritania, Niger, Sierra Leone and Togo. Five of these countries were judged to be ready to develop influenza surveillance capacity. They have chosen to begin surveillance with the following number of ILI and SARI sites: Niger 12/12; Mali 5/1; Mauritania 1/1; Burkina Faso 1/1; Togo 1/1. Niger and Mali have recently begun influenza surveillance. Mauritania, Burkina Faso and Togo are expected to begin in the next 2-3 months. In conclusion, significant progress has been made recently in influenza surveillance in West Africa. The capacity of countries has improved and the number of countries with functional surveillance systems is increasing.

1165

PLANNING FOR RIFT VALLEY FEVER VIRUS: USE OF GIS TO ESTIMATE THE HUMAN HEALTH THREAT OF WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*)-RELATED TRANSMISSION

Sravan Kakani[1], A. Desiree LaBeaud[2], **Charles H. King**[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Childrens Hospital Oakland Research Institute, Oakland, CA, United States

Rift Valley fever virus (RVFV) is a mosquito-borne phlebovirus of the *Bunyaviridae* family that causes frequent outbreaks of severe animal and human disease in sub-Saharan Africa, Egypt, and the Arabian Peninsula. Based on its many known competent vectors, its potential for transmission via aerosolization, and its progressive spread from East Africa to neighboring regions, RVFV is considered a high-priority, emerging

health threat for humans, livestock, and wildlife in all parts of the world. Introduction of West Nile virus to North America has shown the potential for 'exotic' viral pathogens to become embedded in local ecosystems. While RVFV is known to infect and amplify within domestic livestock such as taurine cattle, sheep, and goats, if RVFV is accidentally or intentionally introduced into North America, an important unknown factor will be the role of local wildlife in the maintenance or propagation of virus transmission. We examined the potential impact of RVFV transmission via white-tailed deer (*Odocoileus virginianus*) in a typical Ohio (northeastern United States) urban-suburban landscape, where livestock are rare, but these potentially susceptible ungulate wildlife are highly abundant. GIS modeling results, based on overlap of mosquito, human, and projected deer densities, indicate that a significant proportion (192 / 458 mi2, or 42 %) of the Cuyahoga County urban and peri-urban landscape could be affected by RVFV transmission during the late summer months. Deer population losses, either by intervention for herd reduction or by RVFV-related mortality, would substantially reduce these likely transmission zones to 20.5 mi2, or by 89%. Integrated strategies for vector and animal control, combined with human population preventive measures, will be essential to prevent establishment of novel arboviral pathogens in this typical North American landscape.

1166

INVESTIGATION OF ARBOVIRAL INFECTIONS OF BIRDS IN SÃO PAULO STATE, BRAZIL

Akemi Suzuki[1], Iray M. Rocco[1], Ivani B. Ferreira[1], Luiz E. Pereira[1], Matheus P. Cerroni[1], Patrícia P. Freitas[2], Renato P. Souza[1], Terezinha Lisieux M. Coimbra[1], Selma M. Petrella[1], **Benedito A. Fonseca**[2]

[1]Adolfo Lutz Institute, São Paulo, S.P., Brazil, [2]School of Medicine of Ribeirão Preto, Ribeirão Preto, S.P., Brazil

Arthropod-borne viruses cause diseases of great importance to public health and, for this reason, it is necessary a constant surveillance to detect circulating viruses in a specific area. Among them, West Nile virus (WNV) is an arthropod-borne virus member of the genus *Flavivirus*, family Flaviviridae, which is endemic in Africa and Middle East, but has spread rapidly across Europe and the Americas, causing severe neuroinvasive disease outbreaks in humans and animals. To date, there are no reported cases of WNV isolation in Brazil but a surveillance system has been established by the Ministry of Health, searching for evidence of WNV infections in wild birds captured in different landing sites along the Brazilian territory. Thus, due to the importance of this emerging disease and the lack of a recent epidemiological study of arbovirus circulation, other than dengue and yellow fever, in the state of São Paulo, this study investigated the evidence for West Nile and other arbovirus infections in wild birds captured in the state. Birds were captured in three well established landing sites throughout the state of São Paulo: Central region (21°41'06"S/48°05'03"W), Northwest (20°52'20"S/51°29'15"W) and Southeast (24°42'29"S/47°3319"W). A total of 898 birds were captured during 2005 and 2006; blood samples were collected from all of them and cloacae swabs were collected from 307 wild birds. Most of the analyzed birds belonged to the order Passeriformes (85.3%; 766/898) considered an important viral reservoir. The attempt for virus isolation from swab samples was performed on Vero E-6 and C6/36 and by i.c. inoculation in suckling mice. Culture supernatants and swab samples were also analyzed by RT-PCR for viral RNA detection. Blood samples were tested for antibodies by Hemagglutination Inhibition (HI) assay. In none of the analyzed samples either WNV or other arbovirus was isolated as well the RT-PCR results were also negative in the tested samples. HI results indicated the presence of antibodies against Iguape virus (IGPV), Saint Louis virus (SLEV), and Ilhéus virus (ILHV) with predominance of monotypic reactions. In two samples, heterotypic reaction for WNV was detected; however this result is insufficient to confirm the introduction of the virus in Brazil. Taken together, the results of this study prove that, at least up to 2006, WNV had not been introduced into São Paulo state, and probably into Brazil.

1167

CO-CIRCULATION OF DIFFERENT DOBRAVA HANTAVIRUS LINEAGES IN A*PODEMUS* MICE IN SLOVENIA

Misa Korva[1], Nataša Knap[1], Tomi Trilar[2], Tatjana Avšič-Županc[1]

[1]Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, [2]Slovenian Museum of Natural History, Ljubljana, Slovenia

Hantaviruses are associated with their natural reservoir hosts, either rodents or insectivores. The chronically infected animals excrete the virus in urine, feces and saliva, without developing a disease. Hantaviruses are usually closely associated with one rodent or insectivore species as a result of a co-evolution of the virus and the host. However, deep phylogenetic analyses have shown inconsistence in the pattern of host and virus co-evolution: besides to host switching events, numerous hantaviruses have been reported to be isolated from different sympatric hosts. Such spillover infections are quite common and promoted with complex bio-geographic and anthropogenic pressures on the environment, but their impact on the public health is still undetermined. A severe form of hemorrhagic fever with renal syndrome in Europe is caused by Dobrava hantavirus, carried by *Apodemus flavicollis*. In addition to *A. flavicollis* several other *Apodemus* mice have been shown to carry DOBV-like hantaviruses. In light of monitoring the hantavirus spread in Slovenia in natural environment, the rodent trapping is conducted on several locations twice a year. Using Sherman type live traps several rodent species were caught from 1990 to 2009 and three *Apodemus* mice species were selected: a yellow necked mouse (*A. flavicollis*), a striped field mouse (*A. agrarius*) and a long-tailed mouse (*A. sylvaticus*) for detailed inspection. Both, serologically and molecularly in all three species hantaviral infections were identified. Above that, all *Apodemus* species were positive in several years and on different locations, implying that this is not only a spillover infection as an effect of favouring environmental conditions. Molecular analyses proposes that different DOBV-like hantaviruses circulate in natural reservoir in Slovenia, but only the Dobrava virus prototype, isolated from yellow-necked mouse, is undoubtedly causing a disease in humans.

1168

MONKEYPOX PATHOGENESIS STUDY USING A SERIAL SACRIFICE TECHNIQUE

Christina L. Hutson, Darin S. Carroll, Nadia Gallardo-Romero, Sherif Zaki, Nelva Bryant, Christine Hughes, Victoria A. Olson, Jeanine Bartlett, Jason Abel, Erin McDowell, Sonja Weiss, Scott Smith, Mary S. Keckler, Kevin Karem, Inger K. Damon

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

Previously, we have shown the prairie dog is a valuable model system which closely mimics human systemic orthopoxvirus disease. To further characterize the strains of monkeypox virus (MPXV) and increase our understanding of MPXV progression within an animal host, we challenged groups of prairie dogs with two MPXV strains, one Congo Basin origin and one West African origin (each at 8 X 10^3 PFU), and evaluated disease progression in tissues on days 2, 4, 6, 9, 12, 17 and 24. Viral loads in 28 different tissues from animals were evaluated and compared. Animals challenged with Congo Basin strain had virus recovered on day 4 from nasal cavity, spleen, and submandibular lymph node tissues; earlier than West African challenged animals (day 6). For both MPXV strains, the majority of tissues were positive for virus between days 6-9. Tissues later infected by virus (day 12) included gallbladder, lesion and blood (both strains) and additionally for the West African strain on day 12; brain, heart, mesentery lymph nodes, pancreas, stomach and urine/bladder tissue. Two animals that succumbed to disease on day 12, when evaluated by IHC and histopathology, demonstrated abundant viral antigen in all organs with the exception of the brain. These findings allow for the better understanding of the pathogenesis of MPXV, including identification of sites important during early replication of the virus. This data could

prove useful in the development of therapeutic and biologic agents and in understanding the disease differences observed between the MPXV clades.

1169

SEROPREVALENCE OF ANTIBODIES AGAINST ARBOVIRUSES IN MBEYA REGION, SOUTHWESTERN TANZANIA

Gerhard Dobler[1], Nina Weller[2], Petra Clowes[3], Elmar Saathoff[2], Nyanda Elias[3], Michael Hölscher[2], Norbert Heinrich[2]

[1]Bundeswehr Institute of Micorbiology, Munich, Germany, [2]Department of Infectious Diseases and Tropical Medicine, University of Munich, Germany, [3]Mbeya Medical Research Project, Mbeya, United Republic of Tanzania

Arboviruses cause some of the most important human infections. Although several arbovirus epidemics have originated in East Africa over the last years, few recent data are available on the endemic prevalence of arboviruses in this area, and no data is available from South-West Tanzania. In the current study 1.233 sera were tested that had been collected from participants of the EMINI study in the Mbeya region in South-West Tanzania. Nine sampling sites were selected to cover altitudes from 500 m to 2.300 m above sea level (asl) and different ecologic conditions. Sera were tested for antibodies against Rift Valley Fever virus (RVFV), yellow fever virus (YFV), West Nile virus (WNV), Chikungunya virus (CHIKV), Dengue -1 to -4 viruses (DENV-1 to DENV-4), using an immuno-fluorescence based biochip designed for this study (Fa. Euroimmun, Lübeck, Germany). In two of the nine sites, namely Igurusi (1.100 m asl; east of Mbeya city) and Kyela (500 m asl; on the shores of Lake Malawi), high antibody prevalences against all tested arboviruses were detected. In Kyela anti-CHIKV prevalence was 49%, anti-RVFV was 24%, and anti-flavivirus prevalences ranged from 16 to 27% with higher titers against WNV and DENV-3. The other sites, which were located at mean elevations between 1.300 and 2.300 m asl, showed IgG seroprevalence rates between 0 to 13%. Additional testing of IgM in Kyela site showed preliminary IgM antibody rates of up to 10% against the above arboviruses. Our data demonstrate a non-epidemic circulation of several arboviruses, including CHIKV, WNV, DENV-3 and RVFV, in Mbeya region in South-West Tanzania, none of which had previously been diagnosed in the region. As antibodies against the tested arboviruses were mainly found in areas below 1.100 m, we assume that these arboviruses circulate in regions below 1.100 m in an endemic pattern. The arboviruses circulating should be characterized in detail as they may pose a virus reservoir for future epidemics and pandemics.

1170

SEROLOGIC EVIDENCE FOR MULTIPLE GENERA OF POXVIRUSES CIRCULATING IN THE PERIDOMESTIC RODENT POPULATION IN WESTERN UGANDA

Johanna S. Salzer[1], Thomas R. Gillespie[2], Darin S. Carroll[1], Innocent Rwego[3], Joanna L. Shisler[4], Kevin L. Karem[1], Inger K. Damon[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Emory University, Atlanta, GA, United States, [3]Makerere University, Kampala, Uganda, [4]University of Illinois Urbana-Champaign, Urbana, IL, United States

Rodents are implicated as the likely reservoir host for many zoonotic poxviruses. Little is known concerning the prevalence of poxvirus infections among specific rodent species in the wild. Our current study investigated poxvirus exposure among peridomestic rodents in western Uganda. We identified antibodies to two separate genera of the family *Poxviridae*. Western blot and ELISA studies of rodent serum identified a reactive antibody response to viral proteins found in the genera *Orthopoxvirus* and *Yatapoxvirus*. This implies rodents in western Uganda are exposed to at least two poxviruses. The previous poxvirus exposures in these rodents may be due to known zoonotic human pathogens (such as cowpox, monkeypox, or tanapox virus) and/or currently unknown, novel poxviruses. Endemic zoonotic poxviruses are not known to be circulating in the human population in western Uganda. The current study provides serologic evidence of poxvirus exposure among peridomestic rodents which could represent a concern for human health.

1171

IDENTIFICATION OF A NOVEL ANTIVIRAL DRUG AGAINST BLUETONGUE VIRUS

Qianjun Li, Lin-Lin Gu

University of Alabama at Birmingham, Birmingham, AL, United States

Bluetongue virus (BTV), the prototype virus in the genus *Orbivirus* within the *Reoviridae* family, is transmitted to its vertebrate host by certain species of *Culicoides* biting midges. BTV disease is one of the most important diseases of domestic livestock, causing $3 billion/year loss worldwide. While BTV vaccine is available and has been used to prevent BTV diseases, the recent outbreak of BTV in northern Europe indicates a pressing need for antivirals to fight against this disease. We present the identification and characterization of a novel antiviral against BTV. This novel antiviral small molecule compound belongs to one of the six cluster if antivirals against BTV, as reported previously, identified via a high throughput screening of a 200,000 compound library. This compound showed an IC50 at 0.69 ± 0.13 μM, with very low cytotoxicity (CC50 >50 μM), demonstrated that it is high selective against BTV with a Selective Index (SI50) over 50. This compound also reduced the BTV plaque formation by 2-3 logs in standard plaque assay. The Time-of-Addition assay showed that this compound inhibited the early event of the BTV viral life-cycle. Mechanism of action studies indicated that it might interact with the BTV viral replication machinery. The above results demonstrated that the novel antiviral against BTV could be identified and characterized for future drug discovery and development to prevent BTV diseases.

1172

ARBOVIRUS STRAIN PHENOTYPES CAN BE CHARACTERIZED VIA DYNAMIC ESTIMATES OF VECTORIAL CAPACITY AND THE DISPLACEMENT INDEX

Rebecca C. Christofferson, Christopher N. Mores

Louisiana State University, Baton Rouge, LA, United States

Vectorial capacity (VC) is defined as the number of infections that a specific mosquito population can distribute per case per day, and is used as a measure of the transmission potential of a vector borne pathogen into a susceptible population. Since its original description by McDonald-Ross, an important modification has been to include a transmission capability parameter, vector competence. Vector competence is the intrinsic ability of an arthropod to transmit an infectious agent following exposure to that agent. While traditional comparisons of vector competence are informative, the best method for comparing the transmission potentials of arboviruses uses the rate of change of vector competence over an interval of time meant to represent a range of extrinsic incubation periods. Using published data, we demonstrate the validity of this VC model when comparing intrinsic viral characteristics as related to fitness phenotypes both within a single mosquito species as well as among two species. Our results demonstrate, through calculation of the Displacement Index (DI), that the relative fitness of the West Nile Virus strain that was first introduced into the United States (NY99) was inferior to an emergent strain identified in 2002 (WN02). The DI shows, relative to an established strain of virus, the potential of another strain to overtake and displace that established strain in the system. For example, the DI of WN02 to NY99 is 2.14, indicating that WN02 has a significant fitness advantage for transmission than does NY99. The culmination of this displacement was seen as WN02 spread across the US. We also analyzed the inter and intra-specific relationship of a chikungunya virus (CHIKV) strain before and after a valine substitution that gave rise to the epidemic strain of the 2006 outbreak on La Reunion and Southern India. Comparisons of CHIKV phenotypes were made within and between *Aedes*

aegypti and *Ae. albopictus* mosquitoes. The cumulative VC and DI allow for measures of viral strain differences and relative fitness estimates within a dynamic transmission system.

1173

PRIME-BOOST MALARIA VACCINES IN RHESUS MONKEYS USING PROTEIN IN POLY I:C ADJUVANT AND ADENOVIRUS VECTORS

Andrea McCoy[1], Tupur Husain[1], Evelina Angov[2], David Narum[3], Joseph Bruder[4], Cristina Stoyanov[3], Lauren Trager[3], Barbara Flynn[3], Kavita Tewari[3], Elke Leitner[2], Jack Komisar[2], Christian Ockenhouse[2], Thomas Richie[1], Walter Weiss[1], Robert Seder[3]

[1]Naval Medical Research Center, Silver Spring, MD, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]National Institutes of Health, Bethesda, MD, United States, [4]GenVec, Inc., Gaithersburg, MD, United States

Development of an effective vaccine against malaria is an urgent priority in global public health. Vaccines directed at the liver stage of malaria infection work by inducing both antibody and cellular immune responses. Recombinant protein-based vaccines are strong inducers of antibody responses, while recombinant attenuated adenovirus (Ad) vaccines are strong inducers of T cell immune responses. Both have protected mice against malaria alone and after priming animals with other vaccine constructs. In this study, we evaluated the immunogenicity and protective efficacy of recombinant protein and Ad vaccines using the *P. knowlesi* (Pk)-*M. mulatta* model. The Pk circumsporozoite protein (CSP) was used in both the protein and adenovirus constructs. All protein vaccinations included Poly I:C (PIC) adjuvant. The following vaccine strategies were compared: protein prime/Ad5 boost; Ad5 prime/protein boost; Ad28 prime/Ad5 boost; and protein prime/protein boost. Control groups received Ad not encoding malaria antigen and PIC adjuvant without protein. Four weeks after the final vaccination, all monkeys were challenged with Pk sporozoites by mosquito bite and followed for 30 days for the development of parasitemia by thin and thick blood smears. Blood from all monkeys was collected throughout the study and antibody and T cell responses to the CSP were analyzed. We will present the immunogenicity and parasitemia data from the study, along with correlations between immune responses and protective efficacy.

1174

EVALUATION OF IMMUNE RESPONSES TO A *PLASMODIUM VIVAX* CSP-BASED RECOMBINANT PROTEIN VACCINE CANDIDATE IN COMBINATION WITH SECOND-GENERATION ADJUVANTS IN MICE

Joanne M. Lumsden[1], Saule Nurmukhambetova[1], Jennifer H. Klein[1], Sylvie Bertholet[2], Christopher B. Fox[2], Randall F. Howard[2], Steven G. Reed[2], Mark E. Polhemus[1], Christian F. Ockenhouse[1], Anjali Yadava[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]IDRI, Seattle, WA, United States

Plasmodium vivax is the major cause of malaria outside of sub-Saharan Africa and inflicts debilitating morbidity and consequent economic impact in developing countries. In order to produce a *P. vivax* vaccine for global use, we have previously reported the development of VMP001, based on the circumsporozoite protein (CSP) of *P. vivax*. VMP001 is a novel recombinant protein encompassing the N-terminal and C-terminal regions flanking a chimeric repeat region representing VK210 and VK247, the two major alleles of *P. vivax* CSP. Our interest is to evaluate second-generation vaccine formulations to identify novel combinations of adjuvants capable of inducing strong, long-lasting immune responses. In this study groups of C57BL/6 mice were immunized subcutaneously three times with VMP001 in combination with synthetic TLR4 (GLA) or TLR7 agonist (R848) in stable emulsion (SE), or SE alone. Sera and splenocytes were tested for the presence of antigen-specific humoral and cellular responses, respectively. All groups of mice generated high titers of anti-*P. vivax* IgG antibodies as detected by ELISA and immunofluorescence assay. GLA-SE promoted a shift in the antibody response to a Th1 profile, as demonstrated by the IgG2c/IgG1 ratio. In addition, GLA-SE induced a strong cellular immune response characterized by multi-functional, antigen-specific CD4+ T cells secreting IL-2, TNF and IFN-γ. In contrast, mice immunized with SE or R848-SE produced low numbers of antigen-specific CD4+ T cells and these T cells secreted both IL-2 and TNF, but not IFN-γ. Finally, R848-SE did not enhance the immune response to GLA-SE alone. We conclude that the combination of VMP001 and GLA-SE is highly immunogenic in mice and may serve as a potential candidate second-generation vaccine against vivax malaria.

1175

POLYPEPTIDE NANOPARTICLES GENERATE CD8⁺ T-CELL RESPONSES IN THE ABSENCE OF ADJUVANT

Clara Brando[1], Margaret E. McCoy[1], Stephen A. Kaba[1], Qin Guo[1], Debleena Dasgupta[1], Hannah Golden[1], Christian Mittelholzer[2], Tais A. Pimentel[3], Yongkun Yang[4], Peter Burkhard[5], David E. Lanar[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]M.E. Muller Institute, Biozentrum, University of Basel, Basel, Switzerland, [3]Institute of Material Sciences, University of Connecticut, Storrs, CT, United States, [4]Institute of Material Sciences, University of Connecticut, Storrs, CT, United States, [5]Institute of Material Sciences and Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT, United States

Protection against malaria is considered to rely mostly on antibodies to block or destroy sporozoite and blood stage parasites and primarily on CD8+ T-cells for destruction of the liver stage of infection. A single highly effective malaria vaccine has not yet been formulated that induces both antibody and cellular immune responses. In order to generate CD8+ T-cell responses exogenously derived proteins need to be cross-presented, as the normal processing of exogenous protein will generate mainly CD4+ T-cell responses. It is a common belief that cross-presentation of antigen usually requires either the use of viral vectors or strong adjuvants. We recently described a platform of a Self-Assembling Polypeptide Nanoparticle (SAPN) capable of generating protective antibodies against the CS repeat of the CSP from *P. berghei*. We now report that the same formulation is able to deliver the *P. berghei CSP* CD8+ T-cell epitope SYIPSAEKI to induce an effective cellular immune response. Mice were immunized with 10μg SAPN (Pb2) bearing the peptide SYIPSAEKI 3 times, 2 weeks apart. Two weeks post last immunization, animals were sacrificed and splenocytes harvested from spleens to analyze for Ag-specific CD8+ T-cell activation *in vitro* by ELISpot and ICS analysis. Splenocytes from immunized animals responded positively to *in vitro* stimulation with 10μg/ml of SYIPSAEKI by secreting IFNγ (0.05 % in immunized animals versus 0.005 % in the control). Exposure to SYIPSAEKI also induced significant production of IL-6, Rantes and MIP. These results indicate that SAPN can deliver a CD8+ T-cell epitope to induce antigen specific cellular immune responses. This opens the door to the design of a single nanoparticle that can be delivered without adjuvant to induce a protective antibody response that can be coupled with an effective cellular response. We believe that we can now design and delivery a truly effective single component malaria vaccine.

1176

QUANTIFYING INVASION INHIBITORY ANTIBODIES TO AMA1 IN HUMAN POPULATIONS USING TRANSGENIC *PLASMODIUM FALCIPARUM*: IMPLICATIONS FOR VACCINE DESIGN

Damien R. Drew[1], Danny W. Wilson[1], Nadia J. Cross[1], Salenna R. Elliot[1], Ivo Muller[2], Livingstone Tavul[2], Danielle I. Stanisic[1], Nicolas Senn[2], Jack Tairaka[2], Peter M. Siba[2], Julie Healer[1], Alan F. Cowman[1], Anthony Hodder[1], James G. Beeson[1]

[1]The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [2]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea

Antibodies to merozoite antigens are an important component of acquired immunity to *P. falciparum* and act in part by inhibiting erythrocyte invasion. However, the major targets of protective and invasion-inhibitory antibodies remain unclear. Apical membrane antigen 1 (AMA1) is an essential erythrocyte invasion ligand and leading vaccine candidate. Antibodies raised against AMA1 inhibit the growth of blood stage parasites, but are constrained by strain-specificity. A successful AMA1 vaccine may require the inclusion of multiple AMA1 alleles to overcome this limitation. To quantify the importance of AMA1 as a target of acquired inhibitory antibodies in humans and understand the acquisition of antibodies to different AMA1 alleles, we have successfully generated transgenic *P. falciparum* lines expressing different AMA1 alleles on the same genetic background. Using these parasites in invasion inhibition assays enables the measurement of AMA1-specific inhibitory antibodies distinct from other antigen-specific antibodies. Testing antibodies among a cohort of children and adults in Papua New Guinea revealed that AMA1 is a major target of acquired invasion inhibitory antibodies. Acquired inhibitory antibodies showed substantial strain-specificity, and the prevalence of inhibitory antibodies differed significantly for different AMA1 alleles, suggesting different rates of acquisition of antibodies to different alleles. These results have important implications for vaccine development and understanding the targets of protective and inhibitory antibodies in humans. Transgenic parasites may be valuable for measuring antibody specificity and functional activity in AMA1 vaccine trials.

1177

QUANTIFYING THE IMPORTANCE OF PFEMP1 AND OTHER ANTIGENS EXPRESSED ON THE SURFACE OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES AS TARGETS OF PROTECTIVE ANTIBODIES AGAINST MALARIA

Katherine Howell[1], Jo-Anne Chan[1], Linda Reiling[1], Ricardo Ataide[2], Fiona McCallum[1], Peter C. Bull[3], Alexander G. Maier[4], Joanne Chesson[1], George Warimwe[3], Stephen J. Rogerson[2], Alan F. Cowman[1], Kevin Marsh[3], **James G. Beeson**[1]

[1]Walter and Eliza Hall Institute of Medical Research, Parkville, Australia, [2]Department of Medicine, University of Melbourne, Parkville, Australia, [3]Kenya Medical Research Institute, Kilifi, Kenya, [4]LaTrobe University, Bundoora, Australia

Plasmodium falciparum-infected erythrocytes (IEs) express variant surface antigens (VSAs) that are major targets of immune responses. Antibodies to VSAs develop in a largely variant-specific manner and are associated with protection from symptomatic and severe malaria. Several confirmed or proposed VSAs have been identified, including PfEMP1, RIFIN, STEVOR, and SURFIN protein families, and others. However, the relative importance of these different proteins as targets of acquired antibodies remains unclear. To address this, we have used parasite lines in which surface expression of PfEMP1 and other antigens was inhibited by transfection of parasites with specific constructs that either i) suppress var gene expression or ii) interfere with trafficking of antigens to the IE surface. We developed novel assays to measure antibodies to PfEMP1 and other VSAs on the surface of IEs by comparing antibody reactivity to transfected versus parental parasites. Using this approach with samples from Kenyan children and adults we have quantified the importance of PfEMP1 and other VSAs as targets of acquired antibodies and we have related these antigen-specific responses to protective immunity in cohort studies. Furthermore, to understand the functional relevance of antibodies to different VSAs, we have measured antibody opsonic phagocytosis activity using parasites with modified VSA expression compared to parental parasites. These studies have enabled us to quantify the importance of different VSAs as targets of acquired antibodies, and their likely role in protective immunity to malaria. These findings have significant implications for understanding human immunity to malaria and informing vaccine development.

1178

DIFFERENCES IN HUMORAL IMMUNITY AGAINST *PLASMODIUM FALCIPARUM* MALARIA IN MALIAN CHILDREN CARRYING NORMAL HEMOGLOBIN A OR SICKLE HEMOGLOBIN S

Kazutoyo Miura[1], Mahamadou Diakite[2], Samuel E. Moretz[1], Hong Zhou[1], Ababacar Diouf[1], Gregory Tullo[1], Tatiana M. Lopera-Mesa[1], Jennifer M. Anderson[1], Rick M. Fairhurst[1], Carole A. Long[1]

[1]Laboratory of Malaria and Vector Research/National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States, [2]Faculty of Medicine, Pharmacy and Odontostomatology, University of Bamako, Bamako, Mali

Previous studies have shown that children with sickle-cell trait (HbS heterozygosity) have reduced incidence of *Plasmodium falciparum* malaria compared to normal (HbA homozygosity) children. While we still do not understand the mechanisms responsible for this protection, enhanced immunity by sickle-cell trait has been proposed to play a role. To develop a more comprehensive picture of anti-malarial immunity in these African children, we initiated a 5-year longitudinal cohort study of 73 HbS and 126 HbA children in Mali. As part of this study, we followed these children through a 6-month malaria transmission season, collecting plasma from all children at the beginning (May 2009) and end (December 2009) of the transmission season to compare their immune responses to *P. falciparum* antigens. As expected, HbS children experienced significantly fewer malaria episodes than HbA children during this period. To compare the development of humoral immune responses between the two groups of children, we used a standardized ELISA to quantify antibody titers against 4 erythrocytic-stage antigens (AMA1, MSP1, EBA175, and MSP2). Among the children aged 6-11 years, HbS children showed significantly lower antibody titers to several of the antigens in May compared to HbA children. While these titers increased in both groups during the transmission season, similar differences in titers were found in December (HbS<HbA). We hypothesize that the lower antibody titers in the HbS children were due to fewer malaria episodes and consequently less exposure to parasite antigens. However, there was no correlation between the number of malaria episodes during the transmission season and the increase in ELISA titers in both HbS and HbA children. Therefore, other mechanisms are likely involved in modulating levels of *P. falciparum*-specific antibodies in HbS children. Additional immunological measures (e.g., functional activity of anti-malarial antibodies, etc) will be performed to compare the HbA and HbS populations.

1179

DECLINING BLOOD-STAGE IMMUNITY IN THE SETTING OF DECREASING MALARIA INCIDENCE IN UGANDA

Bryan Greenhouse[1], Lauren Gong[1], Bridget Nzarubara[2], Philip J. Rosenthal[1]

[1]University of California, San Francisco, San Francisco, CA, United States, [2]Makerere University - University of California San Francisco Collaboration, Kampala, Uganda

In endemic areas, protective immunity against *Plasmodium falciparum* usually increases with age and cumulative exposure. However, successful

malaria control efforts are leading to decreased intensity of parasite exposure in many areas. The consequences of decreased exposure on antimalarial immunity are unclear. We recently suggested that an increasing risk of treatment failure in response to amodiaquine + sulfadoxine-pyrimethamine in a closely managed cohort of children in Kampala, Uganda, was due to declining immunity and not increased drug resistance, prompting us to investigate other manifestations of blood stage immunity. 601 randomly selected children from Kampala, aged 1-10, were followed for a median of 1.4 years. Blood smears were read every 30 days and any time a child presented with fever. Children with malaria, defined as asexual parasites on blood smear and fever, were treated after randomization to one of 3 combination therapy regimens. To follow parasite strains within individuals over time, parasitemic samples were genotyped by assessment of polymorphisms in *merozoite surface protein 2* by nested PCR and capillary electrophoresis. We estimated associations between calendar time and two measures of immunity, the ability to avoid clinical illness despite parasitemia and the ability to spontaneously clear a parasite strain without receiving therapy, in both cases adjusting for age and accounting for repeated measures within individuals. During the study the incidence of malaria fell from 1.6 to 0.9 episodes per person year and 375 children (62%) had at least one positive blood smear. The probability of avoiding symptoms despite parasitemia (OR=0.38 per year, 95%CI=0.26-0.56, p<0.001) and the probability of spontaneously clearing an infection (OR=0.29 per year, 95%CI=0.13-0.64, p<0.01) decreased significantly over time. These data suggest that clinically relevant and readily measurable blood stage immunity declined over a short period of time in our cohort, possibly due to improved access to effective therapy and decreasing parasite transmission intensity.

1180

A GENOME WIDE TRANSCRIPTIONAL STUDY INVESTIGATING CANDIDATE GENES IMPORTANT FOR DESICCATION RESISTANCE IN *ANOPHELES GAMBIAE*

Mei-Hui Wang, Guiyun Yan

University of California Irvine, Irvine, CA, United States

Anopheles gambiae plays an important role in malaria transmission. In Africa, the dry season can last 2-3 months and malaria vector come back right after onset of the rainy season. How they survive through the dry season is still poorly understood. It is plausible that *An. gambiae* may have increased tolerance over the dry season while in the egg or larvae stages. Genetic analysis has shown that mosquitoes carrying inversion distribution on 2La and 2Rs (2Rb, 2Rc, 2Rd and 2Rd) are non-randomly correlated to aridity; therefore the genes involved within these inversions are worth to explore desiccation resistance. A genome-wide study of *An. gambiae* can identify transcript profiles of genes and pathways involved in desiccation resistance, shedding light on the possible survival strategies of malaria vectors during the dry season.

1181

INFLUENCE OF 2L*A* CHROMOSOMAL INVERSION ON DESICCATION RESISTANCE OF *ANOPHELES GAMBIAE S.S.* FROM CAMEROON

Caroline Fouet[1], Emilie Gray[2], Nora J. Besansky[2], Carlo Costantini[1]

[1]Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale, Yaoundé, Cameroon, [2]Eck Institute for Global Health, Department of Biological Sciences, Notre Dame, IN, United States

Many natural populations show geographic clines in traits including gene frequencies, morphological characters, and physiological or behavioral responses that can be interpreted as adaptive responses resulting from contrasting selective regimes. The frequency of the 2L*a* chromosomal inversion in *Anopheles gambiae sensu stricto* is associated across Africa with the degree of aridity, resulting in Cameroon in the clinal distribution of carriers of alternative karyotypes for this inversion along a latitudinal gradient: homokaryotypic standard arrangements prevail in the southern humid rainforest, whereas homokarytotypic inverted arrangements predominate in the northern dry savanna. Accordingly, this inversion is believed to capture genes that could be involved with adaptation to more arid conditions. The physiological bases underlying such adaptation, however, are as yet unknown. To study the relationship between the 2L*a* inversion and resistance to desiccation, we subjected the F10-F12 of an *Anopheles gambiae s.s.* molecular form S strain originating from a polymorphic population from Eastern Cameroon to a desiccation stress-test. Newly emerged male and female mosquitoes were put in sealed glass vials containing a desiccant and their survival was followed with an automated video-control system until death. Using a molecular diagnostics for this inversion, we were able to measure differences in survival of alternative 2L*a* karyotypes controlling for confounding covariables such as sex and body size during the test. Homokaryotypic inverted female mosquitoes survived, on average, significantly longer in a desiccated environment than females of the other karyotypes (+120 min), and than males, whatever their karyotype (+180 min).

1182

MOLECULAR EVOLUTION OF GENES INVOLVED IN POST-MATING REPRODUCTIVE MECHANISMS OF THE MALARIA MOSQUITO *ANOPHELES GAMBIAE*

Emiliano Mancini[1], Federica Tammaro[1], Francesco Baldini[2], David Rogers[2], Allegra Via[3], Domenico Raimondo[3], Phillip George[4], Igor Sharakhov[4], Anna Tramontano[3], Flaminia Catteruccia[2], Alessandra della Torre[1]

[1]Department Public Health Sciences, 'Sapienza' University, Rome, Italy, [2]Imperial College, London, United Kingdom, [3]Department of Biochemical Sciences, 'Sapienza' University, Rome, Italy, [4]Department of Entomology, Virginia Tech, Blacksburg, VA, United States

Proteins involved in reproduction evolve rapidly due to positive selection resulting from intersexual interaction and sexual conflict. In *Drosophila*, rapid evolution driven by positive selection has been detected in proteins expressed either in the male accessory glands (MAGs) and in the female lower reproductive tract (LRT). In *Anopheles gambiae* MAG-products are transferred to females as a solid mating plug that induces a series of physiological post-mating responses in females. We here report data on the molecular evolution in five members of the *A. gambiae* complex (*A. gambiae* s.s., *A. arabiensis, A. quadriannulatus, A. melas and A. merus*) of two clusters of 3 LRT- and 3 MAG-specific genes potentially involved in post-mating mechanisms. The 3 LRT-specific genes encode serine-proteases that are down-regulated after mating, two of which are expressed in the atrium (and interact with the mating plug) and one in the spermatheca. Adaptive evolution was detected in several codons of the 3 genes; moreover, episodic selection was inferred in the spermatheca-specific gene along the branch leading to *A. melas*. The particularly high level of replacement polymorphisms in all 3 proteases suggests that, as in *Drosophila*, these duplicated genes might experience relaxed evolutionary constraints that could be important to rapidly explore and eventually fix new advantageous variants. Among the 3 MAG-specific paralog genes, two are conserved, whereas one is highly differentiated among *A. melas*, *A. merus* and *A. quadriannulatus*, due to positive selective pressure and purifying selection maintaining lineage-specific products.Overall, the evolution of these genes appears to be consistent with a model of sexual conflict, in line with their crucial role in *A. gambiae* reproduction. The association of evolutionary and functional analyses might help clarifying their role in post-mating responses and, possibly, in maintaining reproductive isolation among *A. gambiae* species, thus hopefully providing new targets for the development of novel malaria vector control strategies.

1183

IDENTIFYING THE *ANOPHELES GAMBIAE* PROTEIN KINASE C GENE FAMILY AND EXPLORING ITS ROLE IN MOSQUITO INNATE IMMUNITY

Hannah M. Smithers, Win Surachetpong, Shirley Luckhart

University of California at Davis, Davis, CA, United States

With nearly 40 percent of the world's population at risk for malaria infection, it is necessary to explore novel strategies of malaria control. One approach to decrease disease transmission is to target and alter immune genes in the mosquito to reduce the parasite burden and disable vector competence.

The highly conserved protein kinase C (PKC) gene family has been shown to regulate wide-ranging immune functions in a variety of vertebrate and invertebrate species. Based on these observations, we sought to identify and characterize all of the PKC isoforms encoded within the genome of the African malaria mosquito Anopheles gambiae as a prelude to functional studies. A total of 13 PKC isoforms are known from mammals and 6 are known from Drosophila melanogaster. However, prior to our studies, only two PKC-encoding genes had been identified in the genome of A. gambiae. Using Hidden Markov Model (HMM) searches of the translated reading frames of the unannotated A. gambiae genome sequence, we confirmed the identity of the two previously annotated PKC-encoding genes, identified an additional three PKC-encoding genes and a gene encoding a PKC-related kinase 2 (PKN2) ortholog. Subsequently, we identified conserved domains, putative translational start sites, and phosphorylation sites required for catalytic function of the predicted proteins using ClustalX and manual alignments of transcriptionally validated orthologous sequences. Expression data for of all but one of these PKC-encoding genes have been deposited in publically available databases. Phylogenetic analyses were performed using PAUP* 4.0 and PHYLIP, revealing close relationships between the newly identified A. gambiae PKCs with other dipteran PKCs within the same subfamily. It has been shown that insulin signaling can activate PKCs via phosphorylation or cellular translocation in vertebrates. In immortalized A. gambiae cells lines Sua5B and 4a3B, phosphorylated PKCs mu and zeta are upregulated in response to insulin treatment. Interestingly, phospho-PKC mu is translocated both to the cell membrane and to the nucleus in response to insulin treatment. With this new knowledge of the PKC gene family in A. gambiae, we can continue to characterize the functions of these proteins in host physiology.

1184

MOSQUITO IMMUNE CELLS FORM SESSILE FOCI IN RESPONSE TO INFECTION

Jonas G. King, Julián F. Hillyer

Vanderbilt University, Nashville, TN, United States

Mosquitoes counter the threat of microbial infection with a capable innate immune response that relies heavily on the action of immune cells (hemocytes) that are found circulating with the hemolymph or attached to tissues. Changes in sessile hemocyte populations following immune challenge have been reported in other insect taxa, but remain poorly understood and have not been studied in mosquitoes. We have developed an effective method of staining mosquito hemocytes *in vivo* along with a means of consistently perfusing approximately 95% of circulating hemocytes from an adult mosquito and have used these techniques to study the effect of immune challenge on the numbers of sessile and circulating hemocytes in *Anopheles gambiae*. Qualitative studies showed that sessile hemocytes occur throughout the mosquito's body but tend to concentrate in specific regions and increase in abundance following immune challenge. These studies also revealed the identity of previously reported phagocytic foci near the abdominal ostia as large aggregates of hemocytes and showed that formation of these foci can be induced by inoculation with bacteria or inert particles. Lysosomal staining confirms that the foci are engaged in degradation of the phagocytosed materials and in some cases the formation of foci is coupled with a melanization response. Quantitative analyses showed that the number of hemocytes in phagocytic foci increases in a dose dependent manner following immune challenge and that foci form on a consistent time scale. Analyses of the systemic immune response showed that total hemocyte numbers increase significantly following immune challenge and that there is a significant increase in the number of circulating hemocyte aggregates following immune challenge. Together, these data demonstrate a novel cellular immune response in mosquitoes.

1185

WEST NILE VIRUS-BINDING PROTEINS IN THE MIDGUT OF *CULEX PIPIENS QUINQUEFASCIATUS* SAY AND *C. NIGRIPALPUS* THEOBALD (DIPTERA: CULICIDAE)

Sheri L. Anderson, Stephanie L. Richards, Walter J. Tabachnick, Chelsea T. Smartt

University of Florida, Vero Beach, FL, United States

It is known that different species and populations of mosquitoes show differential vector competence, however, the molecular mechanisms that contribute to vector competence variation at the level of the mosquito midgut are largely unknown. Midgut virus-binding proteins may represent a mechanism that contributes to vector competence. We examined virus-binding midgut proteins in two important West Nile virus (WNV) vectors, *Culex pipiens quinquefasciatus* and *Cx. nigripalpus*. Polyacrylamide gel electrophoresis showed that there were at least 15 midgut proteins in *Cx. p. quinquefasciatus*, ten of which bound WNV after a virus overlay binding assay. The proteins that bound WNV ranged in size from 38 kDa to 198 kDa. Polyacrylamide gel electrophoresis of *Cx. nigripalpus* midgut proteins revealed that there were at least 21 midgut proteins, seven of which bound WNV after a virus overlay binding assay. The *Cx. nigripalpus* midgut proteins that bound WNV also ranged in size from 38 kDa to 198 kDa. These results are consistent with midgut virus-binding proteins from *Aedes aegypti* that bind dengue virus, including one, a 67 kDa protein, that has been related to vector competence and may serve as a genetic marker. We provide a protein expression profile from two different vectors of WNV. The involvement of the WNV-binding proteins in virus entry into mosquito midgut epithelial cells will be discussed.

1186

A REGULATORY UNIT OF THE MELANIZATION RESPONSE AFFECTS THE LIFE SPAN OF MOSQUITOES

Chunju An[1], Aidan Budd[2], Michael R. Kanost[1], **Kristin Michel**[1]

[1]Kansas State University, Manhattan, KS, United States, [2]EMBL, Heidelberg, Germany

Melanization is a powerful innate immune response in the arthropods that leads to encapsulation and killing of invading pathogens. This process renders some mosquito species partially or completely resistant to infection with pathogens of global public health significance. However, if not properly controlled, melanization reduces the life span of the mosquito itself. The rate-limiting step in the process of melanogenesis is the activation of prophenoloxidase (PPO), which is controlled by an extracellular protease cascade and associated serpin inhibitors with largely unknown molecular composition in mosquitoes. A notable exception is *Anopheles gambiae* serpin (SRPN)2 and its orthologs in other mosquito species, which were previously identified as key negative regulators of melanization. The aim of this study was to identify the molecular target of SRPN2 in *An. gambiae* and thus identify a regulatory unit of the PPO activation cascade in mosquitoes. Using a combination of reverse genetic and biochemical techniques we identified the *An. gambiae* clip-serine protease CLIPB9 as a PPO-activating protease (PAP). Double-knockdown of SRPN2 and CLIPB9 significantly reversed the pleiotrophic phenotype induced by silencing of SRPN2, including rescue of melanotic tumor formation and shortened life span. Recombinant activated CLIPB9 forms

SDS-stable complexes with SRPN2 *in vitro* and in mosquito hemolymph, which leads to the inactivation of the protease. The association rate constant of this complex and the stoichiometry of inhibition are comparable to known inhibitory serpin-protease interactions. Furthermore, recombinant CLIPB9 cleaved and activated purified insect PPO. This study identifies the first inhibitory serpin-serine protease pair in mosquitoes, thereby defining a regulatory unit of the biochemical cascade that is essential for melanization in the mosquito innate immune response. To the best of our knowledge, CLIPB9 is the first bona fide PAP to be described in a dipteran species, including *Drosophila melanogaster*.

1187

PAN PHYLUM ANALYSIS OF PROTEIN-PROTEIN INTERACTIONS REVEALS POTENTIAL DRUG TARGETS FOR HELMINTHES

Christina M. Taylor, Sahar Abubucker, Zhengyuan Wang, John Martin, **Makedonka Mitreva**

Washington University School of Medicine, St. Louis, MO, United States

Parasitic helminths have deleterious effects on human health, livestock, and plants, costing society billions of dollars annually. Finding new drug targets for parasitic infections would be of great utility for humanity, as there is a large need to develop new drugs to fight parasitic helminth infections due to the developing resistance and side effects of current treatments. This study underlines three major principles: i) proteins that are essential and conserved among species that span a phyla are of greatest value, as they provide foundations for developing broad control strategies, ii) Parasite proteins that share homology to the host counterpart are also of a great value when they posses molecular features that are unique to the parasite therefore are candidates for selective targeting, and iii) proteins rarely act in isolation, and the majority of biological processes occur via interactions with other proteins, so protein-protein interactions offer a realm of unexplored potential drug targets. Here we present a computational approach which which builds on these three principles, utilizing complete proteomes of the model free-living *Caenorhabditis elegans*, 6 parasitic helminthes and 2 of their hosts. Markov clustering of the proteins resulted in orthologous families that could be placed in species specific groups. Protein-protein interactions within these species specific groups were identified by comparisons to evidence based protein-protein interactions. Protein-protein interactions specific to nematodes were prioritized and scored based on RNAi phenotype and homology to the PDB. In addition, investigation of the parasite protein-protein interactions shared with the host resulted in amino acid insertions and deletions specific to the parasites. Developmental gene expression profiles, functional annotation (GO), and druggability were also considered. Several protein-protein interactions unique to nematodes or with nematode specific amino acid insertions and deletions emerged from this study and provide novel potential drug targets for controlling parasitic helminth infections.

1188

THREE NOVEL GUINEA WORM *(DRACUNCULUS MEDINENSIS)* GENOMIC SEQUENCES

Andrey A. Perelygin[1], Norman J. Pieniazek[2], Alexandre J. da Silva[2]

[1]Centers for Disease Control and Prevention and Atlanta Research and Education Foundation, Decatur, GA, United States, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States

Dracunculus medinensis is a parasitic nematode that causes dracunculiasis (Guinea worm disease). In 1986, the World Health Assembly resolved to eradicate dracunculiasis. Sequencing of the *D. medinensis* genome before complete eradication of dracunculiasis and the parasite is a high priority project. The only available genomic sequences of *D. medinensis* are for the 18S ribosomal RNA multicopy gene determined by Bimi et al., 2005 and Wijova et al., 2006. Additional data obtained for single-copy genes will be useful for verification of *D. medinensis* genomic sequencing results. We aligned the available genomic sequences for the nematode order Spirurida to identify conserved regions within the heat shock protein 70 (HSP70) gene and to use them to design PCR primers. Using these primers under low stringency conditions, we amplified and directly sequenced three PCR products of 517, 1952 and 2584 bp. The shortest and mid-size products were found to represent a portion of the utrophin exon with an adjacent intron and three exons flanked with introns of the hammerhead gene, respectively. Their sequences were submitted to GenBank under accession numbers HM131215 and HM131214. The longest product showed 70% similarity with the *Wuchereria bancrofti* HSP70 gene. Starting from that partial HSP70 sequence, we extended it to full length by DNA walking and submitted sequence data to GenBank (HM125969). The alignment of this full length sequence with several Spirurida HSP70 gene sequences identified 11 coding exons including exons 4A and 4B that were separated by an intron of 73 bp. A similar intron was not detected in orthologous HSP70 genes of other Spirurida studied to date; all of them had a single exon 4. The *D. medinensis* HSP70 amino acid sequence showed over 95% similarity to available Nematoda HSP70 protein sequences and phylogenetic analysis revealed significant divergence between *D. medinensis* and other Spirurida HSP70 gene sequences.

1189

UNRAVELING THE BIOLOGY OF AUTOINFECTION BY *STRONGYLOIDES STERCORALIS*: A MICROARRAY BASED ANALYSIS

Roshan Ramanathan[1], David Abraham[2], Thomas J. Nolan[3], James B. Lok[3], Sudhir Varma[1], Thomas B. Nutman[1]

[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

An unusual feature of the life cycle of Strongyloides stercoralis (Ss) is its ability to persist for years in infected hosts, by the process termed autoinfection. During autoinfection, larvae develop precociously within the host of origin to the third stage (L3a), penetrate the colonic wall/perianal skin and migrate via a variety of routes including the lungs. The molecular differences between autoinfective larvae and infective larvae which develop outside the host (L3i) have been uncharacterized to date. We conducted a microarray analysis to compare gene expression of L3a and L3i larvae recovered from experimentally infected animals. Differentially labeled cDNA obtained from RNA extracted from larvae were hybridized to a Ss microarray. Genes that were more highly expressed in either stage (based on a conservative cutoff of 2 fold increased gene expression and microarray signals with $p < 0.01$; false discovery rate of 1%) were examined for differences in gene function based on a novel cDNA annotation system. In a preliminary analysis, 600 of 3571 genes on the array were identified as being differentially expressed. Striking differences in gene expression were found between the two stages with higher numbers of L3a genes involved in transcription (p=0.03), molecular chaperones (p=0.03), signal transduction (p=0.01), vesicular transport (p=0.03) and metabolism (p=0.0002). L3a upregulation of the ubiquitin proteasome system may be critical to Ss larval development and differentiation in the host. In addition, a potential therapeutic target, a highly expressed and abundant L3a nucleoside hydrolase (Ss-contig 2570), was identified. Upregulation of L3i cuticular collagens (p=0.004) likely enable survival in harsh environmental conditions. Increased numbers of L3i ferritin transporters implicates a role for iron metabolism in the response to environmental stress. These data provide valuable insights into how Ss larvae adapt to stress induced by the environment and host immune system that can then be applied to the development of novel therapeutic and vaccine targets.

1190

QUANTITATIVE PCR-BASED ASSESSMENT OF *ANGIOSTRONGYLUS CANTONENSIS* IN *RATTUS RATTUS* FROM HAWAII

Rebecca Bandea[1], Wesley Warashina[2], Arlene Buchholz[2], Sarah Y. Park[2], Yvonne Qvarnstrom[1], Alexandre J. da Silva[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Hawaii Department of Health, Honolulu, HI, United States

Angiostrongylus cantonensis is the most common parasite causing human eosinophilic meningitis worldwide. This nematode's infectious larvae develop in mollusks, but can also be present in paratenic hosts. The major route of infection is thought to be accidental consumption of infected mollusks in fresh produce or other foods. A survey performed in 2007 on mollusks collected in Hawaii main island showed an infective rate ranging from 24 to 78% depending on the mollusk species analyzed. The severe cases of eosinophilic meningitis recently reported could be due to overexposure of humans to highly infected mollusks; there are no measures in place to control the spread of *A. cantonensis* in Hawaii. The aim of this study was to determine the prevalence of *A. cantonensis* in rats, the most efficient definitive hosts for this parasite. A total of 62 rats were trapped in 4 Hawaiian communities. The presence of *A. cantonensis* was initially assessed by morphologic identification of adult worms in lungs and hearts of the animals. DNA was then extracted from 28 randomly selected lung and heart tissue samples obtained from these animals. The DNA extracts were evaluated using a quantitative TaqMan PCR validated for diagnostic detection of *A. cantonensis*. A total of 60 % (n=37) of the rats were positive by morphology, while 100% of the tissue samples examined with the PCR were positive. A quantitative analysis of the PCR results indicated that 32 % (n=9) of the rat samples contained *A. cantonensis* DNA corresponding to more than 100 larvae in approximately 25 mg of tissue. Together with previous studies, this data indicates that angiostrongyliasis may be a more serious public health issue in Hawaii than currently estimated and that measures to control its spread in mollusks and rodents may be warranted.

1191

INCREASED BASOPHIL RESPONSIVENESS AFTER ANTHELMINTIC TREATMENT IN A COASTAL ECUADORAN POPULATION

David Larson[1], Marc P. Hübner[1], Philip J. Cooper[2], Edward Mitre[1]

[1]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]Instituto de Microbiologia, Universidad San Francisco de Quito, Quito, Ecuador

As basophils function as allergy effector cells and may be involved in initiating and perpetuating allergic diseases, it is possible that much of the allergy-protective effect conferred by helminths is through their effect on basophils. Recently, we demonstrated that basophil responsiveness decreases in murine models of chronic helminth infection. In this study, we tested the hypothesis that basophil responsiveness is suppressed during helminth infection of humans. Twenty-three children infected with intestinal helminths were identified by parasitological examination of stool samples in Esmeraldas, Ecuador. Peripheral blood cells from these children were washed twice and stimulated with increasing concentrations of anti-IgE and 5 µg/ml ionomycin. Basophil activation was determined by measuring histamine release into the supernatant using a competitive ELISA. Children were then treated with ivermectin and 2 doses of albendazole and histamine release from blood basophils measured again two weeks later. A two week timepoint was chosen as basophil half-life is estimated to be between 5-8 days. Significantly more histamine was released from basophils after anthelmintic treatment when blood was stimulated with 0.5, 0.125, and 0.031 µg/ml anti-IgE (mean % of total histamine release from infected children before treatment = 40.38, 43.12, 31.49 vs. 55.17, 60.53, 44.89 from children post-treatment, $p < 0.01$, $p < 0.001$, $p < 0.05$). A similar increase in histamine release was seen when stimulating with ionomycin (mean % of total histamine release from infected children before treatment = 49.30 vs. 76.71 from children post-treatment, $p < 0.0008$). These results suggest that the ability of basophils to respond to IgE-dependent and IgE-independent activation is suppressed during intestinal helminth infection of humans. This suppression appears to require the continuous presence of helminths as basophil histamine releasability increases shortly after helminths are eliminated. These findings may explain why helminth infections protect against allergic disease.

1192

SPATIAL PATTERNING OF HEALTH DISPARITIES AND ENVIRONMENTAL FACTORS ASSOCIATED WITH *ASCARIS LUMBRICOIDES* PREVALENCE IN BOLIVIA

Julie A. Clennon, Alexandra D. Huttinger, **Emily R. Smith**, Christine L. Moe

Emory University, Rollins School of Public Health, Atlanta, GA, United States

Infection with *Ascaris lumbricoides*, a soil-transmitted helminth (STH), affects as many as one billion people worldwide, and causes an estimated 10.5 million disability-adjusted life-years (DALYs) lost. STH prevalence data for Bolivia is sparse. A 2009 literature review found only 7 studies in the last 25 years that offer prevalence estimates for any region of Bolivia. A review conducted in 2006, that reported data from 1976-2003, showed that *A. lumbricoides* infection prevalence varies geographically across Bolivia, from 1-28% in the Western high plains to 15-96% in the Eastern tropical lowlands. Although data on STH prevalence in Bolivia is patchy, there is information available on environmental and social risk factors known to be associated with *A. lumbricoides* prevalence. Environmental conditions, particularly temperature and soil moisture, largely determine global distribution of *A. lumbricoides*. Social factors, such as piped water, sanitation and improved housing (absence of dirt floors), are associated with lower STH infection. Using spatial autocorrelation statistics (Moran's I and Local Indicator of Spatial Association [LISA]), we examined the spatial distribution of risk factors associated with increased prevalence of *A. lumbricoides* infection. Subsequently, we created a risk model integrating environmental and social factors with previous community-level *A. lumbricoides* survey data. This model is a cost-effective way to predict areas of high and low STH endemicity in the absence of nationally representative prevalence data. The model can be used to inform efforts to fill information gaps on prevalence and intensity of STH infections and better focus mass drug administration campaigns and water, sanitation and housing infrastructure investments to mitigate STH infections across Bolivia.

1193

HOOKWORM AND *TRICHURIS* INFECTIONS ASSOCIATED WITH ANEMIA DURING PREGNANCY

Theresa W. Gyorkos[1], Nicolas L. Gilbert[1], Renee Larocque[2], Martin Casapia[3]

[1]McGill University, Montreal, QC, Canada, [2]Global Health Research Initiative, Ottawa, ON, Canada, [3]Asociacion Civil Selva Amazonica, Iquitos, Peru

The objective of this study was to assess changes during pregnancy in the association between the intensity of soil-transmitted helminth (STH) infections and hemoglobin (Hb) levels, following iron supplementation and mebendazole administration, in an endemic region of Peru. Data from a randomized clinical trial on the effect of mebendazole on birthweight were re-analyzed. Pregnant women (N=935) recruited in their second trimester provided baseline data on STH infections and anemia. All women were given iron supplements (60 mg/d); half were randomly allocated to receive single dose 500mg mebendazole and half placebo. Hb was also determined in the third trimester. Mean Hb level increased from 11.03 to 11.39 g/dL between the second and the third trimester. Hookworm

(prevalence 46.3%) and *Trichuris* (76.1%) infections, but not *Ascaris* (63.9%), in the second trimester were independently associated with lower Hb in both the second and third trimester. *Trichuris* >1200 epg was also associated with a lower increase of Hb between the second and the third trimester (ΔHb = -0.26 g/dL, p=0.026). The prevalence of anemia (Hb < 11 g/dL) decreased from 46.8% to 34.7% between the second and the third trimesters. Hookworm and *Trichuris* infections were significantly and independently associated with increased risk of anemia in the third trimester. The adjusted OR for hookworms >600 epg was 1.52 (95% CI 1.01 to 2.28), and the adjusted OR for *Trichuris* >1200 epg was 1.72 (95% CI 1.14 to 2.61). Mebendazole treatment was not associated with Hb or with the prevalence of anemia, and did not modify the relationship between helminths and Hb. In conclusion, in pregnancy, both hookworm and *Trichuris* infections are associated with increased risk of anemia. Infected women remain at higher risk of anemia throughout pregnancy, irrespective of mebendazole administration. These data suggest that more effective deworming interventions be targeted to pregnant women in endemic areas.

1194

TUBERCULOSIS (TB) CONTROL AMONG HEALTH CARE WORKERS (HCW), RESEARCHERS, TRAINEES AND TRAVELERS TO A TB-ENDEMIC AREA WITH AN ACADEMIC, INTERNATIONAL, MEDICAL EXCHANGE PROGRAM

Adrian Gardner[1], Ted Cohen[2], E. Jane Carter[1]

[1]Warren Alpert School of Medicine at Brown University, Providence, RI, United States, [2]Brigham and Women's Hospital and Harvard School of Public Health, Boston, MA, United States

Although individuals from low TB burden countries experience an increased risk of TB infection when traveling to high burden countries for medical training or service, degree of risk has not been well quantified. Improved knowledge will aid development of guidelines for TB screening, pre/post-travel education and risk reduction. 608 individuals who traveled to Eldoret, Kenya with the AMPATH medical exchange program between July, 2004 - June, 2009 were invited to complete an online survey. Survey questions included demographic characteristics, pre-travel TB counseling, in-country activities, and post-travel TB testing. 418/608 (69%) responded. The majority were young adults (65% between 22-40 years old) and 55% were female. 7% had chronic medical conditions predisposing to increased risk of TB. Pre-travel, respondents sought travel advice from a number of different sources: travel clinic (54%), CDC travel website (31%). 58% reported that TB prevention was discussed in travel preparations. Pre-travel, 81% reported negative Tuberculin skin test (TST) or Interferon Gamma Release Assay (IGRA); 11% reported unknown infection status. Most commonly reported duration of visits was 5-8 weeks. Most-common activities included direct medical care in adult medical ward (49%) and non-direct medical care activities in a lab or on hospital grounds (38%). Respondents also commonly visited Kenyan homes (65%) and used public transport (40%). 13 individuals (3 under age 21) converted to a positive TST during their travel. One reported an episode of active TB. 113 (28%) of survey participants reported "ideal" care (definition: pre-travel TST (within one year of travel), pre-travel counseling, and a repeat post-travel TST). In conclusion, travelers to TB endemic areas with international, medical exchange programs are at significant risk for TB infection. Many receive inadequate pre and post-travel TB counseling and testing. Efforts should be made to improve TB education for program participants. Further study is needed to quantify the risk of TB infection.

1195

MOLECULAR EPIDEMIOLOGY OF BOVINE TUBERCULOSIS ACROSS THE UNITED STATES-MEXICO BORDER: SUPPORTING STRATEGIES FOR ANIMAL AND PUBLIC HEALTH INTERVENTIONS

James Higgins, Tod Stuber, Patrick Camp, Doris Bravo, David Farrell, Beth Harris

NVSL, APHIS, United States Department of Agriculture, Ames, IA, United States

Bovine tuberculosis, caused by Mycobacterium bovis, is a major global cause of respiratory disease and decreased production in beef and dairy cattle. Human infections with M. bovis represent an emerging public health concern in immigrant communities in the US. The epidemiology of zoonotic M. bovis infection is complex, but may be associated with exposure to contaminated animal products such as soft cheeses and unpasteurized milk. Elucidating the epidemiology of M. bovis prevalence and transmission among cattle in the US, particularly among animals imported from Mexico, can be an important component of strategies to reduce the economic and public health impacts of this disease. Using techniques to genotype M. bovis strains, such as spoligotyping and variable number tandem repeat (VNTR) analyses, we have examined the molecular epidemiology of bovine tuberculosis outbreaks in southwestern states in the US. In the majority of these outbreaks, the data indicate that the M. bovis isolates do not display the genotypic profiles common to isolates present in cervids, which are considered to be a major source of bovine TB infections among cattle in the central and eastern regions of the US. Rather, the genotypic profiles for the M. bovis isolates recovered from US animals are representative of those documented to occur in cattle originating from Mexico. Efforts are underway to expand databases of molecular fingerprinting data for M. bovis isolates obtained from cattle and wild animals to enhance the USDA's abilities to identify foci of the disease in cattle populations, and improve efforts to control bovine tuberculosis within the US.

1196

QUALITATIVE FINDINGS AND IMPLICATIONS FOR SCALING UP AN IMPROVED COOKSTOVE PROJECT IN RURAL KENYA

Bobbie Person[1], Jennifer Loo[1], Mercy Owour[2], Lorraine Ogange[3], Adam Cohen[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Tropical Institute of Community Health, Kisumu, Kenya, [3]Kenya Medical Research Institute, Kisumu-Kisian, Kenya

Traditional indoor, three-stone cooking fire pits, a staple in rural households in resource-poor countries are not without costs to human health and the environment. We conducted a pilot intervention to promote the purchase and use of an improved cookstove in rural Kenya. In order to improve the scaling up of the stove promotion effort to the larger community, we conducted a qualitative inquiry to better understand the activities and strategies vendors used to promote the stove, the motivations of community members to purchase and use the stove, and the perceived benefits and challenges of stove use among very poor Luo women. Purposive sampling was used to recruit 10 health promoter-stove vendors and 30 stove purchasers of the stove in the Luo community. Formative research was conducted through qualitative semi-structured key informant interviews with a topic guide. Data were transcribed and imported into Atlas-ti and a thematic analysis conducted to identify patterns representing a comprehensive picture of personal and collective experiences. Women who purchased the improved cook stove reported the most influential promotion strategies were interpersonal communication through informal social networks, observations of someone using the stove, and actual cooking demonstrations. Luo women reported the need for less firewood, fuel costs savings, reduced smoke, increased cooking efficiency, and reduced eye irritation, lung congestion

and coughing as major benefits. While there were notable financial and health benefits along with a decreased workload for women, the cost of the stove and the associated role of men in decision making for household purchases appeared to be possible barriers to wider spread adoption. In conclusion, qualitative findings noted that the price of the stove will need to be reduced or subsidized if there is a commitment for community-wide access to the health benefits of this newer technology. Promotion activities employing interpersonal communication and cooking demonstrations were critical for scaling up the project.

1197

UNDERSTANDING RESPIRATORY INFECTION IN BANGLADESH: COMMUNITY PERCEPTIONS, SOCIAL NORMS AND CURRENT PRACTICES

Md. Fosiul A. Nizame[1], Sharifa Nasreen[1], Leanne Unicomb[1], Dorothy Southern[1], Emily S. Gurley[1], Shaila Arman[1], Mohammad Abdul Kadir[1], Peter J. Winch[2], Eduardo Azziz Baumgartner[1], Stephen P. Luby[1]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Respiratory infections are the leading cause of childhood death in Bangladesh. Non-pharmaceutical interventions such as respiratory hygiene (covering the nose and mouth when coughing or sneezing and washing hands after having contact with respiratory secretions and contaminated objects) may be effective in reducing infection transmission. This formative study explored community perceptions about respiratory infections, why they occur, how they are spread, and the preventive measures that can be taken to protect themselves and their families. We used semi-structured interviews and focus group discussions with community members, leaders and school children to explore respiratory infection and hygiene related perceptions, social norms and practices. From both the semi-structured interviews and focus group discussions, we found that our informants were not familiar with the term "respiratory disease" as translated into Bengali. When asked to give examples of respiratory diseases, they named diseases some of which had no relation to respiratory function. The informants identified a number of social norms related to respiratory hygiene, including covering coughs or sneezes, turning face away during sneezing and coughing, and not spitting on the ground, but very few people practice these. During semi-structured interviews, all informants cited hot/cold weather changes and using cold water as the cause for catching cold during the previous twelve months. Yet, when asked about modes of transmission, these were associated with close contact with the breath, spit or cough droplets of a sick person. The most effective way to prevent respiratory infection was to avoid these. Although most of our informants perceived that handwashing after coughing and/or sneezing might prevent illness, most of them felt that this was not practical after every event. In conclusion, informants related their personal experience of catching a cold to the local explanatory model of hot-cold imbalance including perceived changes in body temperature and environment. But for transmission and prevention they related to both the biomedical construct and the cultural contagion model. This gap provides useful insights for message development that can be incorporated into "culturally compelling" communication interventions to make people conscious about respiratory hygiene and to increase their self efficacy.

1198

INCIDENCE OF INFLUENZA INFECTION IN EARLY INFANCY IN SOUTH ASIA

Mark C. Steinhoff[1], Emily Henkle[2], Saad B. Omer[3], William J. Moss[2], Shams E. Arifeen[4], Rubhana Raqib[4], Monica McNeal[1], Robert F. Breiman[5], K. Zaman[4]

[1]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, [2]Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, [3]Rollins School of Public Health, Emory University, Atlanta, GA, United States, [4]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [5]Centers for Disease Control and Prevention, Nairobi, Kenya

Young infants 0-6 months of age in the US have a reported influenza incidence of 12% and high rates of 'flu hospitalization. Historically influenza has not been considered a frequent or serious illness of infants in the tropics, partly because of the lack of data. We report the serology and 'flu antigen data from 131 infants of mothers who received pneumococcal vaccine as controls in a 'flu vaccine study. A full description of the Mother'sGift trial has been published. 340 pregnant women in Dhaka were randomized to receive pneumococcal or trivalent inactivated influenza vaccine. From Aug 2004 - Oct 2005, non-'flu vaccine control mothers and their infants were visited weekly from birth to 6 months to record symptoms of febrile respiratory illness. Influenza antigen was tested by rapid antigen-detection test (RADT) in symptomatic infants. A standard hemagglutination inhibition (HAI) assay was done on infant sera collected at birth, 10, and ~ 22 weeks of age. We defined serological infection as a $\geq$ 4-fold increase of the expected declining later titers compared to the earlier titer. Of 131 infants with prospective data 29 experienced 31 serologically-defined influenza infections. Adding 10 distinct RADT-proven infections resulted in a total of 41 influenza infections or a cumulative incidence of 31.3/100 0-6 month old infants (95% CI: 23.6-41.4). Maternal HAI data is being analyzed. In conclusion, additional studies of the incidence of influenza in young infants in Asian and other tropical regions should be carried out to define the burden of preventable influenza illness. Antenatal immunization is a proven prevention strategy in this vulnerable group.

1199

PREVENTIVE MEASURES ASSOCIATED WITH PROBABLE CASES OF A (H1N1) INFLUENZA IN INHABITANTS OF THE FEDERAL DISTRICT OF MEXICO CITY

Ana Flisser[1], Veronica Cruz-Licea[1], Fernando Gonzalez-Dominguez[2]

[1]UNAM, Faculty of Medicine, Mexico City, Mexico, [2]Hospital General "Dr. Manuel Gea Gonzalez, SSA, Mexico City, Mexico

When pandemic A (H1N1) Influenza (flu) broke out in the spring of 2009 in Mexico, the Ministry of Health established and profusely promoted preventive and control measures that could slow or stop transmission. The objective of this study was to evaluate if inhabitants of the Federal District of Mexico City understood and implemented the measures recommended and if there was an association with probable cases of the flu. An ad-hoc questionnaire was given to 4003 subjects who voluntarily agreed to participate in a household survey aimed at detecting cases of influenza between August and September 2009. The average age was 46±16 years of age and 64% were women. Most people reported to wash hands and open windows at home, but only 32% covered their nose with the forearm when sneezing, 34% used alcohol gel and 70% kept shaking hands or kissing when greeting others. The frequency of symptoms of flu like disease occurring from January to September 2009, were: 29% running nose, 25% coughing, 17% head, muscle or joint ache and 7% sudden high fever and 11% had 3 or 4 of these symptoms (probable cases); 62% attended a physician when flu symptoms appeared, 22% self-medicated and 17% did not do anything. Significant associations were found between probable cases and not washing hands, and also for

inadequate techniques when sneezing or greeting. The potential impact of these factors on the frequency of probable cases, calculated as the attributable risk for the exposed, ranged from 29 to 55%. The fact that a third of the population used the appropriate technique to cover their nose while sneezing or used alcohol gel, measures newly recommended by the government, suggests that people follow novel advices, while a similar proportion changed inadequate habits, such as touching or kissing. This study was performed only 3 months after the pandemic started and allowed identifying public health information that should be intensified or modified, especially because the survey was carried out among the general population and not only in diagnosed cases.

1200

OUTBREAK OF PANDEMIC INFLUENZA A (H1N1) IN RWANDA, OCTOBER 2009

Thierry Nyatanyi[1], **Joseph Rukelibuga**[2], Marie Aimée Muhimpundu[1], Richard Nkunda[3], Adeline Kabeja[1], Alice Kabanda[3], John Rusine[3], John Ruyonga[4], Ange Irakoze[1], Noella Umulisa[1], David Lowrance[5], Zara Ahmed[5], Simon Antara[6], Caitlin Biedron[5], David McAlister[5], Jean Baptiste Koama[5], Eugenie Kayirangwa[5], Odette Mukabayire[3], Corine Karema[1], Pratima Raghunathan[5], Justin Wane[7]

[1]Center for Treatment and Reserach on AIDS Rwanda, Kigali, Rwanda, [2]CDC Rwanda, Kigali, Rwanda, [3]National Reference Laboratory Rwanda, Kigali, Rwanda, [4]Ministry of Health, Rwanda, Kigali, Rwanda, [5]CDC-GAP Rwanda, Kigali, Rwanda, [6]African Field Epidemiology Network, Rwanda, Kigali, Rwanda, [7]King Faycal Hospital Rwanda, Kigali, Rwanda

On October 9th, 2009 Pandemic Influenza A (H1N1) outbreak was confirmed in Rwanda. The index case a Rwandan female, 39 years old traveled to US and arrived in Kigali on October 3rd, 2009. She presented with fever, cough and joint pains on October 4th, 2009. We describe the epidemiology of this novel virus in Rwanda. Methods: We undertook epidemiologic investigations around the index case. We collected respiratory specimens from suspect cases using a WHO case definition and tested specimens by real time RT-PCR at the National Reference Laboratory. From October 8th through October 31st, 2009, we tested 810 specimens. Of these, 127 (15.7%) were positive for pandemic H1N1. Of confirmed cases, 81 (63.8%) were children <15 including 17 children < 5 years old, 74 (58.2%) were female, 35 (27.6%) were in a high-risk category. Two (1.6%) cases were hospitalized and subsequently discharged; all other cases were outpatients. One hundred and seventeen (98%) confirmed cases received treatment with oseltamivir and two received post-exposure prophylaxis. Confirmed cases occurred in two referral hospitals, five schools all in Kigali city and in 46 households in Kigali City, northern and western Provinces. The attack rates at the two schools with the largest H1N1 clusters were 2.4% and 3.8%. Twelve (9.4%) confirmed cases of third-generation transmission occurred and 87 (68.5%) cases had unknown exposure to known confirmed cases. In conclusion, transmission of Pandemic influenza A (H1N1) was confirmed in Rwanda and documented in diverse settings, including health facilities, schools, and households. Most confirmed cases were female and children. Disease appears mild, similar to that seen in other East African countries. We recommended mitigation measures, including public education on home care, enhanced training of health care workers, use of targeted laboratory testing and treatment, and robust surveillance.

1201

SPATIAL AND TEMPORAL VARIATION IN VECTOR COMPETENCE OF *CULEX PIPIENS* AND *CX. RESTUANS* MOSQUITOES FOR WEST NILE VIRUS

A. Marm Kilpatrick[1], Dina M. Fonseca[2], Greg D. Ebel[3], Michael R. Reddy[4], Laura D. Kramer[5]

[1]University of California, Santa Cruz, Santa Cruz, CA, United States, [2]Rutgers University, New Brunswick, NJ, United States, [3]University of New Mexico, Albuquerque, NM, United States, [4]Harvard University, Boston, MA, United States, [5]New York State Department of Health, Albany, NY, United States

Vector competence, the probability that a vector will transmit a pathogen after feeding on an infected host, is known to vary among vector species, populations, days since feeding, and temperature during the extrinsic incubation period. However, the extent of spatio-temporal variability and consistency in vector competence of populations is not known. We examined vector competence of *Culex* pipiens Linnaeus and *Cx. restuans* Theobald mosquitoes for West Nile virus (WNV) collected over three years from sixteen sites to measure spatial and temporal scales of variation in vector competence. We found extreme variation with 0-52% of mosquitoes transmitting WNV at a single site between different sampling periods, and similar variation across populations. However, we also found that within a smaller geographic range vector competence varied somewhat synchronously, suggesting that environmental and population genetic factors might influence vector competence. These results highlight the spatio-temporal variability in vector competence and the role of local processes.

1202

WEST NILE VIRUS ENVELOPE PROTEIN GLYCOSYLATION AFFECTS VIRUS-VECTOR INTERACTIONS IN A SPECIES-SPECIFIC MANNER

Robin M. Moudy, Anne F. Payne, Brittany L. Dodson, Laura D. Kramer

Wadsworth Center/New York State Department of Health, Albany, NY, United States

Many, but not all, strains of *West Nile virus* (WNV) contain a single N-linked glycosylation site on their envelope (E) proteins. Previously, we found that a WNV that lacked the glycosylation site (ENONGLY) replicated less efficiently than glycosylated WNV (EGLY) in *Culex pipiens* and *Cx. tarsalis* mosquitoes. Additionally, transmission of WNV by mosquitoes that fed on ENONGLY was associated with viral reversion to a glycosylated phenotype. We have recently expanded these studies to include an additional important WNV vector, *Cx. quinquefasciatus*. To determine if replicative differences were present in this species, we inoculated mosquitoes intrathoracically with 10pfu of either ENONGLY or EGLY and determined titers in the mosquitoes from 1 to 10 days post inoculation. As we previously observed with *Cx. pipiens* and *Cx. tarsalis*, replication of ENONGLY was decreased relative to that of EGLY in *Cx. quinquefasciatus*. Similar decreases in replication were observed in individual mosquito tissues (midguts and salivary glands). To determine whether viral genotype affected infectivity and transmission in *Cx. quinquefasciatus*, we fed mosquitoes a bloodmeal containing either EGLY or ENONGLY and examined infection, dissemination, and transmission rates at various days post-feeding (dpf). At all dpf, infection rates were greater in mosquitoes that had fed on a bloodmeal containing EGLY, similar to previous results in *Cx. pipiens*. However, dissemination and transmission rates were higher at 7 and 14 dpf in mosquitoes that fed on a bloodmeal containing the ENONGLY virus. In contrast to previous results with both *Cx. pipiens* and *Cx. tarsalis*, none of the viruses transmitted by *Cx. quinquefasciatus* following feeding on the ENONGLY bloodmeal exhibited reversion to EGLY phenotype. Taken together, these data suggest that E protein glycosylation

affects WNV-vector interactions differently in different *Culex* mosquito species and confirms earlier studies with WN02 and NY99 showing that *Cx. quinquefasciatus* differs from its sibling species *Cx. pipiens*.

1203

LACK OF GENETIC BOTTLENECKS DURING WEST NILE VIRUS INFECTION OF *CULEX PIPIENS QUINQUEFASCIATUS*

Doug E. Brackney, Ivy K. Brown, Kelly A. Fitzpatrick, Gregory D. Ebel

University of New Mexico, Albuquerque, NM, United States

The introduction and adaptation of arthropod-borne viruses (arboviruses) to new ecological niches pose an ongoing threat to human health. Understanding the evolution of arboviruses in the context of their transmission cycles and how this impacts viral emergence, adaptation and persistence is paramount to effectively minimizing public health risk. Introduced into North America in 1999, West Nile virus (WNV; *Flaviviridae*, *Flavivirus*) has adapted to local transmission cycles and is now considered endemic. Previous studies have shown that WNV exists within hosts as a genetically diverse group of competing viral variants, and that the complexity of the WNV quasispecies is greater in mosquitoes than birds. The mechanism(s) responsible for this observed difference and the impact of mosquito infections on viral evolution are unknown. Therefore, using WNV and *Culex pipiens quinquefasciatus* mosquitoes as a model system, we addressed the role mosquito infections have in shaping arbovirus populations. We offered *Cx. p. quinquefasciatus* mosquitoes an infectious bloodmeal containing a highly genetically diverse WNV population and quantified viral genetic diversity in three well-characterized stages (infection, dissemination, and transmission) from three mosquitoes at three time points (7, 14, and 21 days post infection). WNV RNA corresponding to the E-NS1 coding region (nt 1971-2928) was reverse transcribed, amplified with high fidelity polymerase and cloned. Subsequently, thirty clones from each sample were sequenced. Baseline levels of input viral genetic diversity were determined from whole-body mosquitoes recovered immediately post blood feeding. The genetic diversity of virus obtained from three freshly fed mosquitoes was exteremely high (0.170%). Further, on average, 14.7 haplotypes were identified with many of these haplotypes being shared among individuals. Examination of midguts from later timepoints revealed genetic restriction with a reduced number of haplotypes and an overall genetic diversity of 0.021%. Sampling of the legs (dissemination) and saliva (transmission) revealed that many of the input haplotypes that had been lost in the midgut were recovered, and viral genetic diversity was greater than in the midgut. Overall, these data suggest that the *Culex* mosquito midgut fails to impose a genetic bottleneck on WNV populations and that WNV genetic diversity accumulates during the course of an infection.

1204

INSIGHTS INTO ARBOVIRAL MUTANT SWARM DYNAMICS USING EXPERIMENTAL EVOLUTION OF FLAVIVIRUSES

Alexander T. Ciota, Greta V. Jerzak, Dylan J. Ehrbar, Graham G. Willsey, Anne F. Payne, Laura D. Kramer

Wadsworth Center, New York State Department of Health, Slingerlands, NY, United States

The rapid production of mutants created by the combination of high rates of replication and error prone nature of the RdRp of arboviruses results in a population of variants collectively called the mutant swarm. In order to fully understand how selection acts on these populations one must first fully describe the role of the mutant swarm both within and among hosts. Understanding the role of minority variants in viral fitness and plasticity is particularly important for arboviruses, which require replication in disparate hosts and tissues. In addition, an understanding how bottlenecks within and among hosts work to shape the arboviral mutant swarm is still lacking. Using *in vitro* and *in vivo*-passed strains of West Nile virus (WNV) and St. Louis encephalitis virus (SLEV) we evaluated the importance of the mutant swarm in viral fitness, the distribution of variants in cell-adapted populations, the potential for strain complementation, and the size of the neutral bottlenecks within *Cx. pipiens* mosquitoes. Previous results have demonstrated that passed populations are highly adapted and, that the WNV mutant swarm is important in adaptive phenotypes. WNV viral diversity has been shown to accumulate at high levels with passage and adaptation to both mosquitoes and mosquito cells, while SLEV has not. Preliminary *in vitro* competition assays with WNV suggest that increases in strain co-infection in mosquito cells limit the relative fitness of the adapted strain, suggesting strain complementation in co-infected cells. In addition, preliminary results using equally fit WNV variants have allowed us to quantify the probability that minority variants will survive both dissemination throughout and transmission from *Cx. pipiens* mosquitoes given their starting concentrations in the population. Taken together, these results provide crucial insight into arboviral mutant swarm dynamics which have significant impact on the understanding of arbovirus adaptation, evolution, and epidemiology.

1205

PATTERN FORMATION IN THE DYNAMICS OF WEST NILE VIRUS AMPLIFICATION IN A TRANSMISSION HOT SPOT IN CHICAGO, U.S.A.

Tavis K. Anderson[1], Gabriel L. Hamer[1], Edward D. Walker[2], Luis F. Chaves[3], Uriel D. Kitron[3], Jeffrey D. Brawn[4], Marilyn O. Ruiz[4], Tony L. Goldberg[1]

[1]University of Wisconsin-Madison, Madison, WI, United States, [2]Michigan State University, East Lansing, MI, United States, [3]Emory University, Atlanta, GA, United States, [4]University of Illinois at Urbana-Champaign, Champaign, IL, United States

Untangling the influence of host and vector diversity on West Nile virus (WNV) persistence and transmission is relatively complex given the large numbers of species and interactions. Although the complexity of these networks have been hypothesized to affect transmission, at times positively or negatively, the actual mechanisms that facilitate repeated outbreaks of WNV remain largely unknown. It has been suggested that "super spreaders," may be important in the WNV system, defined as a minority of individuals, or host species, that give rise to the majority of secondary infections. Here we examine this idea, using a series of computer simulations of pathogen transmission. Under most conditions, we find that cascades of WNV transmission are not solely driven by super spreaders, but rather by a critical mass of infected individuals. Although these data do not exclude the possibility that super spreaders are important, they suggest that making assumptions about their influence on pathogen dynamics requires careful testing.

1206

IL-10 AND PD-L1 IMPAIR THE T CELL RESPONSE IN THE CENTRAL NERVOUS SYSTEM DURING PERSISTENT WEST NILE VIRUS INFECTION

Barbara S. Stewart[1], Chrystal M. Chadwick[2], Sharone Green[3], **Kristen A. Bernard**[4]

[1]University at Albany, Albany, NY, United States, [2]Wadsworth Center, Albany, NY, United States, [3]University of Massachusetts Medical School, Worcester, MA, United States, [4]University of Wisconsin-Madison, Madison, WI, United States

West Nile virus (WNV) can lead to a persistent infection in humans and in several animal models. In mouse studies from our laboratory, WNV RNA persists in the central nervous system (CNS) for up to 6 months post-inoculation (p.i.), and infectious virus can be isolated from CNS tissues for up to 4 months p.i. We also showed that plasma cells in the CNS of WNV-inoculated mice secreted WNV-specific antibody for at least 16 weeks p.i. Additionally, we detected epitope-specific CD8+ T cells in the CNS for up to 16 weeks p.i., using a MHC class I dimer assay for a dominant WNV epitope (SSVWNATTA). We hypothesized that CD8+ T cells are

ineffective in clearing the virus from the CNS, resulting in viral persistence. We assessed the function of T cells using intracellular staining for IFN-γ and found that only 20% of the epitope-specific CD8+ T cells in the brain were able to secrete IFN-γ at 2 to16 weeks p.i. In contrast, approximately 95% of the epitope-specific CD8+ T cells in the spleen produced IFN-γ after peptide stimulation. Similar results with TNF-α production were obtained. We examined the possibility that the CD8+ T cells were inhibited by IL-10 and/or programmed death receptor ligand-1 (PD-L1). WNV-inoculated mice were treated with antibodies to IL-10 receptor (1L-10R) and/or PD-L1 every three days for five treatments starting at 2 weeks p.i. Three days after the last treatment, mice were sacrificed, and CD8+ T cell function and WNV persistence were compared to isotype antibody treated controls. The function of the epitope-specific CD8+ T cells was partially restored upon blockade of IL-10R and/or PD-L1. In addition, infectious WNV was found in two of nine mice treated with antibodies to both IL-10R and PD-L1 compared to eight of eight mice treated with isotype control antibodies (Fisher's exact test, *P*=0.002). In summary, these results suggest that CD8+ T cells are impaired in the CNS of mice due to IL-10 and PD-L1, and blockade of these molecules promotes viral clearance.

1207

KERATINOCYTES ARE CELL TARGETS OF WEST NILE VIRUS *IN VIVO*

Pei-Yin Lim[1], Gang Zou[2], Chrystal M. Chadwick[2], Kristen A. Bernard[1]

[1]University of Wisconsin, Madison, WI, United States, [2]Wadsworth Center, New York State Department of Health, Albany, NY, United States

West Nile virus (WNV) is transmitted to humans through the bite of an infected mosquito at the skin. Langerhan cells, resident dendritic cells in the skin, are postulated to become infected with WNV and migrate to the draining lymph nodes. We hypothesized that WNV infects additional cell types in the skin because high levels of infectious virus are detected in both the skin and draining lymph node at 24 hour post-inoculation (hpi) and persist for at least seven days. The goal of this study was to identify the cell target(s) of WNV in the skin. We inoculated mice subcutaneously (SC) in the left rear footpad with WNV replicon particles containing a luciferase reporter and monitored luciferase activities in various tissues. Luciferase activity was detected in the skin at the inoculation site at 24 hpi and increased ~100-fold at 72 hpi. These results suggest that WNV infects non-migrating cells in the skin, which continually support virus replication. We inoculated mice SC in the footpads with WNV and performed immunohistochemical assays on the skin to determine the cell tropism of WNV. WNV-positive cells were found only in the epidermal layer of the skin of the WNV-inoculated mice at 96 hpi, and these cells were identified morphologically as keratinocytes. We inoculated mouse primary skin cells with WNV and analyzed the cells by flow cytometry for expression of WNV E antigen and cell surface markers - the pan-leukocyte marker CD45 and a keratinocyte marker K10. Approximately 4% of the skin cells expressed WNV antigen, and ~64% of these cells expressed K10, further confirming that mouse keratinocytes were susceptible to WNV infection. No WNV-positive cells expressed CD45, but they may not have been detected since there were low numbers of CD45-positive cells (~2.5%) in the skin cell population. In addition, primary human keratinocytes were permissive for WNV and supported virus production for at least six days. In summary, the skin is an initial replication site for WNV, and keratinocytes are cell targets *in vivo* in the skin.

1208

THE IMPACT OF A SENTINEL SITE MALARIA SURVEILLANCE PROGRAM ON IMPROVING CASE MANAGEMENT IN UGANDA

Asadu Sserwanga[1], Ruth Kigozi[1], Hasifa Bukirwa[1], Stella Kakeeto[1], Anne Gasasira[1], Manoj Menon[2], Sussann Nasr[2], Scott Filler[2], Jamal Harris[3], Grant Dorsey[3], Ebony Quinto[4], Moses Kamya[5]

[1]Uganda Malaria Surveillance Project, Kampala, Uganda, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]University of California at San Francisco, San Francisco, CA, United States, [4]Ministry of Health, Kampala, Uganda, [5]Makerere University College of Health Sciences, Kampala, Uganda

Malaria surveillance is critical for monitoring trends in malaria morbidity and mortality, but can also be used as a tool for improving case management. The WHO now recommends ACTs for the treatment of uncomplicated *falciparum* malaria along with prompt laboratory confirmation in all patients suspected of malaria before treatment is started. From August 2006 - January 2007 we implemented a sentinel site malaria surveillance system at the outpatient departments of 5 government health centers in Uganda with a 6th site added in September 2008. This system was established by the Uganda Malaria Surveillance Project in collaboration with the Ministry of Health and Health Management Information System. Individual patient data is entered electronically onsite using a standardized case record form and sent monthly to a core facility using cellular technology. Through February 2010, a total of 397,407 patients were seen of which 54% (range 47-63% at the 6 sites) were suspected of having malaria. We compared data from the first two months of surveillance with data from the most recent two months (Jan-Feb 2010) to evaluate key indicators in malaria case management. The proportion of patients with suspected malaria for whom a laboratory test was done increased from 44% (range 32-64%) to 97% (range 93-99%). The proportion of patients with a laboratory test done who were appropriately prescribed an antimalarial drug (negative test not prescribed an antimalarial, positive test prescribed an antimalarial) increased from 64% (range 51-78%) to 94% (range 87-98%). The proportion of patients with a positive laboratory test who were prescribed an ACT increased from 50% (35-88%) to 74% (35-91%), although these results were highly variable across the sites. The implementation of sentinel site malaria surveillance system in the context of the existing government system was associated with dramatic improvements in utilization of laboratory services and rationale antimalarial treatment decision making. However, further improvement is needed in ACT prescribing practices.

1209

MATERNAL AND PLACENTAL MALARIA AND LOW BIRTH WEIGHT AFTER INTRODUCTION OF INTERMITTENT PREVENTIVE TREATMENT PROGRAM USING SULFADOXINE-PYRIMETHAMINE IN PREGNANT WOMEN IN BAMAKO, MALI, WEST AFRICA

Mahamadou Diakite, Aminata Famanta, Seidina A. Diakité, Sory Ibrahim Diawara, Saibou Doumbia, Karim Traoré, Drissa S. Konaté, Mory Doumbia, Abdoul Salam Keita, Daouda Thiéro, Sékou Fantamady Traoré, Seydou Doumbia

University of Bamako, Bamako, Mali

In 2006, the Malian government established a program for free insecticide-treated net (ITNs) and intermittent preventive treatment during pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) for pregnant women. In March to November of 2009, we conducted a cross-sectional study in periurban areas of Bamako, Mali to determine the malaria prevalence among pregnant women and their newborn children. We included 379 pregnant women aged 15 to 45 years. At delivery, malaria was diagnosed using peripheral thick smears in mothers and newborns, as well as

umbilical cord blood and placental blood. The prevalence of *P. falciparum* malaria was 2.4%, 1.6% and 0.5% respectively in mother, placenta and cord samples. Approximately 77% of our parturient were housewives. The illiteracy rate among this group was 72.3%. Of the 379 women, 72.8% had at least three prenatal visits, 82.8% had received at least one free ITNs, and 71.8% had received IPTp-SP during antenatal visit. Among them, 80.7% claimed to have complied with IPTp-SP. We observed a low birth weight rate of 12.1%. We did not find any congenital malaria. The prevalence of malaria in both mother and newborn has show a significant decrease in Bamako, compared with previous studies before the implementation of IPTp-SP policy in Mali. This study shows a high rate of coverage in use of IPTp-SP and ITNs correlated with low malaria prevalence in pregnant women.

1210

MALARIA RESURGENCE IN WESTERN KENYA IN THE ERA OF MALARIA INTERVENTIONS

Guofa Zhou[1], Yaw A. Afrane[2], Anne M. Vardo-Zalik[1], Guiyun Yan[1]

[1]University of California at Irvine, Irvine, CA, United States, [2]ICIPE, Nairobi, Kenya

Despite significant increases in the supply of mosquito bednets since 2006, the link between interventions and their impact is not always clear in malaria endemic south Sahara Africa. To monitor the impacts of insecticide treated bed nets on malaria prevalence and mosquito density, we conducted monthly surveys in three sites in Western Kenya beginning in January 2007. Our results illustrate a resurgence of malaria in all three of our study sites (2 highland and one lowland) after an initial decline in 2006. Despite a high household bed net coverage rate of 50-80%, asymptomatic malaria prevalence and mosquito density have increased in all study sites since 2007, with an approximate 4-10 fold increase in *Anopheles* funestus and *An. gambiae* densities. Upon examination of households that used bednets, the majority of nets were in good condition, but only 60% were regularly used. Additionally, almost half of the bed nets were 4 or more years old, so their efficacy was waning. We also found a decrease in the prescription of ACT to treat malaria cases, even though ACT is the most effective drug at the moment. The increase in malaria prevalence and vector densities suggests that the current control methods are not sufficient and that future control effort should include a more intensive distribution of long-lasting insecticidal nets and the provisions for a sustainable supply of effective antimalarial drugs.

1211

SIGNIFICANT DECREASE IN ANEMIA IN AREAS OF VERY LOW MALARIA TRANSMISSION AFTER INTERRUPTION OF TRANSMISSION

Gregory S. Noland[1], George Ayodo[1], James S. Hodges[2], John M. Vulule[3], Chandy C. John[1]

[1]Center for Global Pediatrics, University of Minnesota Medical School, Minneapolis, MN, United States, [2]Department of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, MN, United States, [3]Kenya Medical Research Institute, Center for Global Health Research, Kisumu, Kenya

The potential effect of malaria control measures on prevalence of anemia in areas of low and unstable malaria transmission has not been well characterized. In the adjoining highland areas of Kapsisiywa and Kipsamoite, Kenya, areas of low and unstable malaria transmission, we assessed hemoglobin levels and frequency of anemia in a cohort of randomly selected asymptomatic individuals in May 2007 and July 2008 (Kapsisiywa, n=910, Kipsamoite, n=787). Widespread indoor residual spraying of insecticide led to a 12- month interruption of malaria transmission in both areas starting in April 2007, and none of the individuals in the cohort had clinical malaria between May 2007 and July 2008. In May 2007, anemia was common in both sites, with 57.5%, 21.7% and 22.7% prevalence in Kapsisiywa for individuals <5 years, 5-14 years and >15 years of age, respectively, and 47.2%, 19.3% and 17.5% prevalence in Kipsamoite for individuals <5 years, 5-14 years and >15 years of age, respectively. Fourteen months later, malaria interruption was associated with significant increases in hemoglobin levels and consequent decreases in the prevalence of anemia for individuals in Kapisisiywa <5 years of age (34.1% reduction of anemia, $P<0.0001$), 5-14 years of age (51.6%, $P<0.0001$) and >15 years of age (26.9%, $P=0.004$), and in Kipsamoite for individuals <5 years of age (33.7%, $P=0.001$). Insecticide treated net (ITN) use was infrequent in both sites (16.8% in Kapsisiywa, 11.3% in Kipsamoite), and was associated with an increase in hemoglobin level only in pregnant women. Even in areas of very low malaria transmission, anemia occurs frequently, particularly in children. Reduction or interruption of malaria transmission in these low transmission areas is associated with highly significant decreases in the prevalence of anemia.

1212

INTERMITTENT PREVENTIVE THERAPY IN THE POST-DISCHARGE MANAGEMENT OF SEVERE MALARIAL ANAEMIA IN PRE-SCHOOL CHILDREN; A MULTI-CENTRE RANDOMIZED PLACEBO CONTROLLED TRIAL IN SOUTHERN MALAWI

Kamija Phiri[1], Michael Esan[2], Michael Boele van Hensbroek[3], Brian Faragher[4], Sarah White[1], Feiko O. ter Kuile[4]

[1]Malawi-Liverpool Wellcome Trust Research Programme and Haematology Department, College of Medicine, Blantyre, Malawi, [2]Malawi-Liverpool Wellcome Trust Research Programme, Blantyre, Malawi, [3]Global Child Health Group, Emma Children's Hospital, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands, [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Despite adequate in-hospital treatment with blood transfusions and antimalarials, young children admitted with severe malarial anaemia in malaria endemic regions in southern Malawi and western Kenya are at high risk of dying or being re-admitted in the first few months after discharge. They may represent a particularly vulnerable group due to a combination of parasite, host, environmental and socio-behavioural factors. In highly endemic areas, failure to clear the initial malaria infection and the acquisition of new infections in the first few months after discharge may negate the initial improvements in haemoglobin concentrations resulting from the blood transfusion. Interventions that result in radical cure of the initial infection and prevention of subsequent malaria episodes will provide a time-window in which the bone marrow can recover to restore haemoglobin levels. We have completed a randomized placebo controlled trial of the efficacy and safety of Intermittent Preventive Therapy post-discharge (IPTpd) in children aged 4-59 months admitted for severe malarial anaemia requiring blood transfusion in 4 hospitals in southern Malawi. Convalescent children who had successfully received a blood transfusion and quinine were randomized to receive either 3 treatment courses of artemether-lumefantrine given at discharge, and again at 1 and 2 months post-discharge, or a single treatment course of AL at discharge followed by placebo at 1 and 2 months. Children were followed for a total of 6 month to compare the rates of rebound severe malaria, severe anaemia, or death 1-3 months after discharge (the intervention period) and during the subsequent 3 months. Between June 2006 and Aug 2009, 1435 children aged 4-59 months were recruited from the Queens Elizabeth Central Hospital in Blantyre and 3 adjacent district hospitals within 1 hour drive from Blantyre; over 90% were followed-up successfully. Analysis is ongoing and study results will be presented.

1213

THE IMPACT OF INSECTICIDE-TREATED NETS ON ACQUIRED HUMORAL IMMUNITY TO *PLASMODIUM FALCIPARUM*

Sandra Chishimba[1], Tamaki Kobayashi[2], Musapa Mulenga[1], Masiliso Phiri[1], Sungano Mharakurwa[1], Philip Thuma[1], William J. Moss[2]

[1]Malaria Institute at Macha, Choma, Zambia, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Acquisition of immunity to malaria is attributed to frequent exposure to the parasite. Lack of exposure can result in loss of immunity, leaving individuals at higher risk for severe disease and death. Intensified malaria control interventions have played a major role in reducing malaria incidence and mortality. An important intervention has been the mass distribution of long-lasting, insecticide-treated bed nets (LLINs). Following implementation of these effective control programs, population immunity is expected to decrease. It is, therefore, important to monitor population immunity to measure the impact of control measures and anticipate future epidemics upon reintroduction of the parasite and vector. We investigated the relationship between the distribution of insecticide-treated bed nets and acquired humoral immunity to *Plasmodium falciparum* in a low malaria transmission area of Macha, Southern Province, Zambia one year after the widespread distribution of LLINs. IgG antibody levels were measured using an enzyme immunoassay against whole, asexual stage *P. falciparum* antigens derived from NF54 strain schizont cell lysate. Seropositivity was defined as an optical density value > 0.57 based on the mean plus three standard deviations from 10 individuals never exposed to malaria. Plasma was extracted from whole blood samples stored as dried blood spots. Samples were collected between March and November 2008 from 313 individuals residing in randomly-selected households in southern Zambia. 56.5% of the study participants were seropositive for antibodies to whole *P. falciparum* antigens. 31% of seropositive individuals reported sleeping under a bed net, compared with 39% of seronegative individuals. Within one year of introducing LLINs for malaria control in southern Zambia, we found no significant reduction in seropositivity to whole P. faliciparum antigens in those who reported sleeping under a bed net compared to those who did not. Further monitoring of changes in population immunity to malaria is warranted in regions implementing accelerated control efforts.

1214

A SURVEILLANCE SYSTEM TO MEASURE CHILDHOOD MORTALITY AND DRUG RELATED ADVERSE EVENTS IN THREE DISTRICTS IN SENEGAL

BA K. Elhadji[1], J.L. Ndiaye[2], C. Sokhna[1], B. Cisse[2], J.F. Gomis[1], A. Marra[1], E. Faye[2], B. Faye[2], O. Faye[2], B.M. Greenwood[3], J.F. Trape[1], O. Gaye[2], P.J.M. Milligan[3]

[1]IRD Institut de Recherche pour le Developpement, Dakar, Senegal, [2]Universite Cheikh Anta Diop de Dakar, Dakar, Senegal, [3]London School of Hygiene and Tropical Medicine, London, United Kingdom

A simplified Demographic Surveillance System (DSS) was established in three health districts in Senegal to measure childhood mortality and for monitoring the incidence of serious adverse events in an area where seasonal IPTc is being implemented. The DSS covers a population of 602,000 people living in rural and semi-urban communities served by 54 health posts in three districts, and includes the area of the long-standing Niakhar DSS. Births deaths and migrations, ITN use and hospitalizations are recorded in 6-monthly household rounds. Verbal autopsies are performed on all deaths in the population served by 12 of the health posts. Mothers of children under 10 years are issued with a DSS card bearing details of all the children in their care. This card is used to record any health interventions delivered at village level including malaria IPT, and to confirm child identity when the child visits a health facility. Incidence of malaria confirmed by rapid diagnostic test was recorded by passive case detection at health facilities. A cluster sample survey of the under-5 population was done at the end of the transmission season to estimate the prevalence of malaria parasitaemia and anaemia. In 2008 there was no evidence of the seasonal peak in deaths from September to November which was characteristic of the pattern of mortality in previous years. A dramatic reduction in under-5 mortality observed in the population maintained under long term surveillance in the Niakhar DSS is borne out in the much larger area covered by the new surveillance system. Incidence of malaria among children <5yrs was less than 1 per 1000. The prevalence of *P. falciparum* parasitaemia at the end of the 2008 transmission season was less than 4%. Very low incidence of malaria in 2008 was associated with substantial improvement in child survival and disappearance of the usual seasonal peak in under 5 deaths associated with malaria transmission, in a large rural population not previously kept under demographic surveillance.

1215

NOVEL MEMBRANE-ASSOCIATED PROTEIN KINASES IN TRYPANOSOMES

Bryan C. Jensen[1], John A. Flaspohler[2], **Marilyn Parsons**[1]

[1]Seattle Biomedical Research Institute, Seattle, WA, United States, [2]Concordia College, Moorhead, MN, United States

Protein kinases modulate cellular responses to signals from the environment or from within the cell. Their ATP binding pocket renders them sensitive to small molecule inhibitors, making kinases attractive drug targets. *Trypanosoma brucei* possesses over 150 protein kinases; less than one-fourth of these have been studied in any detail. We have identified nine kinases that bear predicted transmembrane domains and hence could function to regulate processes spanning compartments within the parasite or between the parasite and its external environment. The presence of multiple transmembrane domains in the predicted structures of these kinases is unprecedented, indicating a novel means of signal transduction between the putative sensing and catalytic domains. Surprisingly, these kinases are localized to diverse structures within the parasite, indicating that they have varied cellular functions. One modulates the biogenesis of lipid bodies, organelles than function in intracellular lipid homeostasis. Two kinases localize to the secretory system, although these kinases do not resemble host cell ER kinases. Several of the kinases appear to be essential for bloodstream form viability, suggesting their potential use as drug targets.

1216

T CELL RESPONSES IN INDIVIDUALS WITH DISCORDANT *TRYPANOSOMA CRUZI* SEROLOGY

Diana Martin[1], Gerson Galdos-Cardenas[2], Michael Z. Levy[3], Brook Goodhew[1], Jenny Ancca[4], Cesar Naquira[4], Manuela Verastegui[5], Patricia Escalante-Mejia[4], Michelle Kaplinski[6], Katty Borini[4], Juan G. Cornejo del Carpio[7], Cesar Bocangel, Jr.[6], Cesar Bocangel, Sr.[7], Caryn Bern[1], Robert Gilman[2]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Johns Hopkins University, Baltimore, MD, United States, [3]University of Pennsylvania, Philadelphia, PA, United States, [4]Universidad Peruana Cayetano Heredia, Arequipa, Peru, [5]Universidad Peruana Cayetano, Lima, Peru, [7]PRISMA, Arequipa, Peru, [7]Oficina regional del Ministerio de Salud, Arequipa, Peru

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is a major cause of morbidity and mortality in Central and South America. Serological diagnosis of *T. cruzi* infection requires positive testing on two of three independent immunoassays. Geographic variations in the concordance of these diagnostic assays suggest that local parasite strain diversity may play a role in the strength of the anti-*T. cruzi* antibody response. Peru in particular has a high rate of discordant serology. To determine whether T cell responses could be used as a surrogate diagnosis in individuals with discordant serology, IFNγ production from peripheral blood mononuclear cells were stimulated with *T. cruzi* antigen was measured by ELISPOT assay. The studies were conducted in the

city of Arequipa and the town of LaJoya in the Peruvian Andes. Of the 103 subjects enrolled, 24 were seronegative, 53 were concordant by conventional serology ("seropositive"), and 26 were discordant by conventional serology ("discordant"). Peripheral blood mononuclear cells were stimulated with five different strains of *T. cruzi* - a laboratory strain (Brazil), two strains isolated from LaJoya, and two isolated from Bolivia - in order to determine if patients responded differently to parasites isolated from distinct geographic regions. Seronegative patients did not respond to the *T. cruzi* antigen. In the discordant and seropositive groups, the percent producing IFNγ in response to antigen stimulation was similar (approximately 67% in each group), and the frequency of IFNγ-producing cells was not statistically different in the discordant (mean = 39.7 spot-forming units/400,000 cells) and seropositive groups (mean = 42.7). Similar frequencies of IFNγ producing cells were detected regardless of the stimulating parasite strain, although responses were slightly lower in response to the Brazil strain. These data suggest that patients with discordant serology are likely infected with *T. cruzi* and that T cell responses may have utility as diagnostic tools in instances of inconclusive serology.

1217

ROLE OF HLA CLASS-II ALLELES (HLA DR AND DQ) IN PROTECTION/SUSCEPTIBILITY TO VISCERAL LEISHMANIASIS IN SUDAN

Mona E. Elfaki[1], Eltahir A. Khalil[1], Ahmed M. Musa[1], Brima M. Younis[1], Ahmed M. El- Hassan[1], Kerstin Muller[2], Rainer Blasczyk[2]

[1]Institute of Endemic Diseases/University of Khartoum, Khartoum, Sudan, [2]Institute for Transfusion Medicine, Hanover Medical School, Hannover, Germany

The antigen-presenting molecules of the HLA class-II (DR, DQ and DP) have a possible role in the susceptibility to autoimmune and infectious diseases. In this study the possible role of these alleles in susceptibility/protection against visceral leishmaniasis (VL), a potentially fatal parasitic disease was investigated. Following informed consent, fifty four individuals with parasitologically confirmed history of VL together with forty individuals with no history of VL were enrolled. The volunteers were mostly from the Masalit (Nilo-Saharan; Zurga) and the Fellata (Bargo/Maba/Wadians) tribes of eastern Sudan. Genomic DNA was extracted from peripheral blood mononuclear cells and buccal wash deposit using the Phenol-Chloroform Isoamyl technique. The HLA-Class II alleles were identified using high resolution PCR-Sequencing Based Typing (PCR-SBT) technique. The DRB1*1101 allele was detected in 28.6% of patients with VL and in 17.7% of those in those with no history of VL (*p=0.5).* DRB1*1001-DQA1*0501-DQB1*0501/DRB1*1001-DQA1*0102- DQB1*0501 haplotypes were detected with highly significant frequencies in individuals who contracted VL compared to those who did not (*p=0.01-p=000012* respectively*).* On the otherhand DRB1*0804-DQA1*0101 haplotype was significantly more predominant in individuals who did not develop the disease compared to those who did (*p=000000)*In conclusion: the DRB1*0804-DQA1*0101 haplotype is a probable protection haplotype, while DRB1*1001-DQA1*0501-DQB1*0501/DRB1*1001-DQA1*0102-DQB1*0501 haplotypes are probable susceptibility haplotypes for visceral leishmaniasis in the some ethnic groups of Sudan. Study findings could be helpful in peptide vaccine development for VL.

1218

LEISHMANIA MEXICANA INFECTED *PHEBOTOMUS YUCATNICUS*: NO PRODUCTION BY LYMPHOCYTES-MACROPHAGES

Fernando J. Andrade-Narvaez, Elsy N. Loria-Cervera, Liliana E. Villanueva-Lizama, Erika I. Sosa-Bibiano, Nicole R. Van Wynsberghe

Universidad Autonoma de Yucatan, Merida, Yucatan, Mexico

The Yucatan peninsula of Mexico is a well known endemic area of cutaneous leishmaniasis (CL) caused predominantly by *Leishmania (Leishmania) mexicana*. Studies of CL in the murine model (C57BL/6 infected with *L. major*) have documented the nitric oxide (NO) role in the elimination of the parasite through the classical macrophage activation by IFN-γ produced by CD4 Th1 lymphocytes. *Phebotomus yucatanicus* a primary reservoir of *L. mexicana* has been adapted to captivity and a colony for experimental studies has been developed. The main purpose was to compare NO production between cocultured macrophages and lymphocytes from *P. yucatanicus* infected with *L. mexicana* stimulated with or without SLA with and macrophages alone. Group A was inoculated with 1x102 promastigotes of *L. mexicana*; group B with 2.5x106 promastigotes; and group C inoculated with RPMI (n=14, per group). They were followed-up for 12 weeks to register clinical signs. At the end they were sacrificed to determine indirect nitrite oxide production employing Griess reaction. No one of the group A (subclinical) develops signs of CL, whereas in 13/14 (92.9%) of Group B show signs of CL. Nitrite oxide production was significative higher ($p<0.05$) in both Groups A and B (co-cultured lymphocytes and macrophages with or without SLA). Results support the employment of *P. yucatanicus* as a new experimental model to study CL.

1219

CHARACTERIZATION OF UNKNOWN PREDICTED GPI ANCHORED PROTEINS IN *TRYPANOSOMA BRUCEI BRUCEI*

Amy F. Savage[1], Gustavo C. Cerqueira[2], Yineng Wu[1], Sandesh Regmi[1], Najib El Sayed[2], Serap Aksoy[1]

[1]Yale University, New Haven, CT, United States, [2]University of Maryland, College Park, MD, United States

African trypanosomes of the *Trypanosoma brucei* complex undergo several differentiation steps during development in the tsetse fly, including differentiation to the procyclic form in the fly midgut, epimastigote forms in the proventriculus, and ultimately to mammalian infective metacyclic trypomastigotes in the salivary glands. Finally, metacyclics develop into the bloodstream form in the mammalian host. An in-silico screen of the *T. brucei* genome yielded 111 unknown proteins containing glycosylphosphatidylinisotol (GPI) anchor structures. GPI anchors typically bind proteins to cell membranes and as such, these hypothetical proteins may be expressed on the parasite cell surface. To determine if these unknown genes were preferentially expressed in particular developmental stages, expression analysis was performed on parasite infected salivary glands, proventriculus, and midguts, as well as bloodstream parasites. cDNAs were prepared, normalized, and expression levels were tested with gene-specific primers using a semi-quantitive assay. This revealed that the majority (66%) of the unknown genes were expressed in parasite stages infecting the proventriculus or salivary glands. Results were validated using quantitative PCR analysis. Twenty-one genes were determined to be specifically expressed in the salivary glands, and 15 of these were found to be expressed in the mammalian infective metacyclic form collected from tsetse saliva. A few of these genes were selected for further characterization at the RNA and protein level. Antibodies to the recombinant protein corresponding to one of these gene products have been used to demonstrate the expression profile of this protein in the both the immature (attached) salivary gland stages and free metacyclics. These

data can identify novel targets for blocking trypanosome transmission, and increase our broader understanding of this complex host-parasite interaction.

1220

INCREASED INTERFERON-Γ LEVELS OCCUR IN THE CEREBRAL SPINAL FLUID DURING THE CHRONIC PHASE OF *TRYPANOSOMA BRUCEI BRUCEI* INFECTION IN CATTLE

Monica Namayanja[1], Rosemary Flores[2], Ann Nanteza[1], Zachariah Nsadha[1], Sam Eyanu[1], Roger Prichard[3], Elisabeth Knapp[2], Vidadi Yusibov[2], George W. Lubega[1]

[1]Department for Veterinary Parasitology and Microbiology, Makerere University, Kampala, Uganda, [2]Fraunhofer USA Inc., Center for Molecular Biotechnology, Newark, DE, United States, [3]Institute of Parasitology, McGill University, Montreal, QC, Canada

Trypanosoma brucei brucei, one of the causative agents of nagana in animals, is a tissue invasive parasite. Monitoring of *T.b.brucei* infection in cattle is hampered by the lack of a clear parameter or marker that correlates with disease severity. In turn, this has made it difficult to measure benefits of disease intervention efforts such as immunization. *T.b.brucei* infection in cattle appears to mimic sleeping sickness in humans whereby it occurs in two phases, an acute or early phase where trypanosomes are readily seen in blood and a chronic or late phase where trypanosomes remain in tissues including the brain. In humans interferon gamma (IFN-γ) has been shown to be up-regulated in the cerebral spinal fluid (CSF) during the second stage of the disease and has been proposed as one of the markers of disease severity. However, the pathophysiological role of this cytokine in cattle has not yet been investigated. To substantiate this role, we carried out an experimental cattle infection under controlled indoor conditions. We analyzed the (IFN-γ) levels in the CSF during the acute phase and chronic phase and found that IFN-γ levels were elevated during the chronic phase of the disease. In addition to this, the levels of IFN-γ in the CSF were proportional to the dose used to initiate the infection. These data suggest that brain involvement plays a major part in the pathogenesis of *T.b.brucei* infection and IFN- γ is a marker for disease severity in cattle as has been proposed in humans

1221

DEVELOPMENT AND *IN VIVO* TESTING OF AN EFFECTIVE NOVEL DELIVERY VEHICLE FOR HETEROLOGOUS PROTEINS TO BOVINE ANIMALS

Glenn A. Mott[1], Ray Wilson[1], Anu Fernando[1], Dick Schaap[2], Theo Schetters[2], Jacqueline Matthews[1], Keith Matthews[1]

[1]University of Edinburgh, Edinburgh, United Kingdom, [2]Intervet Schering Plough Animal Health, Boxmeer, The Netherlands

We have developed a novel protozoan vehicle for the expression and delivery of heterologous proteins to bovines. Conditions for growth of the vehicle in a cell-free culture system have been developed and expression constructs engineered which permit the stable expression of a range of vaccine candidates or other proteins. These have been stably introduced and successfully expressed in the delivery vehicle, generating significant levels of the expressed protein. To evaluate the delivery-vehicle *in vivo*, the expression and immunogenicity of a known protective vaccine candidate against *Babesia divergens* has been evaluated. Inoculation in cattle resulted in the rapid establishment and stable maintenance of the vehicle over 9 weeks, without any adverse consequence for cattle welfare. Monitoring of the reactivity of the sero-response of the inoculated cattle by quantitative ELISA analysis demonstrated that specific antibodies to the delivered antigen were generated which progressively increased over time, achieving antibody titres known to be in the protective range. This novel delivery system offers potential as a flexible system, for example, for the generation of protective immune responses in cattle to a wide range of potential pathogens.

1222

COMPARATIVE GENOMIC HYBRIDISATION OF PHENOTYPICALLY DISTINCT *SCHISTOSOMA JAPONICUM* GEOGRAPHICAL ISOLATES

Geoffrey N. Gobert

Queensland Institute of Medical Research, Brisbane, Australia

Geographical isolates of Schistosoma japonicum from China and the Philippines exhibit a number of morphological and phenotypic differences, including pre-patent period, tegument topography, adult worm size, virulence and the subspecies of snail intermediate host infected. However, major genotypic differences have yet to be identified between the two strains. To address this deficit we have utilised microarray analysis to identify copy number variations within the genome of the two S. japonicum strains.

We have designed a Schistosoma japonicum custom microarray using EST data to examine 14,171 contigs. Since we intended to use the microarray to probe gDNA instead of mRNA, each probe was checked for relative specificity against the S. japonicum (Chinese) genomic supercontig assembly. We identified 8,310 microarray probes (of the total 14,171) with greater than 95% homology to only one region of the genome. This sub-group of probes present a high degree of specificity to the genome, since 60mer oligonucleotide probes with lower homology to the target sequence are known to result in significantly lower hybridisation efficiencies. The microarray platform was used to comparatively hybridise genomic DNA from Chinese mainland strain, and Philippine strain S. japonicum, in two colour experiments. Genomic DNAs from separate adult female or male parasites were used for comparison. We identified 306 contigs with differential hybridisation between the two S. japonicum isolates in both male and female parasites, which may represent a copy number difference. Validation and further characterisation of a subset of differentially hybridised genome regions is currently underway.It is hoped that these new data will provide insights into key aspects of the biology of this important human pathogen.

1223

GENETIC DIFFERENTIATION OF *SCHISTOSOMA MANSONI* LABORATORY POPULATIONS

Walter A. Blank[1], Matthew R. Test[1], Shi Fan Liu[1], Fred A. Lewis[2], Ronald E. Blanton[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Biomedical Research Institute, Rockville, MD, United States

Changes in the composition of schistosome populations under differing environmental conditions, in differing hosts, and between developmental stages can be studied in parasite lab strains. A longitudinal study was performed with archived samples collected over 16 years from a population of *Schistosoma mansoni* maintained at CWRU and samples collected over 9 years from a related strain at the Biomedical Research Institute (BRI). Pooled samples of worms or cercariae were genotyped to obtain allele frequencies at 14 microsatellite loci and analyzed for the genetic differentiation measure *D*. Low levels of differentiation (first sample vs. other samples, $D<0.05$) were observed throughout the period examined for the BRI strain and for most of the CWRU samplings, with the exception of two time periods. The first was the result of an admixture event when the CWRU life cycle was supplemented with cercariae from an external source (first sample vs. pre-mixture, $D = 0.07$, vs. post-mixture, $D = 0.13$). The BRI strain was shown to be the external source (pre-mixture CWRU vs. BRI, $D = 0.35$; $D = 0.07$ post-mixture). The second event was a reduction in the number of mice used to maintain the CWRU life cycle, which increased genetic drift (prior to reduction mean *D* per generation = 0.002; 0.014 after reduction). To investigate how immunological background of the host influences differentiation, mice from 4 strains (C57, CF1, BALB/c, and BALB/c IFNg KO) which had been previously infected with *S. mansoni* were reinfected. Worms were

perfused, pooled by host strain, and genotyped. Negligible differentiation (mean $D < 0.01$) was seen between host strains. Very low differentiation was also observed between all life cycle stages in previously uninfected CF1 mice (for cercariae vs. worms, cercariae vs. eggs, and worms vs. eggs mean $D < 0.01$). Although the laboratory is a simpler and more controlled environment, these studies demonstrate the potential for monitoring the dynamics of field populations.

1224

PHYSICAL CONTACT MODULATES LARGE-SCALE GENE EXPRESSION IN *SCHISTOSOMA MANSONI* ADULT MALES AND FEMALES

Giulliana T. Almeida, Sergio Verjovski-Almeida

Departamento de Bioquímica, Instituto de Química Universidade de São Paulo, Sao Paulo - SP, Brazil

A continuous pairing with male is essential for *Schistosoma mansoni* female sexual maturation and to maintain reproductive activity and egg production. Our aim was to identify genes that are modulated by either physical contact or by the possible diffusion of proteins and/or hormones in the medium. Males and females from mixed infections were recovered by perfusion and triplicates of adult worms were maintained *in vitro* at 5 different culture conditions during 13 days (paired, separated males or females isolated from the opposite sex, females kept in the presence of males without physical contact, and couples remated for 7 days after separation for 6 days). We used oligonucleotide microarrays with ~44,000 probes. Statistical multiclass analysis of female data (paired, separated, females in the presence of male, and remated females) identified 1010 differentially expressed genes (FDR = 0.01%). This analysis revealed that remating restored gene expression profile of separate females to a similar profile as that of paired females. Furthermore, we observed that females in the presence of males, without physical contact showed a different gene expression from that of females in the absence of males. Statistical multiclass analysis of male data (paired, separated and remated males) identified 277 differentially expressed genes (FDR 1%). Males' gene expression was modulated by direct contact with females in paired couples, since paired male expression profile was significantly different from that of isolated males. Remating of separated males led to a gene expression profile similar to that of paired males. These results provide strong evidence for the influence of physical contact on large-scale gene expression of male and female adult worms. They also show that some genes, for which the change in expression does depend on the presence but not on direct contact between male and female, are possibly regulated by the diffusion of proteins and/or hormones released by the parasites in the medium.

1225

CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF *SCHISTOSOMA MANSONI* U6 PROMOTER

Mary A. Ayuk[1], Sutas Suttiprapa[2], Gabriel Rinaldi[2], Victoria H. Mann[2], Clarence M. Lee[1], Paul J. Brindley[2]

[1]Howard University, Washington, DC, United States, [2]The George Washington University, Washington, DC, United States

The recent release of draft genome sequences of two of the major human schistosomes has underscored the pressing need to develop functional genomics approaches for these significant pathogens. The use of small interfering RNA (siRNA) molecules to achieve double stranded RNA-mediated interference (RNAi) has emerged as a powerful approach to sequence-specific gene knockdown. Vector based methods using siRNA expressing plasmid are used to induce RNAi in cells of eukaryotes and provide a long-term knockdown effect. We are developing vector based RNAi in *Schistosoma mansoni* using the U6 promoter to drive short hairpin RNA targeting reporter firefly luciferase. An upstream region of the U6 snRNA gene predicted to contain the U6 promoter was amplified from genomic DNA of *S. mansoni*, cloned and sequenced. A short hairpin RNA construct driven by U6 promoter targeting luciferase was engineered and cloned into pXL-Bac II (a *piggyBac* transposon donor construct). One day after mechanical transformation from cercariae, schistosomules of *S. mansoni* were exposed to the short hairpin construct by square wave electroporation. Short interfering RNAs (siRNA) specific for luciferase and a scrambled sequence short hairpin construct were used as positive and negative controls, respectively. 48h after exposure to short hairpin RNA constructs, schistosomules were electroporated with luciferase mRNA, harvested 3 hours later, and luciferase activity assayed. Short hairpin driven by *S. mansoni* U6 successfully knocked down luciferase activity by 20 to 50%. We are now transferring this technique to knock down endogenous genes in *S. mansoni* and to establish retrovirus and transposon based vector RNAi in transgenic schistosomes.

1226

TRANSCRIPTOMAL ANALYSES OF *SCHISTOSOMA MANSONI* PR1 TREATED WITH PRAZIQUANTEL

Jarrett Hines, Pauline M. Cupit, Anthony D. Aragon, Charles Cunningham

University of New Mexico, Albuquerque, NM, United States

Schistosoma mansoni is one of the most common etiological agents of human schistosomiasis and is estimated to infect more than 83 million people in 54 countries. Praziquantel (PZQ) is the least expensive, easiest to use and most readily available of all current anti-schistosomal drugs. One problem associated with PZQ treatment is that it does not kill schistosomes for a period of 2-4 weeks after they infect the human host. A second potential problem is the presence of drug resistance traits in natural populations of worms. As a result there is an urgent need to develop a new generation of anti-schistosomal drugs, a task that will be made easier by understanding the mechanism of action of PZQ. As yet, neither the molecule to which PZQ binds nor the means by which it kills mature schistosomes is known. The overarching aim of our study is to understand the molecular basis of PZQ sensitivity in *S. mansoni.* We hypothesize that PZQ sensitivity is a reflection of the differential expression of a gene that encodes either the PZQ binding partner or a downstream component of a biochemical pathway whose activity is influenced by PZQ binding to its partner molecule. Four and six week post infection (p.i.) *S. mansoni* PR1 have been treated *in vitro* with multiple sub-lethal as well as lethal doses of praziquantel. mRNA was extracted from replicate samples, cRNA prepared and labeled with Cy5 for transcriptomal analysis using a 44K *S. mansoni* microarray. All samples were compared against a common reference sample labeled with Cy3. Our initial analyses suggests that a number of genes associated with programmed cell death including cathepsins, Bax Interacting Factor 1 and death associated protein kinase (DAPK) are induced in 6 week (p.i.) praziquantel treated but not untreated schistosomes. DAPK has also been implicated in the phosphorylation of myosin light chains which has been reported to lead to vucuolation of cells and membrane blebbing - an often observed effect of PZQ on the mature but not juvenile worm tegument that may provide a more direct route to worm death.

1227

EXPRESSION LEVELS OF *SCHISTOSOMA MANSONI* MULTIDRUG RESISTANCE TRANSPORTERS INCREASE IN RESPONSE TO PRAZIQUANTEL AND ARE CORRELATED WITH REDUCED DRUG SUSCEPTIBILITY

Ravi S. Kasinathan, William M. Morgan, Robert M. Greenberg

University of Pennsylvania, Philadelphia, PA, United States

P-glycoprotein (Pgp) and the multidrug resistance-associated proteins (MRPs) are members of the ATP-binding cassette (ABC) superfamily of proteins involved in transport of toxins and xenobiotics from cells. These transporters likely play important physiological roles in the parasite's excretion of wastes and metabolites and provide attractive candidate targets for novel antischistosomal agents. Additionally, these

transporters are associated with development of drug resistance, and have been implicated in resistance to anthelmintics. We have previously shown that expression of *Schistosoma mansoni* Pgp (SMDR2) is altered in worms exposed to praziquantel (PZQ), the current drug of choice against schistosomiasis, and is expressed at higher levels in worms from isolates with reduced PZQ susceptibility. We have also shown that PZQ inhibits SMDR2, and also appears to be a substrate of SMDR2. Here, we examine the relationship between PZQ and SmMRP1, a *S. mansoni* homolog of mammalian MRP1, a transporter of anionic and GSH-detoxified compounds. SmMRP1 RNA is differentially expressed in adult males and females, which also show differences in the distribution of MRP1 immunoreactivity. Levels of SmMRP1 RNA increase transiently following exposure of adult worms to sub-lethal concentrations of PZQ. A corresponding, though delayed, increase in anti-MRP1 immunoreactive protein also occurs following exposure of worms to PZQ. PZQ-insensitive juvenile worms express higher levels of both SmMRP1 and SMDR2 RNA than mature adults, consistent with the hypothesis that increases in levels of schistosome multidrug transporters may be playing a role in development or maintenance of reduced susceptibility to PZQ. We are currently using molecular genetic and pharmacological approaches to define the physiological roles played by these transporters and to dissect the mechanisms by which they interact with PZQ and may modulate responsiveness to the drug.

1228

FORWARD GENETICS FOR *SCHISTOSOMA MANSONI*: QTL MAPPING OF OXAMNIQUINE RESISTANCE

Claudia Valentim[1], Claudia Queiroz[1], Matthew Berriman[2], Philip T. LoVerde[1], Timothy J. Anderson[3]

[1]University of Texas Health Science Center, San Antonio, TX, United States, [2]Wellcome Trust Sanger Institute, Hinxton, United Kingdom, [3]Southwest Foundation for Biomedical Research, San Antonio, TX, United States

Schistosomes show heritable variation in biomedically important traits such as drug resistance, host specificity, and virulence. Our central aim is to develop forward genetic methods (i.e. linkage mapping) to identify parasite genes that underlie this phenotypic variation. This approach is well suited to *S. mansoni* as the lifecycle can be maintained in the laboratory and clonal propagation of parasites within snails generates large numbers of genetically identical parasites. To provide proof-of-principal that this approach is feasible and powerful, we have mapped a genome region that underlies resistance to oxamniquine (OXA). Resistance to OXA has arisen in nature, has a simple recessive basis and results in ~500-fold reduction in drug sensitivity. We crossed resistant and sensitive parasites (parents) and then crossed two F1 individuals to generate multiple F2 progeny, at each stage isolating individual parasite genotypes by infecting snails with single miracidia. We measured OXA-resistance by monitoring death of cultured worms following drug exposure and genotyped parents, F1 and F2 progeny using 64 microsatellite markers distributed at ~20cM intervals across the genome. As expected trait segregation in the cross was consistent with recessive inheritance as F1s were sensitive and ~25% of F2 progeny were resistant. We used the *S. mansoni* linkage map developed by our group to locate quantitative trait loci (QTL) underlying OXA resistance. We found a strong QTL (LOD = 5.43) on the p arm of chromosome 6 where microsatellite markers segregate closely with OXA resistance. The two parents of this cross have now been sequenced, simplifying fine mapping and identification of candidate genes. Successful identification of gene(s) that underlie OXA-resistance will provide insights into mode of drug action, allow development of modified compounds that kill resistant parasites, generate selectable markers for genetic manipulation, and set the stage for forward genetic analyses of a range of biomedically important traits including praziquantel resistance.

1229

BURDEN OF MALARIA IN HIV-POSITIVE PREGNANT WOMEN IN IBADAN, SOUTHWEST NIGERIA: AN ONGOING STUDY

Catherine O. Falade[1], Olusegun G. Ademowo[2], IkeOluwapo O. Ajayi[3], Olubukola A. Adesina[4], Hannah O. Dada-Adegbola[5], Isaac F. Adewole[6], Phylis Kanki[6]

[1]Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria, [2]IMRAT, University of Ibadan, Ibadan, Nigeria, [3]EMSEH, University of Ibadan, Ibadan Nigeria, [4]Department of Obstetrics and Gynecology, University of Ibadan, Ibadan Nigeria, [5]Department of Medical Microbiology, University of Ibadan, Ibadan, Nigeria, [6]APIN Plus (PEPFAR) Project, College of Medicine, University of Ibadan, Ibadan, Nigeria

Pregnancy and HIV infection individually increase susceptibility to malaria. This gives the HIV-positive pregnant woman a double burden. Malaria transmission is intense and occurs all year round in southwest Nigeria. We evaluated the prevalence of malaria parasitemia by expert microscopy of Geimsa stained thick blood smear among HIV-positive (+ve) women at the PEPFAR Clinic and uninfected pregnant women at the antenatal clinic at booking both located at the University College Hospital Ibadan in southwest Nigeria. The prevalence of malaria parasitemia was 18.8% (24/128) among HIV +ve women and 5.9% (24/406) HIV -ve women (ρ = <0.0001, OR 3.673; 95% CI 2.004 -6.732). The geometric parasite density was higher among HIV +ve women but this was not significant. Mean hematocrit was significantly lower among HIV +ve women compared with their normal counter parts (30.37% ± 4.11 versus 34.5% ± 3.76; ρ <0.0001; *f-statistics* 107.129). The use of opportunistic infection chemoprophylaxis or antiretroviral therapy did not significantly influence the prevalence of malaria parasitemia. HIV -ve women booked significantly earlier than the HIV +ve women (21.26 ± 8.71 week versus 19.44 ± 7.64 weeks; ρ <0.029; *f-statistics 4.834)*. All socio-economic indicators (level of education of the women and their spouses, occupation of the women and their spouses, type of wall in accommodation, type of toilet facility, type of portable water and ownership of various gargets) were significantly (ρ <0.0001) lower among HIV +ve women. In conclusion, malaria exerts significant burden on HIV positive women in southwest Nigeria. These findings underscore the need for an adequate regimen of IPTp as well as other malaria control efforts (ITN, IRS and prompt case management) specifically targeted at HIV +ve women.

1230

HUMAN IMMUNODEFICIENCY VIRUS (HIV) TYPES WESTERN BLOT PROFILES AS SURROGATE MARKERS OF HIV DISEASE PROGRESSION

Kerina Duri[1], Fredrik Müller[2], Felicity Z. Gumbo[1], Munyaradzi P. Mapingure[1], Mike Z. Chirenje[1], Babill Stray-Pedersen[2]

[1]University of Zimbabwe, Harare, Zimbabwe, [2]University of Oslo, Oslo, Norway

A nested case-control study within a PMTCT cohort of antiretroviral therapy naive pregnant women. HIV-1/-2 Western blot test and surrogate markers of HIV disease progression were determined at 36 gestational weeks. Among the 64 pregnant women with HIV infection 98.4 % had pure HIV-1 infections and one woman (1.6%) had dual HIV-1/HIV-2 infections, with 100% band reactivity to both the envelope and polymerase proteins. However reactions to the *gag* core protein genes varied, being 100%, 90%, 70% and 63% for the p24, p17, p39 and p55 respectively. Lack of antibody reaction to *gag* p39 protein was significantly associated with disease progression among women with chronic HIV-1 infection as demonstrated by the presence of lymphadenopathy, anemia and higher viral load, p=0.010, 0.025 and 0.016 respectively. Although not statistically significant, women who had *gag* protein p39 missing were 1.4 times more likely to transmit the virus to their infants. In conclusion, absence of *gag* p39 and *pol* gene bands was significantly associated with disease progression and sero-conversion respectively. This data emphasises the importance of considering both the *env* and *pol* genes

proteins in the intepretation of positive Western blot HIV test results. Band profiles and simple laboratory tests like differential counts together with clinical symptoms could be useful in establishing and evaluating disease progression prior to antiretroviral therapy.

1231

MARKED REDUCTION IN THE PREVALENCE OF MALARIA AND ANEMIA IN HIV-INFECTED PREGNANT WOMEN TAKING COTRIMOXAZOLE WITH OR WITHOUT SP-IPT IN MALAWI

Atupele Kapito-Tembo[1], Steven R. Meshnick[1], Michaël Boele van Hensbroek[2], Kamija Phiri[3], Margaret Fitzgerald[4], Victor Mwapasa[5]

[1]University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC, United States, [2]Emma Children's Hospital AMC, University of Amsterdam, Amsterdam, The Netherlands, [3]Malawi–Liverpool–Wellcome Trust Clinical Research Programme, College of Medicine, Blantyre, Malawi, [4]Médecins Sans Frontières (Belgium), Thyolo, Malawi, [5]Department of Community Health, College of Medicine, Blantyre, Malawi

HIV-infected women are at risk for both malaria and other opportunistic infection. Current WHO guidelines recommend cotrimoxazole for all HIV-infected pregnant women. However, it is not known whether this regimen is as effective as sulfadoxine-pyrimethamine (SP) intermittent preventive therapy (IPTp) in preventing malaria. Here, we compare the effectiveness of cotrimoxazole and SP-IPTp in protecting against malaria in a cohort of HIV-infected pregnant women in Malawi. From 2005 to 2009, we conducted a cross-sectional study of HIV-infected pregnant women attending routine antenatal services at Thyolo district hospital, Malawi. Peripheral blood was tested for anemia (hemoglobin<11g/dl) and malaria by both microscopy and PCR. We also collected data on use of anti-malaria interventions and other potential risk factors. CTX prophylaxis for HIV-infected pregnant women was introduced as policy in 2007, but implementation problems resulted in some women receiving CTX alone, SP-IPTp or both. We enrolled 1,142 women of whom 1,121 had data on CTX and/or SP-IPTp intake. Of these, 49.7%, 29.8%, and 15.4% reported taking SP-IPTp only, CTX only and SP-IPTp plus CTX respectively. Compared with women taking SP-IPTp only, those taking CTX (with or without SP-IPTp) were less likely to have microscopic malaria (OR, [95%CI]: 0.09, [0.01-0.66] and 0.43, [0.19-0.97] respectively) or PCR-detected malaria infections (OR 0.24; 95%CI 0.10-0.62 and 0.44; 95%CI 0.25-0.78 respectively) or anemia (PR, [95% CI]: 0.67, [0.54-0.83] and 0.72, [0.61-0.83] respectively). In conclusion, in HIV-infected pregnant women, CTX with or without SP-IPTp reduces the risk of malaria and anemia compared to SP-IPTp only. Longitudinal studies need to assess the effects of these drugs on birth outcomes and toxicity.

1232

INCIDENCE OF MALARIA EPISODES IN WEST AFRICAN ADULTS HIV-1 INFECTED PATIENTS EXPOSED OR NOT TO COTRIMOXAZOLE: MALHIV COHORT STUDY

Frédéric N. Ello[1], Mariama Ouiminga[1], Lise Denoeud[2], Adrien Sawadogo[3], Alassane Hamar[4], Soumaré Masserigne[5], Serge P. Eholié[1], Emmanuel Bissagnene[1]

[1]CHU Treichville, Abidjan, Côte D'Ivoire, [2]Hopital saint-antoine, Paris, France, [3]Hopital Sourou Sanon, Bobo-dioulasso, Burkina Faso, [4]Hopital Point G, Bamako, Mali, [5]CHU de Fann, Dakar, Senegal

A key issue of the interrelation between HIV/AIDS and malaria is the impact of cotrimoxazole chemoprophylaxis on the natural history of malaria among HIV-infected patients. The objective of this study was to determine the incidence of malaria among HIV-1 infected patients, receiving daily cotrimoxazole or not. West African adults HIV-1 infected patients, receiving or not cotrimoxazole were enrolled in the 30 months MALHIV cohort study. Patients were divided into two groups: Group A CD4 < 350/mm^3 exposed to cotrimoxazole and group B CD4 ≥ 350/ mm^3 non exposed to cotrimoxazole. The cumulative incidence of clinical and parasitological malaria episodes was evaluated at baseline, every six months and in case of fever. Comparison of incidence in the two groups was used to assess the influence of cotrimoxazole primary chemoprophylaxis. At inclusion, the 548 enrolled patients [mean age=35.1 year, sex ratio: 0.29] had a CD4 mean of 198/µl and a viral load mean of 5, 2 $\log_{10}$ 368 patients (68%) were included in group A and 168 (32%) in group B, of whom 23 (4%) were lost to follow-up and 11 (2%) died. 325 patients received antiretroviral therapy. Asymptomatic parasitemia was diagnosed in 13 patients (2, 5%). Overall 35 malaria episodes (6.4%) occurred in 30 patients (5 in group A, 30 in group B). One case of severe malaria was noted. Malaria episodes were less frequent in group A. (1.3% versus 17% [RR= 0.03, IC=0.07-0.19, P< 0.05]). Fever episodes were less frequent in group A. (18, 9 %versus 63% [RR= 0,45; IC=036-0, 55 and P<0.05]. In conclusion, there is a trend of protection against malaria and fever episodes in HIV infected patients receiving cotrimoxazole in stable malaria transmission areas, even at lower CD4 cell counts.

1233

UTILITY OF PARACHECK-*PF*™ MALARIA RAPID DIAGNOSTIC TEST FOR THE DIAGNOSIS OF MALARIA AT POINT OF CARE AMONG ADULT HIV-POSITIVE PATIENTS IN IBADAN SOUTH WESTERN NIGERIA: AN ONGOING STUDY

Catherine O. Falade[1], George O. Ademowo[2], Olubukola A. Adesina[3], Isaac F. Adewolw[3], Phylis Kanki[3]

[1]University of Ibadan, Ibadan, Nigeria, [2]Institute for Advanced Medical Research and Training, University of Ibadan, Ibadan, Nigeria, [3]APIN Plus (PEPFAR) Project, College of Medicine, University of Ibadan, Ibadan, Nigeria

Malaria and HIV are major public health problems in sub-Saharan Africa. Febrile illnesses occur frequently among HI+ve patients and these febrile episodes are often first treated presumptively as malaria in endemic areas. In other to eliminate unnecessary treatments; reduce drug-drug interactions and the chances for emergence of drug resistant *Plasmodium,* the World Health Organization recommends a parasite-based diagnosis of malaria as much as possible. We evaluated 301 HIV+ve patients with febrile episode suspected of being malaria by expert microscopy and Paracheck *pf*™, (a histidine-rich protein-2 based malaria rapid diagnostic test, Orchid Biomedical Systems, Goa India) at the PEPFAR Clinic of the University College Hospital Ibadan, Nigeria. The mean age was 36.7 years (±9.2). About 80% (240/301) enrollees were female and 26.9% (81) had received various antimalarial drugs in the preceding two weeks. The prevalence of malaria parasitemia was 18.9% (357/301) by microscopy of thick blood smear with a geometric mean parasite density of 470/µL (range 39 - 204,000/µL). The prevalence of parasitemia by Paracheck *pf*™ was 18.5% (55/297) and four samples which were all blood smear negative gave indeterminate result for RDT testing. Sensitivity and specificity of Paracheck *pf*™ when compared with microscopy at all parasite densities were 54.4% and 90% while corresponding figures at parasite densities ≥ 200/µL were 93.9% and 91.7% respectively. Negative and positive predictive values at all parasite densities were 90% and 54.4%. Corresponding negative and positive predictive values for parasite density ≥ 200/µL were 99.2% and 58.5% respectively. The overall test accuracy was 83.2% while test accuracy at parasite density ≥ 200/µL was 91.9%. In conclusion, Paracheck *pf*™ malaria rapid diagnostic test accurately ruled malaria "in or out" at the point-of-care, facilitating appropriate clinical management and averting unnecessary antimalarial therapy.

1234

DIAGNOSIS OF IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME IN SCHISTOSOMIASIS PATIENTS UNDERGOING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY IN WESTERN KENYA

Erick M. Muok[1], Diana Huis in 't Veld[2], Anali Conesa-Botella[2], Zipporah W. Ng'ang'a[3], George O. Ogola O. Ogola[4], Katja Polman[2], Luc Kestens[2], Diana M. Karanja[1], Robert Colebunders[2], Pauline N. Mwinzi[1]

[1]Kenya Medical Research Institute, Kisumu, Kenya, [2]Institute of Tropical Medicine, Antwerp, Belgium, [3]Jomo Kenyatta University, Nairobi, Kenya, [4]Maseno University, Maseno, Kenya

The immune reconstitution inflammatory syndrome (IRIS) is a frequent complication seen in HIV-TB co-infected patients during successful highly active antiretroviral therapy (HAART). IRIS is common in resource-limited countries where the underlying prevalence of opportunistic infections is high, and where patients initiating HAART are more likely to have advanced immunodeficiency. We conducted a clinical study to investigate the immunopathogenesis, clinical aspects and manifestation of IRIS in HIV-schistosome co-infected patients undergoing HAART along the shores of Lake Victoria in western Kenya. Adults who were exposed to snail-infested lake water and are dually infected with HIV and *Schistosoma mansoni* were enrolled into the study. Baseline and follow-up indicators included CD4 counts measured by four-color flow cytometry, viral load measured by PCR, *S. mansoni* egg counts by the Kato-Katz method and evaluation of liver pathology by ultrasound. The prevalence of *S. mansoni* infections in this population was over 90% while that of HIV infection was 16%, and 67.9 % of those that were HIV positive had dual infections. Only 4% had detectable liver pathology at baseline. The intensity of schistosome infection had no influence on either the percentage of T helper cells (CD4+) ($p = 0.3640$) or the frequency of viral load copies ($p = 0.8470$). However, individuals with *S. mansoni* infection only had significantly higher mean egg production compared to those with dual infections. Clinical monitoring of dually infected patients getting started on HAART is ongoing to determine the case definition and the prevalence of IRIS.

1235

ALANYL-GLUTAMINE SUPPLEMENTATION PROTECTIVE EFFECTS ON NELFINAVIR-INDUCED INTESTINAL EPITHELIAL INJURY

Manuel B. Braga-Neto[1], Cirile A. Warren[2], Bruna M. Oliveira[1], Jamilly G. Maciel[1], Jesus E. Sevilleja[2], Aldo A. Lima[1], Richard L. Guerrant[2]

[1]Federal University of Ceara, Brazil, Fortaleza, Brazil, [2]University of Virginia, Charlottesville, VA, United States

HIV protease inhibitors (PI) remain a crucial component of highly active therapy (HAART) contributing to reduce mortality and morbidity related to Human Immunodeficiency Virus (HIV) infection. However, discontinuation of therapy remains an important issue, which may be related to various side-effects, especially diarrhea. The aim of this study was to evaluate the effects of nelfinavir, an HIV PI, and of alanyl-glutamine supplementation, on intestinal cell migration, proliferation, apoptosis, necrosis, using a rat intestinal cell line, IEC-6, and on crypt depth, villi length, mitotic index and apoptosis using swiss mice. Migration was evaluated at 12 and 24h after injury, using a wounding assay. Proliferation was measured indirectly at 24 and 48h, using tetrazolium salt WST-1. Apoptosis and necrosis were measured by flow cytometry, using the Annexin V assay. We measured intestinal morphometry, mitotic index and apoptosis in mice following a seven day treatment with 100mg/kg of nelfinavir, given orally. Nelfinavir decreased IEC-6 cell proliferation and migration and increased apoptosis and necrosis. Alanyl-glutamine supplementation enhanced intestinal cell migration both at 12 and 24h and proliferation at 24 and 48h, but did not decrease apoptosis and necrosis after nelfinavir treatment. Nelfinavir decreased duodenal villus height and villus surface area, which was reversed with the addition of alanyl-glutamine and increased apoptosis. Alanyl-glutamine also increased crypt depth in the duodenum, jejunum, and ileum and duodenal mitotic index in nelfinavir-treated mice. In conclusion, alanyl-glutamine reversed nelfinavir-induced intestinal epithelial damage *in vitro* and *in vivo*. Alanyl-glutamine supplementation may be beneficial in the prevention or treatment of diarrhea induced by PIs during HAART.

1236

A NATIONAL MALARIA TRAINING MODEL FOR ACHIEVING RATIONAL USE OF ANTIMALARIAL MEDICINES IN SUB-SAHARAN AFRICA

Nancy L. Blum[1], Kelly Willis[1], Umaru Ssekabira[2]

[1]Accordia Global Health Foundation, Washington, DC, United States, [2]Infectious Diseases Institute, Makerere University, Kampala, Uganda

Presumptive treatment of malaria is a common practice in Africa resulting in widespread overtreatment, misuse of costly artemisinin-combination therapy (ACT), and inappropriate treatment of non-malarial febrile illnesses. Accordia Global Health Foundation's Integrated Management of Malaria (IMM) course and an on-site training program using Rapid Diagnostic Tests (RDT), developed in partnership with the Infectious Diseases Institute (IDI) at Makerere University and others, target multidisciplinary clinical teams and achieve substantial declines in the unnecessary use of antimalarials. Research conducted in partnership with the Uganda Malaria Surveillance Project (UMSP) demonstrated that the improvement was achieved at no detriment to patient health outcomes. Recently-issued WHO guidelines for malaria treatment will substantially increase demand for training in correct laboratory and RDT diagnosis of malaria, for the many African countries who will strive to achieve them. To enable high quality training delivered cost-effectively to a national scale, Accordia complemented its IMM and RDT training programs with field-based and peer-facilitated versions of those courses, led by graduates of the IDI-based courses at a fraction of the cost of centralized training. Following completion of additional coursework at IDI, IMM graduates conducted "Cascade Training" and achieved reductions in antimalarial use similar to those shown in IDI-based training: in three sites that received Cascade Training, the probability of an antimalarial drug being prescribed to someone who had a negative blood smear decreased by 28%; there was a 25% decline at eight sites that received the IDI-based training. The resulting National Malaria Training Model, reinforced by periodic site visits by Mobile Teams and a toll-free call center for clinical consultation, presents a cost-effective approach to build national capacity in the appropriate diagnosis of malaria and rational use of antimalarial medicines in sub-Saharan African nations.

1237

LARGE-SCALE IMPLEMENTATION OF INTERMITTENT PREVENTIVE TREATMENT IN INFANT WITH SULFADOXINE PYRIMETHAMINE FOR MALARIA IN RURAL AREAS OF HIGH TRANSMISSION IN SENEGAL

Mouhamed Ndiaye[1], Jean Louis Ndiaye[1], Doudou Sow[1], Youssoupha Ndiaye[2], Magatte Ndiaye[1], Sylvain Faye[3], Roger Tine[1], Babacar Faye[1], Alexandra De Sousa[4], Oumar Gaye[1]

[1]Laboratory of Parasitology, Faculty of Medicine, UCAD, Dakar, Senegal, [2]District Medical Office, Ministry of Health and Prevention, Saraya, Senegal, [3]Sociology Department, Faculty of Human Sciences UCAD, Dakar, Senegal, [4]Unit of Policy and Evidence, Health Section, UNICEF, New York, NY, United States

Intermittent Preventive Treatment in infants (IPTi) is a new promising intervention for malaria control in Sub Saharan Africa which can be delivered through the existing routine Expanded Programme on Immunization (EPI). Although the efficacy of IPTi is proven, there is

limited economic evaluation data on scaling up IPTi using sulfadoxine pyrimethamine (SP) as a strategy for malaria control in Senegal. Implementation scale-up cost was calculated by estimating IPTi incremental costs in the start-up year and in subsequent years. Costs included were the financial costs of delivering IPTi to infants (drugs and equipment) and of programme activities. In recurrent years, because a survey in IPTi areas showed high acceptability and health care worker knowledge, programme costs included only IPTi administration and safety surveillance. To estimate IPTi cost-effectiveness (IPTi net cost per case of malaria averted, per death averted, per year of life saved, and per disability adjusted life years) we calculated the intervention's economic costs in recurrent years using a pooled efficacy analysis. A time and motion study was also conducted to assess staff time on IPTi delivery and the associated costs. In order to delivery IPTi-SP to 17,500 infants the total amount of the financial expenditure of programme implementation was $40,753.

IPTi incremental financial costs on start up year (3.31 $US/infant) were substantially higher than in recurrent years (1.043 $US/infant). In startup years communication activities mobilized the most important expenditure (30%) meanwhile, half of the total programme resources is allocated to capacity building development of professional staff. In routine, the part of programme costs was US$ 0.81 per infant, and patient costs (drug and utensils) only 23.7 US cent/infant. The time needed to administer IPTi was an average of 2.19 min/child, representing 7% of the time spends by health workers in immunization clinics. The net cost per averted case of malaria was $22.11.The cost per death averted was $ 447, per year of life saved $ 23.84 and per DALYs $ 25.39. In conclusion, IPTi with SP through EPI can be scaled up successfully at a low cost. Using pooled efficacy results from previous IPTi trails we can see that it is a highly cost effective intervention in the study sites selected. But, the IPTi cost-effectiveness in the other areas in Senegal where malaria transmission is not high, worth assessing.

1238

ARTEMETHER-LUMEFANTRINE VERSUS DIHYDROARTEMISININ-PIPERAQUINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA: LONGITUDINAL OUTCOMES IN A COHORT OF YOUNG UGANDAN CHILDREN

Humphrey Wanzira[1], Taylor Sandison[2], Abel Kakuru[1], Victor Bigira[1], Emmanuel Arinaitwe[1], Jaco Homsy[3], Jordan W. Tappero[4], Moses Kamya[5], Grant Dorsey[6]

[1]Infectious Diseases Research Collaboration, Kampala, Uganda, [2]Department of Medicine, University of Washington, Seattle, WA, United States, [3]Institute for Global Health, University of California, San Francisco, San Francisco, CA, United States, [4]GAP, Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]Department of Medicine, Makerere University, Kampala, Uganda, [6]Department of Medicine, University of California, San Francisco, San Francisco, CA, United States

There are limited comparative data on the long term effects of artemisinin-combination therapies (ACTs) for the treatment of malaria. In a cohort of young Ugandan children living in a highly endemic area, we previously reported that artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP) were both highly efficacious, but DP was associated with a longer post-treatment prophylactic effect. Here we aimed to compare longitudinal outcomes in this cohort. Children were given a long-lasting insecticide treated bednet (LLITN) at enrollment and followed for all their healthcare needs. 305 children with a median age of 10 months (range 4-40) were randomized to AL (n=155) or DP (n=150) at the time of their first episode of uncomplicated malaria. The same treatment was given for all subsequent episodes of uncomplicated malaria and episodes of complicated malaria were treated with quinine. After randomization, children were followed a median of 22 months and a total of 2,592 treatments for malaria were given. The incidence of malaria following randomization was higher in the AL arm (5.44 episodes PPY) compared to the DP arm (4.74 episodes PPY), although this difference did not reach statistical significance (IRR=1.13, p=0.06). After randomization, only 27 treatments with quinine (1%) were given for complicated malaria (17 with convulsions, 10 with severe anemia). The incidence of complicated malaria was significantly higher in those randomized to AL compared to DP (IRR=4.84, p=0.006). All 6 early treatment failures were due to the development of complicated malaria within 2 days of initiating treatment with AL. There was one death due to malaria in a child randomized to AL. In this cohort of young children living in a highly endemic area the incidence of malaria was very high despite the use of LLITNs. However, treatment with AL or DP was highly efficacious and the incidence of complicated malaria was relatively low. DP was associated with a trend towards a modest decrease in the incidence of malaria and may lower the risk of complicated malaria compared to AL.

1239

COMMUNITY BASED MALARIA CONTROL IN SARAYA, SOUTHEASTERN SENEGAL

Demetri Blanas[1], Youssoupha Ndiaye[2]

[1]Mount Sinai School, New York, NY, United States, [2]Senegalese Ministry of Health, Dakar, Senegal

In 2009, Senegal's National Malaria Control Program (NMCP) initiated a new Home-based Malaria Management (HMM) program, in which community health workers (CHWs) are trained to diagnose *Plasmodium falciparum* with rapid diagnostic tests and to treat appropriate patients with artemisinin-based combination therapy (artesunate + amodiaquine). The intention is to provide care to 30 villages of 200 to 500 inhabitants, which are all located 5 to 90 km from the nearest government health facility in the Saraya district of southeastern Senegal. The NMCP designed and implemented the 3-day training, which covered: modes of transmission and strategies for prevention; the clinical presentation of malarial infection; and the protocol to diagnose and treat uncomplicated malaria. The training was conducted in French and translated into Malinké. The training materials included a PowerPoint presentation and an instructional illustrated packet. This study evaluated the effectiveness of the training of CHWs. The same self-administered printed multiple-choice questionnaire was administered before and after the training. The questionnaire included questions to assess modes of transmission and strategies for prevention, the clinical presentation of malarial infection, and the protocol to diagnose and treat uncomplicated malaria. Scores in these three areas were determined. Before and after scores were evaluated using a two-tailed t-test. A P-value ≤ 0.05 was considered significant. Twenty-six CHWs participated in the training. Six CHWs were unable to read the training materials or questionnaires. The CHWs who could read and write assisted the others. The mean before and after scores were 52% vs. 73% (p=0.02) for CHWs understanding of transmission and prevention; 37% vs. 47% (p=0.21) for recognition of the clinical presentation of malaria; and 52% vs. 75% (p<0.01) for the ability to understand and carry out the HMM protocol. This study suggests that the training was successful in improving CHWs knowledge of transmission and prevention and ability to follow HMM protocol. Improvements should be made in the training the CHWs in disease recognition and future training should take the literacy level of CHWs into account. Further studies should be done to see how this knowledge translates into field practice and the need for training to reinforce these skills.

1240

PHARMACOVIGILANCE AND ANTIMALARIAL TREATMENT IN UGANDA: RESULTS AND EXPERIENCE FROM A PILOT ACTIVE SURVEILLANCE SYSTEM

Hasifa Bukirwa[1], Charles Karumuna[1], Susan Nayiga[1], Sarah G. Staedke[2], Ambrose O. Talisuna[3]

[1]Uganda Malaria Surveillance Project, Kampala, Uganda, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom, [3]Medicines for Malaria Venture, Kampala, Uganda

The safety of artemisinin combination therapies (ACTs) under large-scale operational use is not fully known. Continued careful monitoring after registration and release onto the market is therefore essential to establish ACT safety under routine conditions. An effective reporting system is a key requirement. Adverse event (AE) reports can be collected though passive spontaneous reports by users and prescribers and through active surveillance of patients receiving treatment. Active follow-up of patients to prospectively assess for AEs after treatment is ideal for detecting safety signals much more quickly and for estimating the incidence of AEs. Between November 2008 and October 2009 we actively followed up 842 patients treated with an antimalarial at public health facilities to assess the incidence of AEs at intervals until 28 days after treatment. Although artemether-lumefantrine (AL) is the recommended first-line treatment of uncomplicated malaria in Uganda, frequent stock-outs of AL resulted in use of many alternative antimalarials during the study period, including chloroquine, amodiaquine, sulfadoxine-pyrimethamine, artesunate monotherapy and quinine. Of 443 AEs reported, 271 (61%) were reported in association with ACT use. Cough, loss of appetite, flu-like symptoms, abdominal pain and weakness/fatigue were commonly reported but no serious AEs were reported. Drug stock-outs at the public health facilities forces patients to fill prescriptions outside formal systems, and affected the quality of collected information. Frequent changes of participants' addresses as well the high resource intensity of the system were major challenges. Effective active adverse event surveillance would require strengthening existing health infrastructure including health worker skills, diagnostic capacity for investigating and managing suspected adverse drug reactions and a good patient referral system.

1241

PHARMACOVIGILANCE OF ARTEMETHER-LUMEFANTRINE IN PREGNANT WOMEN FOLLOWED UP UNTIL DELIVERY IN RWANDA

Stephen Rulisa[1], Nadine Kaligirwa[2], Steven Agaba[3], Petra F. Mens[4], Adriana Tami[4], Peter J. De Vries[5]

[1]Kigali University Teaching Hospital, National University of Rwanda, Kigali, Rwanda, [2]Centre for Treatment and Research on AIDS, Kigali, Rwanda, [3]Centre for Treatment and Research on AIDS (Trac-Plus), Kigali, Rwanda, [4]Royal Tropical Institute/KIT Biomedical Research, Amsterdam, The Netherlands, [5]Academic Medical Center, Division of Infectious Diseases, Tropical Medicine and AIDS, Amsterdam, The Netherlands

Antimalarial drugs that are considered safe during pregnancy become increasingly ineffective. Many countries shifted their treatment guidelines to artemisinin combination treatment (ACT). WHO recommends ACT for uncomplicated *falciparum* malaria in the second and third trimester of pregnancy. In Rwanda, the current policy recommends treatment with artemether-lumefantrine (AL) for malaria during the second and third trimester of pregnancy. However, safety data on ACTs in pregnancy are still limited and more data are warranted.In this proactive pharmacovigilance study we followed pregnant women treated with AL and a matching control group (CG) of healthy pregnant women not exposed to AL in the current pregnancy. Routine antenatal and peripartum data, pregnancy outcomes, congenital malformation and adverse events (AEs) were captured during acute episodes of malaria, routine ante-natal visits, at delivery in the health center or within 48hrs after a home delivery, by history taking and physical examination of the mother and the newborns at delivery. The data for 1783 women (AL n=881; CG n=902) showed: abortions: AL 8 (0.9%), CG 5 (0.6%); perinatal mortality:AL 28 (3.2%), CG 20 (2.2%); comprised stillbirth :AL 25 (2.8%), CG 16 (1.8%); neonatal death ≤7 days after birth :AL 3 (0.3%), CG 4 (0.4%), premature delivery AL :6 (0.7%), CG 3 (0.3%) and congenital malformations: AL 1 (0.1%), CG: 2 (0.2%). A total of 73 AEs were reported (AL 43 (4.9%), CG 30 (3.3%). The most common AEs were still birth. These data showed that the two arms were comparable in terms of pregnancy outcomes and AEs and did not show any specific safety concerns with AL treatment in pregnancy. The slight difference in AEs and pregnancy outcomes between the groups may be due to malaria itself but needs to be assessed further.

1242

EFFECTS OF STEADY-STATE LOPINAVIR/RITONAVIR ON THE PHARMACOKINETICS OF QUININE IN HEALTHY VOLUNTEERS

Myaing M. Nyunt[1], Yanhui Lu[2], Mahmood El-Gasim[1], Theresa Parsons[2], Brent G. Petty[2], Craig W. Hendrix[2]

[1]Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States, [2]Johns Hopkins University School of Medicine, Baltimore, MD, United States

Many antiretroviral and antimalarial drugs share overlapping cytochrome P450 (CYP) -mediated metabolic pathways, indicating potential for drug-drug interactions and clinically important changes in the efficacy or toxicity of these drugs. We conducted a phase I, multiple dose, sequential design pharmacokinetic study to assess the impact of chronic administration of ritonavir-boosted lopinavir (LPV/r), a fixed-dose combination of two HIV protease inhibitors increasingly used in developing countries, on the safety and pharmacokinetics of quinine sulfate in healthy volunteers. Thirteen adult volunteers received a single oral dose of quinine sulfate on Day 1 and Day 15, and LPV/r on Days 4-17. Blood samples were collected to measure plasma concentrations of total and free quinine and 3-hydroxy-quinine metabolite using HPLC-florescence detection. Adverse effects were common, mild-moderate in severity, and self-limited. EKG changes were seen in 7 of 13 participants. Day 15 to Day 1 geometric mean ratios (90% confidence interval) showed: free quinine area under the curve (AUC) 0.40 (0.74-0.22), maximal concentration (Cmax) 0.40 (0.58-0.27), terminal half-life (t1/2) 0.67 (1.0-0.45), apparent volume of distribution (V/F) 1.66 (1.15-2.40), apparent total clearance (Cl/F) 2.5 (1.34-4.65); total quinine AUC 0.49 (0.47-0.51), Cmax 0.51 (0.50-0.52), t1/2 0.79 (0.74-0.85), V/F 1.61 (1.59-1.63), Cl/F 2.0 (1.96-2.13). The addition of LPV/r caused a significant decrease in exposure of both total and free quinine and 3-hydroxyquinine, a decrease in protein binding and metabolite/parent ratio, and an increase in the apparent volume of distribution and total clearance. The study showed significant modification in quinine pharmacokinetics in the presence of LPV/r through a complex interplay of effects on CYP3A4, p-glycoprotein, and protein binding. Further studies are needed to assess changes in quinine pharmacokinetics and pharmacodynamics in individuals with malaria-HIV co-infection who are taking LPV/r-containing antiretroviral therapy.

1243

SUSTAINABILITY OF INTERVENTION FOR HOME MANAGEMENT OF MALARIA: THE NIGERIAN EXPERIENCE

Ikeoluwapo O. Ajayi[1], Samuel A. Jegede[2], Catherine O. Falade[1]

[1]College of Medicine, Ibadan, Nigeria, [2]Department of Sociology, University of Ibadan, Ibadan, Nigeria

An intervention was carried out between2005-2007 to improve home management of malaria using artemeter lumefantrine [Coartem®] in Ona-Ara Local Government Area (LGA) of Oyo State, Nigeria. At the expiration of the project, the community was implored to sustain the program and provide support to the trained community based medicine distributors (CMDs). This study evaluated the sustainability of the HMM project two years after its expiration. A community-based study was

conducted among CMDs and stakeholders in Ona-Ara LGA. A total of 12 FGDs was conducted among CMDs, mothers of children aged 0-5 years and community members. Ten Key Informant Interviews were conducted with community leaders, Primary Health Care Coordinator, Rollback Malaria Manager, and PHC unit staff. After transcribing and word processing the data, Word Atlas Ti software was used to analyse data according to themes. Most of the FGD participants and Key Informants indicated that they were aware of the home management of malaria project in the area. In fact, there was consensus that communities selected their CMDs who distributed Coartem®. Data revealed that the treatment guideline poster given to households was of good assistance for treating children with malaria and some participants could still recapitulate the required dosages for treating malaria among children of different ages. The participants were of the opinion that the occurrence and severity of malaria has reduced in the area. While some CMDs have abandoned the assignment, few continue to provide care to febrile children and distribute Coartem® when available as their own contribution to the good of the community. Source of Coartem® was still the nearest government health facilities but supply was irregular and hindered by incessant transfer of health workers who were acquaintances on the project. All the CMDs mentioned they did not receive any support from the community. Most of the participants wanted the project to continue to support treatment of malaria in the community. In conclusion, while the project has proved to be life transforming, community support for CMDs is a serious challenge to sustainable HMM.

1244

NOVEL SYNTHETIC OZONIDE OZ439: TOLERABILITY AND PHARMACOKINETICS IN HEALTHY VOLUNTEERS

Joerg J. Moehrle[1], Sarah Arbe-Barnes[2], Christoph Siethoff[3], J. Carl Craft[1], Stephan Duparc[1], Susan A. Charman[4], Maria Gutierrez[5], Sergio Wittlin[6], Jonathan L. Vennerstrom[7]

[1]Medicines for Malaria Venture, Geneva, Switzerland, [2]Fulcrum Pharma, Hemel Hempstead, United Kingdom, [3]Swiss BioQuant, Reinach, Switzerland, [4]Centre for Drug Candidate Optimisation, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Australia, [5]Comprehensive Phase One, Miramar, FL, United States, [6]Swiss Tropical Institute, Basel, Switzerland, [7]University of Nebraska, Omaha, NE, United States

OZ439 is a synthetic ozonide (1,2,4-trioxolane) that has potential value as a peroxide antimalarial agent. The clinical protocol design of a randomised, double-blind, placebo controlled study combined the multi-objectives of sequential single (SRD) and multiple dose rising (MRD) as well as a food-effect sub-study. The study was conducted under US-IND. Dose levels of OZ439 investigated in the SRD ranged from 50mg to 1600mg and in the MRD ranged 200mg to 800mg. The food effect (FE) component was studied with 800mg OZ439 in a placebo cross-over design. Healthy male and female subjects aged 18-55 years were recruited for the study. A total of 63 subjects (26 in SRD, 13 in FE, and 24 in MRD) were enrolled. Dose escalation was subject to approval from a Safety Review Board. Safety assessments consisted of vital signs, 12-lead and Holter ECG, laboratory tests, adverse events (AE), audiometry/BAEP parameters. Samples were taken for assay of OZ439 and its metabolites from blood drawn up to 96 hours post drug administration. OZ439 was shown to be safe and well tolerated at the doses studied in the SRD, MRD and fed/fasted state. The most common AEs for OZ439 (2 or more subjects) were headache, nausea, diarrhea, constipation, blood CPK increase, flushing, throat irritation and gastrointestinal hypermotility. One subject dosed with OZ439 (800mg) was prematurely discontinued due to syncope vasovagal (with ECG changes). There were no significant changes in laboratory values or mean changes in vital signs, audiometry/BAEP and ECG/QTc. On SRD and MRD, exposure to OZ439 and its metabolites (based on Cmax and AUC) increased with increasing dose, this being dose-proportional. Following MRD, mean plasma concentration-time profile of OZ439 was characterised by a median tmax of 3.00 hours followed by a multi-phase elimination. Mean t1/2 of OZ439 ranged from 37.8 to 41.7 hours. The plasma concentration-time profiles of the metabolites resembled that of parent compound. Accumulation of OZ439 was less than 2-fold.

The study results justify pursuing the development of OZ439 in a combination therapy for uncomplicated malaria.

1245

CHANGES IN *PLASMODIUM FALCIPARUM* ASEXUAL AND SEXUAL POPULATIONS IN CHILDREN WITH ACUTE INFECTIONS FOLLOWING TREATMENT WITH ARTEMISININ-BASED COMBINATION THERAPIES

Akintunde Sowunmi, Titilope M. Okuboyejo, Grace O. Gbotosho, Christian T. Happi, Obaro S. Michael, Onikepe A. Folarin

Department of Pharmacology and Therapeutics and Institute for Medical Research and Training, University of Ibadan, Ibadan, Nigeria

Artemisinin-based combination therapies (ACTs) may influence malaria transmission but the mechanisms of their effects have not been totally elucidated. The changes in *Plasmodium falciparum* asexual and sexual populations in the first 16 h following treatment with artemether-lumefantrine (AL) or artesunate-amodiaquine (AA) were evaluated in 435 children with acute infections. In 162 children there were significant increases in peripheral asexual parasitaemia at 1 h, and in 9 of these, a simultaneous but insignificant increase in gametocytaemia at 1 h, followed thereafter by a precipitous and significant fall in all patients suggesting mobilization into peripheral circulation before a lethal effect. A fifth of mobilized gametocytes were young with a peak at 1 h. Young gametocytes were not found in peripheral blood after 16 h. Time-course of gametocyte sex ratio (GSR) showed a female-male-female-biased cycle at 0 h, 4 h and 8 h, respectively suggesting a selective lethal or removal effect on male gametocytes. Time-course of GSR was independent of density in another cohort of 52 gametocyte carriers treated with AL or AA. All dynamic effects were similar in AL- and AA-treated children. AL or AA acutely mobilize asexual and sexual parasites and alter GSR before a lethal effect, and may reduce transmission by these mechanisms.

1246

CONSTRUCTION OF AN ANTIMALARIAL SET OF COMPOUNDS: A PUBLIC TOOL TO BOOST LEAD DISCOVERY

Laura Sanz[1], Francisco-Javier Gamo[1], Cristina de Cózar[1], Jaume Vidal[1], Sara Prats[1], Emilio Alvarez[2], Jose F. Garcia-Bustos[1]

[1]Diseases of the Developing World (DDW), Medicines Development Campus, GlaxoSmithKline, Tres Cantos (Madrid), Spain, [2]Centro de Investigacion Basica (CIB), Medicines Development Campus, GlaxoSmithKline, Tres Cantos (Madrid), Spain

Drug resistance to current antimalarials is widespread and no new class of antimalarials has been introduced into clinical practice since 1996. Target-based lead discovery has produced disappointing results, generally for lack of whole-cell activity of new compounds. To secure that property in all chemical starting points for new antimalarial leads, we have tested the approximately 2 million-compound library used for high throughput screening at GlaxoSmithKline for inhibitors of *Plasmodium falciparum* intraerythrocytic stages. Using a modified assay based on *P. falciparum* LDH activity as surrogate of parasite growth, we have found ca. 31K primary hits inhibiting *Plasmodium* growth more than 50% at a final compound concentration of 2 mM. To maximize the chances of selecting submicromolar inhibitors only those hits inhibiting more than 80% were retested to confirm activity (ca. 19K compounds). As result of these experiments a set of 13K compounds has been selected as confirmed inhibitors. This set has been named TCAMS (Tres Cantos Antimalarial set). Additional experiments to add knowledge to TCAMS have been performed and will be described. In order to encourage further research by the larger malaria community on the cellular targets and mode of

action of the compounds, we have made public the chemical structures, together with the above mentioned data. All the information is available at the next URL: http://www.ebi.ac.uk/chemblntd and ideally, this could catalyse a world-wide chemical genomics approach to better understand the drugable genome of *P. falciparum.*

1247

ADVERSE EVENT PROFILE OF SEVEN-DAY COURSES OF ARTESUNATE IN PATIENTS WITH ACUTE *FALCIPARUM* MALARIA

Delia Bethell[1], Youry Se[1], Chanthap Lon[1], David Saunders[1], Sea Darapiseth[2], Sabaithip Sriwichai[1], Jessica Lin[1], Ans Timmermans[1], Mali Itveerakul[1], Ses Sarim[3], Stuart Tyner[1], Duong Socheat[2], Mark Fukuda[1]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [3]Tasanh Health Center, Samlot, Cambodia

In a clinical trial of artesunate monotherapy for the treatment of uncomplicated *P. falciparum* malaria in otherwise healthy adults, 143 patients were randomized to receive one of three artesunate regimens: 2, 4 or 6 mg/kg/day for 7 days (n=75, 40 and 28). Adverse events (AEs) were recorded daily for the first 7 days, then weekly for a further 5 weeks. Symptoms present at baseline were not classified as AEs unless they worsened significantly. Overall artesunate was well-tolerated; only 1 patient had to have a dose repeated. One patient in AS6 was withdrawn on Day 1 after developing signs of severe malaria. In total 375 separate AEs were recorded, of which 149 (40%) occurred during the week of artesunate treatment, 67 in AS2, 46 in AS4 and 36 in AS6. During week 1 gastrointestinal disturbances were relatively common and reported by 12%, 35% and 36% patients in AS2, AS4 and AS6 respectively; in contrast, neurological AEs (5%, 3% and 1% of patients) and rashes (3%, 3% and 5%) were relatively uncommon and did not appear to be affected by artesunate dose. Laboratory abnormalities were detected in 44, 53 and 50% patients in the 3 dosing groups; however the majority of these were due to eosinophilia, which was probably attributable to treatment of the malaria infection. The most significant laboratory abnormality was a reduction in absolute neutrophil count which was detected after 3 doses of artesunate in 2 patients (1 in AS4 and 1 in AS6), and in a further 4 patients in AS6 one week after artesunate monotherapy had been completed. This finding led to closure of the high-dose artesunate arm and early termination of the trial. In conclusion these findings confirm that artesunate remains a well-tolerated drug even at high daily doses given for 7 days. However the occurrence of neutropenia in the high-dose arm 7 days after completion of treatment indicates that artesunate has a significant myelosuppressive effect and should be used with caution when high cumulative doses are given.

1248

A PHASE 4 RANDOMISED STUDY TO ASSESS THE TOLERABILITY OF ARTESUNATE-AMODIAQUINE (ASAQ) AND ARTEMETHER-LUMEFANTRINE (AL) FIXED DOSE COMBINATIONS (FDCS) FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* (PF) MALARIA IN LIBERIA

Birgit Schramm[1], Parastou S. Valeh[1], Elisabeth Baudin[1], Charles S. Mazinda[1], Richard Smith[1], Loretxu Pinoges[1], Timothy Sundaygar[1], Yah M. Zolia[2], Joel J. Jones[2], Eric Comte[3], Vincent Jullien[4], Gwenaelle Carn[5], Jean-Rene Kiechel[5], Elizabeth A. Ashley[1], Philippe J. Guerin[1]

[1]Epicentre, Paris, France, [2]National Malaria Control Programme, Ministry of Health and Social Welfare, Monrovia, Liberia, [3]Médecins Sans Frontières, Geneva, Switzerland, [4]Saint-Vincent de Paul, Paris, France, [5]Drugs for Neglected Diseases initiative, Geneva, Switzerland

Thorough assessment of the tolerability of new artemisinin-based combination therapies (ACTs) in the post-registration phase is needed, especially for the detection of adverse events (AEs) of potential concern such as haematological, liver or neurological toxicities. Our objective was to provide information on ASAQ and AL tolerability in patients >= 6 years (a less studied population), in a highly endemic area. An open-label, randomized controlled trial was conducted in 1000 patients with uncomplicated Pf malaria in Nimba County, Northern Liberia. Drug allocation was 1:1 (ASAQ Winthrop® : AL Coartem®) to a 3-day observed oral regimen, dosed by weight (ASAQ once daily; AL twice daily with fatty food). Treatment emergent clinical signs and symptoms (open questions) and laboratory events were collected until Day28. PCR corrected cure rates were measured. Overall, 92.1% (ASAQ) and 90.2 % (AL) of patients reported at least one AE. Severe AEs were rare and mostly asymptomatic (ASAQ: 3.4%; AL: 1.6%; p=0.064). Derangement of liver function tests (ASAT/ALAT increases) classified as severe was infrequent (ASAQ: 0.6%; AL: 0.2 %). Few severe but asymptomatic neutropenia AE were reported (ASAQ: 0.4%; AL: 0.2%), and only one patient with moderate anemia at baseline developed severe anemia (ASAQ arm). Some clinical AEs, i.e. fatigue (ASAQ: 39.8%, AL: 16.3%; p<0.001), vomiting (ASAQ: 7.1%, AL: 1.6%; p<0.001), nausea (ASAQ: 3.2 %, AL: 1.0 %; p=0.015) as well as anemia (ASAQ: 14.9%, AL: 9.8%; p=0.013) were reported with significantly higher frequency in the ASAQ arm. Clinical AEs were almost exclusively mild to moderate, occurred mainly during the first 3 days and did not lead to treatment discontinuation. No significant neurological AE was reported. Day28 PCR-corrected efficacy rates were high (ASAQ: 98%, AL: 100%) (No follow-up between Day7-28). Both ASAQ and AL were well tolerated in this large >= 6years population sample, with no AE-related treatment discontinuation. Notably, hepatotoxicity, neutropenia, anemia or clinically significant neurological toxicities were of no major concern in this study. Efficacy was high with both treatments.

1249

A SURVEY ON CLINICAL SAFETY OF 8-AMINOQUINOLINES

Hla Yin Myint[1], Colin Ohrt[1], Larry Walker[2], Nick White[3], Alan Magill[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]University of Mississippi, University, MS, United States, [3]Mahidol-Oxford Research Unit, Mahidol University, Bangkok, Thailand

The 8-aminoquinoline (8-AQ) antimalarials are the only class effective against *Plasmodium vivax* relapse and *P. falciparum* gametocytes. It has long been known that the patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency developed hemolysis and hemolytic anemia (HA) after the administration of 8-AQs. The purpose of this review is to compile all safety data in humans to better define risk-benefit of this class in an era of malaria elimination. We collected and reviewed the unpublished and published literature on safety of 8-AQs studies in human

by contacting research experts in the field, exploring and extracting database from libraries where data are available. Papers in languages other than English were also accessed. Data were entered and analyzed in Excel (2007) and SPSS for Windows, version 16. Between 1926 and December 2009, a total of 589 clinical series of 8-AQ in humans (N=469,375) were identified by the searches. An extensive literature on the safety of 8-AQs, mainly primaquine and pamaquine in humans has been identified. Primaquine receiving patients (N=435,626; 92.8%) were the highest proportion among 8-AQs receiving patients, followed by pamaquine (N=22,735; 4.8%). Among pamaquine receiving patients, 170 (0.7%) had HA resulting in 60 patients required blood transfusion and 10 patients had discontinuation of the drug. Six patients (0.3%) in pentaquine receiving patients had HA and 5 patients had to stop the drug. In primaquine receiving patients, 304 (0.07%) had HA resulting in 25 patients required blood transfusion and 43 patients needed to stop primaquine. Two patients (2/2395; 0.08%) in tafenoquine reported HA and one required blood transfusion. In overall literature, 11 deaths had been identified with pamaquine and only 4 deaths attributable to primaquine were identified in G6PD deficient children. No deaths were reported in other 8-AQs. In conclusion, this database will help public health official make risk-benefit decisions about of the use of the class of drugs for routine malaria management and in elimination operations.

1250

A RANDOMISED, DOUBLE-BLIND PLACEBO CONTROLLED TRIAL OF TWO DIFFERENT DOSING REGIMENS OF DIHYDROARTEMISININ-PIPERAQUINE FOR INTERMITTENT PREVENTIVE TREATMENT OF ADULTS AT HIGH RISK OF MALARIA IN THAILAND

Khin Maung Lwin[1], Julien Zwang[1], Aung Phyae Phyo[1], Mya Ohn[1], Stephane Proux[1], Mara Leimanis[1], Joel Tarning[2], Niklas Lindegardh[2], Phaikyeong Cheah[2], Pratap Singhasivanon[3], Nicholas J. White[2], **Francois Nosten**[1]

[1]Shoklo Malaria Research Unit, Mae Sot, Tak, Thailand, [2]Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, [3]Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Piperaquine is an effective antimalarial with a long elimination half life coformulated with dihydroartemisinin (DP). This ACT is efficacious, well-tolerated and has a long post treatment prophylactic effect. DP is a promising candidate for use as intermittent preventive therapy.In areas of low unstable transmission like the Thai-Myanmar border it is often young adult males who bear the brunt of malaria,as their occupation involves travel and exposure to foci of higher transmission. Between October 2006 and June 2008 a double-blind, randomised, placebo-controlled trial was performed in 1000 adult males at risk of acquiring malaria but with a negative malaria smear, attending the clinics of the Shoklo Malaria Research Unit in northwest Thailand.They were randomised into 1 of 3 groups: i) monthly DP treatment (DPm) ii) alternate month treatment (DPalt)and iii) monthly placebo. The primary endpoint was the protective efficacy at 36 weeks of follow-up, defined as one minus the rate ratio of the incidence in the treatment group compared to placebo. The incidence rate of malaria on DPm was 32 episodes per 1000 person-year at risk(PY),compared to 1068 episodes per 1000 PY in the placebo group giving a protective efficacy of 97% (95% CI 95-99, p=0.001). The incidence in the DPalt group was 239 per 1000 PY giving a protective efficacy of 78% (95% CI 72-80, p=0.001)compared to placebo. The number needed to treat(NNT)to prevent an additional case of malaria with DPm and DPalt compared to placebo would be 1 and 4.8 subjects respectively. The adverse event (AE) risk ratio in the DPm group was higher compared to the placebo group for dizziness and diarrhoea (p=0.001 for all comparisons).The mean (SD) number of episodes of AEs was 1.6 (0.5) in the DPm, 1.5 (0.4) in the DPalt, and 1.4 (0.3) in the placebo group. There was a significant difference (p<0.001) in average monthly drug concentrations for the DPm group [29.9 (29.1-30.6) mean (95% CI) ng/mL] compared to the DPalt group [15.4 (15.0-15.9) mean (95% CI) ng/mL]. The 52 subjects contracting malaria had significantly (p<0.001) lower average monthly piperaquine concentrations [11.4 ng/ml (10.3-12.4)] compared to subjects without malaria episodes (n=747) [23.1 ng/ml (22.6-23.6)]. Monthly DP treatment brought about a dramatic reduction in the incidence of clinical episodes of malaria in adults compared to placebo and may be considered as an alternative malaria control strategy in high risk groups.

1251

SOCIOCULTURAL FACTORS THAT INFLUENCE THE IMPLEMENTATION OF ACT INTERVENTIONS IN TANZANIA: BASELINE RESULTS FROM A QUALITATIVE EVALUATION

Denise Roth Allen[1], Emmy Metta[2], Catherine Goodman[3], Salim Abdulla[2], S. Patrick Kachur[1]

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

In 2010 Tanzania will begin providing subsidized antimalarial combination therapy (ACT) through retail drug outlets and supplying all government health facilities with malaria rapid diagnostic tests (RDT). A multidisciplinary evaluation to assess the effectiveness of these interventions on access and targeting of ACTs is currently underway in three regions. One component is examining sociocultural factors influencing implementation in two focus districts. We are using rapid ethnographic methods to document provider and community experiences with malaria diagnosis and treatment. Data will be collected at baseline and post-intervention and timed to capture seasonal differences. Baseline data collection in one site began in November 2009 and included 7 in-depth interviews with health facility and retail drug providers about their experiences with malaria case management, 4 focus groups with community members about their care seeking behaviors and experiences with ACT, and 15 in-depth interviews with people who experienced a recent fever episode to document actual care-seeking behaviors. Artemether-Lumefantrine (ALu) was viewed in a positive light at 3 of the 4 health facilities visited but was perceived as no longer efficacious at the fourth. Although retail providers also had a positive opinion of ALu, none were legally able to sell it. Some community members spoke of ALu's ability to cure malaria or cause side effects as dependent on an individual's body or blood group rather than on the drug itself**.** Although many viewed ALu in a positive light, complaints about "too many" tablets in the treatment dose were common. Both providers and community members decried the lack of diagnostic tests in government dispensaries. Community members referred to clinical diagnosis as "treating by guessing" and voiced concerns that ACTs were being prescribed for non-malarial illnesses. Despite these concerns, community members expressed a preference for health facilities over retail outlets for malaria treatment. In conclusion, provider and community perceptions of ALu are mostly positive, although some concerns about its efficacy and treatment regimen were noted. Widespread complaints about the lack malaria diagnostics suggest that the implementation of RDTs will be positively received, although acceptance of RDT results remains to be seen.

1252

INVESTIGATING THE RELATIONSHIP BETWEEN PRIMAQUINE HEMOTOXICITY AND ANTI-MALARIAL EFFICACY THROUGH METABOLIC PROFILING I: METABOLITE IDENTIFICATION

Brandon S. Pybus, Jason Sousa, Xiannu Jin, Dustin Carroll, ThuLan Luong, Rebecca Barnhart, NeCole Reese, Vanessa Collazo, Charlotte Lanteri, Hans Wei, Michael P. Kozar, Victor Melendez

Walter Reed Army Institute of Research, Silver Spring, MD, United States

The 8-aminoquinoline drug primaquine (PQ) is currently the only approved drug for the treatment of relapsing malaria, yet PQ is known to cause hemolytic anemia in patients with G6PD deficiency. Proposed mechanisms of toxicity suggest a role for transient reactive oxygen species formed

as a byproduct of metabolism. Interestingly, evidence also points to an active metabolite playing a vital role in the drug's antimalarial efficacy. A complete understanding of the overall metabolic profile of PQ is necessary to identify potential routes to circumvent toxicity while maintaining efficacy. Our lab is currently seeking to fully characterize the role of biotransformation in both the efficacy and toxicity of PQ. Accordingly, we have initiated a series of experiments to detect previously postulated metabolites, identify and characterize previously unknown species, and determine the role of individual enzymes in PQ's metabolic pathway. We have begun to explore metabolite formation in a series of *in vitro* systems, including microsomal and hepatocyte incubations, as well as in *in vivo* samples from collaborators' pharmacokinetic studies. These initial studies indicate the presence of several previously postulated metabolites, such as carboxyprimaquine, 5-hydroxyprimaquine, and 6-desmethylprimaquine, as well as suggesting the presence of species that have not been previously reported, such as aldehyde, acetyl, and ketone metabolites. In conjunction with enzyme phenotyping studies (see "Investigating the Relationship Between Primaquine Hemotoxicity and Anti-malarial Efficacy Through Metabolic Profiling II: Enzyme Phenotyping"), we seek to better understand interspecies differences in PQ metabolism that may enhance interpretation of animal models, correlate metabolites with specific enzyme pathways, and provide insight into the relationship, if any, between PQ hemotoxicity and anti-malarial efficacy.

1253

INVESTIGATING THE RELATIONSHIP BETWEEN PRIMAQUINE HEMOTOXICITY AND ANTI-MALARIAL EFFICACY THROUGH METABOLIC PROFILING II: ENZYME PHENOTYPING

Xiannu Jin, Brandon S. Pybus, Jason Sousa, Dustin Carroll, ThuLan Luong, Rebecca Barnhart, NeCole Reese, Vanessa Collazo, Michael P. Kozar, Victor Melendez

Walter Reed Army Institute of Research, Silver Spring, MD, United States

The 8-aminoquinoline drug primaquine (PQ) is currently the only drug in use against the persistent malaria caused by the hypnozoite forming strains *Plasmodium vivax* and *P. ovale*. However, the widespread use of PQ is complicated by its known hemotoxicity in patients with a genetic deficiency in G6PD, an enzyme implicated in cells' ability to combat oxidative stress. To date, the exact mechanisms and metabolic species responsible for PQ's hemotoxic and anti-malarial properties are not well understood. A better understanding of the metabolic profile of PQ may enable the development of analogs that maintain efficacy while minimizing or eliminating hemotoxicity. In the present study, the metabolism of PQ was evaluated in several *in vitro* systems, including: human hepatocytes and pooled human liver microsomes, with and without enzyme inhibitors; and recombinant metabolic enzymes from the cytochrome P450 (CYP), monoamine oxidase (MAO), and flavin-containing monooxygenase (FMO) families. PQ metabolism was measured as the decline of the parent compound peak in LC-MS chromatograms. As a result of this work, CYPs 1A2, 2D6, and 3A4, as well as MAO-A, MAO-B, and FMO-3 have been implicated as the key enzymes associated with PQ metabolism. Enzyme kinetics studies are currently in progress and will be presented. The incubation mixtures were also examined as part of our metabolite identification studies (see "Investigating the Relationship Between Primaquine Hemotoxicity and Anti-malarial Efficacy Through Metabolic Profiling I: Metabolite Identification"). Correlation of metabolites with specific enzymes will allow for a more complete understanding of the pathways associated with PQ metabolism and the relationship between efficacy and toxicity.

1254

EVALUATION OF THE COMPARATIVE EFFICACY AND SAFETY OF ARTEMETHER-LUMEFANTRINE, ARTESUNATE PLUS AMODIAQUINE AND ARTESUNATE PLUS AMODIAQUINE PLUS CHLORPHENIRAMINE (ARTEMOCLO™) FOR ACUTE UNCOMPLICATED MALARIA IN NIGERIAN CHILDREN

Catherine O. Falade[1], Hannah O. Dada-Adegbola[2], Olusegun G. Ademowo[3], Oyekanmi Nash[3], Oluwatoyin O. Ogunkunle[4]

[1]Department of Pharmacology and Therapeutic, University of Ibadan, Ibadan, Nigeria, [2]Department of Medical Microbiology, University of Ibadan, Ibadan, Nigeria, [3]IMRAT, University of Ibadan, Ibadan, Nigeria, [4]Department of Pediatrics, University of Ibadan, Ibadan, Nigeria

Artemisinin based combination therapy (ACT) is the current gold standard for the treatment of acute uncomplicated malaria. Amodiaquine (AQ) plus artesunate (AS) is one of the preferred ACTs. Chlorpheniramine (CP) has been shown to enhance the efficacy of amodiaquine. In an open labeled randomized trial, the comparative efficacy and safety of artemether-lumefantrine (AL), artesunate-amodiaquine (ASAQ) and artesunate-amodiaquine-chlorpheniramine (AQC) was evaluated in 159 Nigerian children aged 6months to 10years with acute uncomplicated malaria. Enrollees were randomized to receive AL, ASAQ or AQC at standard doses over 3 days. (AQC: 100mg AS + 300AQ + 4mg CP/tablet using AQ 10mg/kg/day for dosing). Assessment was by 28day WHO 2003 efficacy test (PCR unadjusted cure rates only). 144/159 (90.6%) completed the study. Mean fever and parasite clearance times for AL, ASAQ and AQC were similar (ρ=0.94 and 0.12 respectively). Day 14 ACPR was 100% for AL and AQC while that for ASAQ was 98% (ρ=0.39). Day 28 ACPR were 91.1%, 92% and 95.9% for AL, ASAQ and AQC respectively (ρ=0.62). ACPR at day 42 for 115/144 (79.9%) evaluable children were similar (ρ=0.48). AQC gave the best parasitemia clearance and hematological recovery on day2 (ρ=0.022 and 0.018 respectively). The three ACTs were well tolerated. In conclusion, the three drugs were efficacious and safe. AQC gave non-significant higher ACPR than the other two on days 28 and 42. The better hematological recovery and parasite clearance with AQC on day 2 may be a fine indication of the enhancement ASAQ effect.

1255

INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE IN PREVENTING MALARIA AND ANEMIA IN PREGNANCY AMONG WOMEN VISITING KORLE-BU TEACHING HOSPITAL, ACCRA, GHANA

Nana O. Wilson[1], Fatou Ceesay[1], Yassa Ndjakani[1], Naomi Lucchi[2], Jonathan Stiles[1]

[1]Morehouse School of Medicine, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention, Malaria Branch, Atlanta, GA, United States

Malaria and anemia takes a great toll on women in sub-Saharan Africa in terms of maternal morbidity and adverse birth outcomes. Intermittent preventive treatment during pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) is currently recommended for prevention of malaria in pregnancy in endemic areas. However, the effectiveness of this approach in preventing malaria and anemia during pregnancy is unclear. The objective of the study was to evaluate the effectiveness of IPTp-SP in preventing malaria and anemia among pregnant women attending antenatal clinic (ANC) at Korle-Bu Teaching Hospital (KBTH) in Accra, Ghana. A cross-sectional study comparing malaria and anemia incidence among pregnant women using IPTp-SP with non-IPTp-SP users was conducted. A total of 363 pregnant women were recruited of which 202 were users of IPTp and 161 were IPTp non-users. Malaria parasites and hemoglobin levels were determined. Thirty-one (15.3%) women using IPTp had malaria compared to 72 (44.7%) of women who did not use IPTp, $p < 0.001$. The number of anemic women not utilizing IPTp was significantly higher (58.4%, 94/161) than women using IPTp (22.8%, 46/202), $p < 0.001$. Controlling for

age and other variables, the difference in the incidence of malaria (odds ratio (OR) = 0.26, 95% confidence interval (CI) = 0.15 - 0.44, p < 0.001) and anemia (OR = 0.19, 95% CI = 0.11 - 0.34, p < 0.001) remained significant. Regardless of reported resistance to SP for malaria treatment, the IPTp-SP regime is effective in preventing malaria and anemia among pregnant women visiting ANC at KBTH. The implementation of the IPTp-SP strategy holds great promise for reducing the burden of malaria and anemia in pregnancy in Ghana.

1256

COST AND COST-EFFECTIVENESS OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN INFANTS WITH SULFADOXINE PYRIMETHAMINE IN SENEGAL

Mouhamed Ndiaye[1], Jean Louis Ndiaye[1], Abdou Diop[1], Doudou Sow[1], Ousmane Sy[1], Alexandra De Sousa[2], Oumar Gaye[1]

[1]Faculty of Medicine UCAD, Dakar, Senegal, [2]UNICEF, New York, NY, United States

Intermittent Preventive Treatment in infants (IPTi) is a new promising intervention for malaria control in Sub Saharan Africa which can be delivered through the existing routine Expanded Programme on Immunization (EPI). Although the efficacy of IPTi is proven, there is limited economic evaluation data on scaling up IPTi using sulfadoxine pyrimethamine (SP) as a strategy for malaria control in Senegal. Implementation scale-up cost was calculated by estimating IPTi incremental costs in the start-up year and in subsequent years. Costs included were the financial costs of delivering IPTi to infants (drugs and equipment) and of programme activities. In recurrent years, because a survey in IPTi areas showed high acceptability and health care worker knowledge, programme costs included only IPTi administration and safety surveillance. To estimate IPTi cost-effectiveness (IPTi net cost per case of malaria averted, per death averted, per year of life saved, and per disability adjusted life years) we calculated the intervention's economic costs in recurrent years using a pooled efficacy analysis. A time and motion study was also conducted to assess staff time on IPTi delivery and the associated costs. In order to delivery IPTi-SP to 17,500 infants the total amount of the financial expenditure of programme implementation was $40,753. IPTi incremental financial costs on start up year (3.31 $US/infant) were substantially higher than in recurrent years (1.043 $US/infant). In startup years communication activities mobilized the most important expenditure (30%) meanwhile, half of the total programme resources is allocated to capacity building development of professional staff. In routine, the part of programme costs was US$ 0.81 per infant, and patient costs (drug and utensils) only 23.7 US cent/infant. The time needed to administer IPTi was an average of 2.19 min/child, representing 7% of the time spends by health workers in immunization clinics. The net cost per averted case of malaria was $22.11.The cost per death averted was $ 447, per year of life saved $ 23.84 and per DALYs $ 25.39. In conclusion, IPTi with SP through EPI can be scaled up successfully at a low cost. Using pooled efficacy results from previous IPTi trails we can see that it is a highly cost effective intervention in the study sites selected. But, the IPTi cost-effectiveness in the other areas in Senegal where malaria transmission is not high, worth assessing.

1257

MALARIA HIGH THROUGHPUT SCREENING ON A GLOBAL SCALE

Sandra Duffy[1], Ian C. Bathurst[2], Vicky M. Avery[1]

[1]Discovery Biology, Griffith University, Brisbane, Australia, [2]Medicines for Malaria Venture, Geneva, Switzerland

This poster provides a brief summary of the principles and performance of an Anti-Malarial Imaging Assay utilized to screen more than 2.5million compounds. In collaboration with MMV, chemical libraries, which are structurally highly diverse, have been sourced from pharmaceutical, biotech and academic partners world-wide. Data is presented on significant aspects of assay development and HTS performance, including assay throughput, precision, reproducibility, sensitivity, and hit-rate. In addition, retest confirmation in dose response against at least one *Plasmodium* parasite strain and the mammalian cell line, HEK293, performed to ascertain the selectivity index, is discussed. The data described, demonstrates the variation of library formats from the different sources, including library storage plate specifications/descriptions, compound concentration and volume, plate seals and in-plate control well availability. All of these aspects have to be considered when processing the plates for HTS, as well as the handling of large data sets. An overview of the compound libraries screened to date and how they performed in the Anti-Malarial Imaging Assay is presented.

1258

PLASMODIUM BERGHEI ANKA: SELECTION OF RESISTANCE TO PIPERAQUINE AND LUMEFANTRINE IN A MOUSE MODEL

Daniel M. Kiboi

Kenya Medical Research Institute, Nairobi, Kenya

We have selected piperaquine (PQ) and lumefantrine (LM) resistant *Plasmodium berghei* ANKA parasite lines in mice by drug pressure. Effective doses that reduce parasitaemia by 90% (ED90) of PQ and LM against the parent line were 3.52 and 3.93 mg/kg respectively. After drug pressure (more than 27 passages), the selected parasite lines had PQ and LM resistance indexes (I90) [ED90 of resistant line/ED90 of parent line] of 68.86 and 63.55 respectively. After growing them in the absence of drug for 10 passages and cryo-preserving them at -80 °C for at least 2 months, the resistance phenotypes remained stable. Cross-resistance studies showed that the PQ-resistant line was highly resistant to LM, while the LM-resistant line remained sensitive to PQ. Thus, if the mechanism of resistance is similar in *P. berghei* and *P. falciparum*, the use of LM (as part of Coartem®) should not select for PQ resistance. The stability of these phenotypes indicates that underlying mechanisms of resistance are probably coded into the cell genome and hence form the platform for studies on mechanisms of resistance of LM and PQ in *P. berghei*.

1259

EFFICACY OF CHLOROQUINE OR ARTEMETHER-LUMEFANTRINE AGAINST *PLASMODIUM VIVAX* AND ARTEMETHER-LUMEFANTRINE AGAINST UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CENTRAL ETHIOPIA

Bereket Hailegiorgis[1], Jimee Hwang[2], Zenebe Melaku[1], Samuel Girma[1], Takele Teshi[1], Sintayehu Gebresellasie[1], Leykun Demeke[1], David Hoos[3], Kedir Gobena[4], Moges Kassa[5], Daddi Jima[6], Richard Reithinger[7], Joseph Malone[8], S. Patrick Kachur[2], Scott Filler[2]

[1]International Center for AIDS Care and Treatment Programs, Addis Ababa, Ethiopia, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]International Center for AIDS Care and Treatment Programs, New York, NY, United States, [4]Oromia Regional Health Bureau, Ethiopia, Addis Ababa, Ethiopia, [5]Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia, [6]Federal Ministry of Health, Addis Ababa, Ethiopia, [7]United States Agency for International Development, Addis Ababa, Ethiopia, [8]Centers for Disease Control and Prevention, Addis Ababa, Ethiopia

In-vivo efficacy assessments of the first-line treatments for both *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv) are essential for effective case management in Ethiopia. In Ethiopia, first-line treatment is artemether-lumefantrine (AL) for Pf and chloroquine (CQ) for Pv. However, with stock-outs of chloroquine and limited laboratory confirmation of species, AL becomes the default treatment for all malaria infections. Between October-November 2009, we conducted a 42-day, three arm, open label study of AL for Pf, AL for Pv, and CQ for Pv in individuals >6 months age at two sites in Oromia Region who had documented Pf or

Pv mono-infection according to the standard WHO protocol. The primary and secondary endpoints were PCR uncorrected and corrected adequate clinical and parasitological response on days 28 and 42, respectively. Tests for drug levels, genotyping, and molecular markers of resistance for all three arms are currently in process to differentiate recrudescence from new infection. Of 4426 patients tested, those with confirmed *falciparum* malaria were enrolled and treated with AL (n=120); patients confirmed with vivax malaria were enrolled, randomized and treated with AL (n=122) or CQ (n=120). The uncorrected adequate clinical and parasitological response for Pf patients treated with AL was 99% on day 28 and 42. Eight Pf patients (7%) presented with Pv infection during follow-up and were excluded from the per protocol analysis. The cure rates for Pv patients treated with AL were 76% on day 28 and 58% on day 42; those for Pv patients treated with CQ were 91% on day 28 and 68% on day 42. There were very few day 3 positives with one case each in the Pf and Pv-CQ arms. There were no serious adverse events. Nausea/ vomiting and oral lesions were the most common adverse events. AL remains a highly effective treatment for uncomplicated *falciparum* malaria in the study setting. Both treatments of vivax malaria were complicated by high rates of recurrent parasitemia. Molecular studies may help to differentiate recrudescence from new infections, but in either case, CQ was more effective than AL in delaying the recurrent parasitemia. Effectively managing Pv relapse poses a major challenge to malaria control.

1260

NOVEL HIGH THROUGH-PUT MOLECULAR DIAGNOSTIC ASSAY DETECTS INCREASED *PLASMODIUM VIVAX* ANTIMALARIAL RESISTANCE IN PAPUA NEW GUINEA

Lincoln Timinao[1], Céline Barnadas[1], Serej D. Lee[1], Ore Topurua[1], Jonah Iga[1], Peter A. Zmmerman[2], Peter M. Siba[1], Ivo Mueller[1]

[1]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea, [2]Center for Global Health and Diseases, Case Western Reserve University, Cleaveland, USA, Goroka, Papua New Guinea

Amodiaquine or chloroquine plus sulfadoxine pyrimethamine (SP) have been the first line antimalarial treatments in Papua New Guinea (PNG) since 2000. This regime was designed for treating *Plasmodium falciparum* infections but because four species of human malaria parasites are transmitted in PNG, potential for development of drug resistance exists for each individual species. In numerous studies, *P. falciparum* resistance to 4-aminoquinolines and SP has been associated with mutations in *dhfr* (dihydrofolate reductase), *dhps* (dihydropteroate synthase), *crt* (chloroquine resistance transporter) and *mdr1* (multidrug resistance 1) genes. Orthologs of these genes and similar patterns of sequence polymorphisms have been identified in *P. vivax*. Following development of a novel molecular diagnostic multiplex strategy (Polymerase Chain Reaction - Ligase Detection Reaction- Fluorescent-Microsphere based Assay), we conducted a study to screen *P. vivax* for molecular markers of resistance in *pvdhfr*, *pvdhps* and *pvmdr1*. A total of 2381 blood samples collected from Madang and East Sepik Provinces were first evaluated for *Plasmodium* species infection status. Of these, 590 *P. vivax* infected samples were screened for single nucleotide polymorphisms (SNPs; 26 different alleles) using the multiplex drug resistance diagnostic assay. High rates of double and triple mutant parasites at *pvdhfr* codons 57-58-61 and mutant 117T were detected. We also detected a high rate of mutation at *pvdhps* codon 647 (S-->P) and at *pvmdr1* codon 976 (Y-->F). Patients from Madang were significantly more often infected with highly mutant parasites than in East Sepik Province possibly reflecting a different evolution of parasites populations ($p<0.01$, *dhfr* triple mutant allele). Genotyping methods developed here will improve our understanding of drug resistance in *P.vivax* and help monitor the effectiveness of new artemisinin-based combinations in PNG.

1261

MONITORING *EX VIVO* MALARIA DRUG SUSCEPTIBILITY IN SENEGAL AFTER INTRODUCTION OF ARTEMISININ-BASED COMBINATION THERAPIES

Papa Diogoye Sene[1], Daouda Ndiaye[1], Daria V. Tyne[2], Amanda Lukens[2], Kayla Barnes[2], Vishal Patel[3], Michelle LeRoux[2], Omar Ndir[1], Souleymane Mboup[1], Sarah Volkman[2], Johanna Daily[4], Dyann Wirth[2]

[1]Faculty of Medicine and Pharmacy, Cheikh Anta Diop University, Dakar, Senegal, [2]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA, United States, [3]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, United States, [4]Department of Medicine, Albert Einstein College of Medicine, Bronx, NY, United States

Malaria remains an important public health issue in developing countries, despite efforts to reduce morbidity and mortality from this disease. Drug treatment with artemisinin-based combination therapy (ACT) is critical to achieving reduction of malaria burden. With increasing drug resistance, malaria drug policies changed in Senegal in 2006 and ACTs were introduced. In this study we determined the *ex vivo* drug resistance profile of ~250 field isolates from Thies, Senegal since 2007 using a non-radioactive DAPI assay to better anticipate resistance and ensure that ACTs are still effective. Testing each drug individually serves as an "early warning" system for reduced responses to either artemisinin compounds or the partner drugs before any clinical failures may be observed. Blood samples were collected from patients with clinical malaria during the three-month (September to December) transmission season in years 2007 through 2009. Blood samples containing 0.1 - 1% parasitemia were incubated with various drugs to determine IC50 values. For 2007, 2008 and 2009 there were 44, 110, and 90 samples analyzed, respectively. Parasites were found resistant (data by year: 2007, 2008 and 2009) to chloroquine (36.4%, 32%, 14%); amodiaquine (9%, 8%, 0%); pyrimethamine (52.27%, 68%, 74%); and tolerant to artemisinin (19%, 18%, 30%). In 2007 and 2009 quinine was tested with 27.7% and 4% resistance respectively and mefloquine showed 54% and 80% resistance in 2008 and 2009 respectively. Artesunate was only tested in 2009 and showed good correlation with artemisinin response. Several parasites stains demonstrated multiple drug resistance, with no evidence of cross-resistance between chloroquine and amodiaquine. Drug sensitivities were observed for all compound classes, however some parasites displayed significant levels of multi-drug resistance. With changing drug use, sensitivities to some drugs (such as chloroquine) are increasing. Decreasing responses to artemisinin suggests that parasites may become less responsive to these compounds and that careful monitoring is required. The decrease of artemisinin sensitivity and the increase of chloroquine sensitivity need to be continually monitored in order to inform drug policy makers.

1262

MOLECULAR SURVEILLANCE OF DRUG RESISTANCE MARKER GENES IN *PLASMODIUM VIVAX* ISOLATES CAUSING SEVERE MANIFESTATIONS FROM INDIA

Shilpi Garg[1], Vishal Saxena[1], Dhanpat K. Kochar[2], Sanjay K. Kochar[3], Ashis Das[1]

[1]Birla Institute of Technology and Science, Pilani, Pilani, India, [2]Kothari Medical and Research Institute, Bikaner, Rajasthan, India, [3]S.P. Medical College, Bikaner, Rajasthan, India

India is a major contributing country to the worldwide burden of *Plasmodium vivax* contributing to almost 70% of the total cases in South East Asia with more than 50% owing to *P. vivax*. With the recent reports of Chloroquine resistance in *P. vivax* and the parasite causing severe manifestations like Cerebral Malaria, ARDS, Acute Renal Failure, Hepatic Dysfunction, etc. (as reported previously) the studies on this parasite

has become important. Due to the lack of information on the resistance pattern of various antimalarial drugs in severe *P. vivax* population, we studied the degree of polymorphism in the drug resistance marker genes that can hypothetically lead to an indication of the state of resistance of these parasites. In *P. falciparum*, it has been suggested that the increase in morbidity and mortality is due to rise in prevalence of parasite resistance to various antimalarial drugs. But in *P. vivax*, it is not known whether it is driven by drug failure or some other changes in intrinsic parasite factors that enhance parasite multiplication and virulence. In this study, the analysis of various drug resistance marker genes (mdr-1 and crt-o for CQ resistance and dhfr and dhps for SP resistance respectively) showed mainly the wild type genotype. However, in contrast to the reported limited polymorphism in the PvDHPS gene, 4 novel mutations were observed in our severe *P. vivax* isolates. But the homology modeling and molecular docking studies showed no effect of these mutations on Sulfadoxine binding. Thus it appears to indicate the absence of a drug resistant status in these severe *P. vivax* samples. The recent reports have also suggested an increased expression of the chloroquine resistance marker genes in severe vivax malaria. The microarray expression data for our severe *P.vivax* isolates also showed any one of these genes; Pvcrt or Pvmdr-1 to be upregulated. These finding on increased expression levels of genes likely involved in antimalarials drug resistance supports to further explore potential of *these genes as* molecular markers of severe disease in *P. vivax.*

1263

TOWARDS VALIDATION OF GENOTYPE: RESISTANCE INDEX AS A MOLECULAR TOOL IN MALARIA DRUG RESISTANCE SURVEILLANCE IN MALI

Mamadou Wele, Hamidou Niangaly, Aldiouma Guindo, Abdoul H Beavogui, Aboubacar Sadou, Blaise Dackouo, Cheik Omar Papa Sangare, Ogobara Doumbo, Abdoulaye Djimde

MRTC, Bamako, Mali

The resistance of *Plasmodium falciparum* to the chloroquine is conferred by point mutations on PfCRT, a protein located on the digestive vacuole membrane. PfCRT 76 T mutations was identified as a molecular marker of P *falciparum in vivo* and *in vitro* resistance to chloroquine. The prevalence of mutant allele Pfcrt 76T was used by Djimdé and collaborators in Mali to calculate the Genotype: Resistance Index. Given this importance, (GRI). We aimed in this work to enhance the use of this molecular marker in epidemiological surveillance of drug resistance in other sites in Mali. We conducted a prospective chloroquine efficacy studies in Kangaba, Kella in 2001/03 and Pongonon in 2007. Malaria is hyper-endemic in all villages with seasonal peaks of transmission. The protocol was reviewed and approved by the Institutional Review Board of the faculty of Medicine, Pharmacy and Stomatology, University of Bamako and the US national institut of Allergy and Infectious Disease. The informed consent was obtained from children parents or tutors prior to their enrollment in the study. The malaria cases were recruited according to WHO 2000 protocol. The cases were treated with chloroquine and followed on days 3, 7, and 14. P *falciparum* DNA was extracted from finger prick blood blotted onto filter paper and the Pfcrt K76T genotypes were analyzed by nested PCR. For analysis purposes, mixed infections were categorized as mutant Pfcrt 76T and GRI was calculated dividing the PfCRT 76T prevalence by the therapeutic failure rate. We included 418 cases in Kella, 336 cases in Kangaba and 259 cases in Pongonon a total of 1,013 subjects with uncomplicated malaria. We determined the IGR for children lees than 5 years, aged between 6 and 10 years and more than 11 years. We found that the parasitological failure rate was 2-3 times less than the prevalence of the mutant allele Pfcrt 76T. The IGR means were 1.2; 1.25 and 1.6 respectively in Pongonon, Kangaba and Kella in children less than 5 years. In the general population, they were 1.9; 1.7 and 2.2 respectively in Pongonon, Kella and Kangaba. Our data have shown that the prevalence of mutant allele Pfcrt 76T could be used to estimate a potential chloroquine treatment failure in Mali.

1264

CHLOROQUINE RESISTANCE HAPLOTYPES IN THE *PFCRT* AND *MDR1* GENES OF GHANAIAN ISOLATES USING PCR AND SEQUENCE SPECIFIC OLIGONUCLEOTIDE PROBE-ENZYME LINKED IMMUNOSORBENT ASSAY (SSOP-ELISA)

Sena A. Matrevi[1], Charles A. Brown[2], Patrick F. Ayeh-Kumi[3], Kwadwo A. Koram[1]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]School of Allied Health Sciences, Accra, Ghana, [3]University of Ghana Medical School, Accra, Ghana

Despite decades of research to reduce the burden of malaria disease, malaria remains a major threat to humanity causing about 1.5 to 3 million deaths a year. Prompt diagnosis and effective treatment of malaria remain the main strategies to reduce the intolerable burden of the disease in the absence of an effective vaccine. Due to the widespread resistance of *Plamodium falciparum* to the commonest and cheapest antimalarial drug, chloroquine (CQ), it is important to provide effective antimalarial drug resistance surveillance system in the country. The aim of this study was to determine the single nucleotide polymorphisms (SNPs) in the *crt,* and *mdr1* genes using PCR and SSOP-ELISA and to capture any SNPs that might have been missed by the use of PCR and restriction fragment length polymorphism (RFLP) in a previous work. The study was carried out in the Hohoe District and Navrongo War Memorial hospitals. Children <5years (male and female) reporting at the outpatients departments at the two hospitals with uncomplicated malaria were recruited. Nested PCR followed by SSOP-ELISA was performed to determine the presence of SNPs responsible for CQ resistance. Fifty-nine participants (29 males and 30 females) were recruited. The SSOP-ELISA results showed the baseline prevalence of the common CQ resistance haplotype CVIET observed for all resistant parasites to be 60% at Navrongo and 56% at Hohoe and the less common haplotype SVMNT (found sporadically in Africa) was 10% at Navrongo and 30.8% at Hohoe. The amino acid change at codon 86 for the *mdr1* genes had a prevalence of 90% at Navrongo and 95% and Hohoe. Using PCR and SSOP-ELISA, the SVMNT mutation which was missed in an earlier work was captured for the first time using only PCR and RFLP. PCR and SSOP-ELISA has a great potential to be used as a tool for general drug resistance surveillance in Ghana since it is faster, provides results comparable to those obtained in previous work and can also capture new point mutations.

1265

HIGH THROUGHPUT GENOTYPING OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE *PLASMODIUM FALCIPARUM DHFR* GENE BY ASYMMETRIC PCR AND MELT-CURVE ANALYSIS

Rochelle Cruz[1], Sandra Shokoples[2], Dammika Manage[1], Stephanie K. Yanow[1]

[1]University of Alberta, Edmonton, AB, Canada, [2]Provincial Laboratory of Public Health, Edmonton, AB, Canada

Mutations within the *Plasmodium falciparum dhfr* gene confer resistance to sulfadoxine-pyrimethamine (SP). Single nucleotide polymorphisms (SNPs) within codons 51, 59, 108 and 164 in the *Pfdhfr* gene are associated with SP treatment failure. We developed an assay using asymmetric real-time PCR and melt-curve analysis to genotype clinical samples. Unlabeled probes specific to each SNP hybridize differentially to mutant and wild-type sequences within the amplicon, generating distinct melting curves. Analytical validation was performed using plasmids, genomic DNA from reference strains, and parasite cultures. Correct genotypes were identified with 100 copies of template. The performance of the assay was evaluated with a blind panel of clinical isolates with low parasitemias. Concordance with DNA sequencing ranged from 84% to 100%. Our assay provides a number of technical improvements that facilitate high throughput screening of patient samples to identify SP resistant malaria.

1266

PREVALENCE OF *PFMDR1* AMPLIFICATION IN *PLASMODIUM FALCIPARUM* FROM FIVE SITES IN CAMBODIA

Rithy Sem[1], Dysoley Lek[1], Sinuon Muth[1], Socheat Duong[1], Frederic Ariey[2], **William O. Rogers**[3]

[1]National Center for Parasitology, Entomology, and Malaria Control, Phnom Penh, Cambodia, [2]Institut Pasteur du Cambodge, Phnom Penh, Cambodia, [3]Naval Medical Research Unit #2, Jakarta, Indonesia

Artemisinin resistance is a potentially serious threat to current artemisinin combination therapies (ACT) for *Plasmodium falciparum* malaria. In Cambodia, resistance to artesunate-mefloquine (A+M), the current first-line therapy for uncomplicated *P. falciparum* malaria in most of the country, may pose a significant problem. Since resistance to A+M is likely to arise on a background of mefloquine resistance, we sampled parasite genomes from five sites around Cambodia and assayed for amplification of the *pfmdr1* gene, a mutation associated with mefloquine resistance. We measured *pfmdr1* copy number using a previously described quantitative RT-PCR assay using the single copy *P. falciparum* beta-tubulin gene as an internal control. Among 776 cases the preliminary estimated prevalences and 95% confidence intervals of parasites with >1.5 copies of *pfmdr1* at the sites were, Chumkiri, 0.64 (0.49, 0.77); Trapaing Prasat 0.51 (0.47, 0.55); Kratie 0.09 (0.04, 0.19); Khsim 0.27 (0.12, 0.46); and Rattanakiri, 0.09 (0.04, 0.17). The estimated means and standard deviations of the *pfmdr1* copy number at the sites were, Chumkiri, 2.8 (2.1); Trapaing Prasat, 2.0 (1.4); Kratie, 1.0 (0.5); Khsim 1.3 (0.6); Rattanakiri, 1.1 (0.7). The finding of elevated *pfmdr1* copy number in Chumkiri is expected; A+M failure rates of approximately 15% were found in Chumkiri in 2006-2007. The lack of widespread *pfmdr1* amplification in the eastern sites, Kratie, Khsim, and Rattanakiri, is consistent with the reported continued efficacy of mefloquine in eastern Cambodia. The relatively high rate of *pfmdr1* amplification at Trapaing Prasat, Oddarmeanchey Province, may represent spread of mefloquine resistance from the nearby provinces on Cambodia's western border, and may represent a threat to the current first-line ACT, A+M, in this area; an *in vivo* efficacy study is underway to evaluate this possibility.

1267

RECTAL AND INTRAVENOUS ARTESUNATE FOR SEVERE MALARIA: MODELLING THE IMPACT OF ARTEMISININ RESISTANCE

Richard J. Maude[1], Borislava Mihaylova[2], Marian Warsame[3], Jan Singlovic[4], Wirichada Pongtavornpinyo[1], Sompob Saralamba[1], Arjen M. Dondorp[1], Nicholas J. White[1], Melba Gomez[5], Lisa J. White[1]

[1]Wellcome Trust Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, [2]Health Economics Research Centre, Department of Public Health, University of Oxford, Oxford, United Kingdom, [3]Case Management and Research, Global Malaria Programme, World Health Organization, Geneva, Switzerland, [4]National Institute for Medical Research, Dar es salaam, United Republic of Tanzania, [5]UNICEF/UNDP/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases, Geneva, Switzerland

Treatment of adult severe malaria with intravenous artesunate greatly reduces mortality when compared to quinine and is thus recommended first-line treatment for severe malaria in adults worldwide. A large study is underway to verify if the same is true in children. Pre-referral treatment with rectal artesunate for severe malaria was shown in a recent large study to reduce mortality in those whose treatment would be otherwise delayed. As these treatments become more widely available across the tropics there is great concern that the newly discovered artemisinin resistance in Cambodia may spread and compromise their effectiveness. A mathematical model for the spread of artemisinin resistance at the population level was developed based on data from two large community-based trials of rectal artesunate, two large hospital-based trials of intravenous artesunate and recent data on artemisinin resistance from Cambodia. Various scenarios were considered including the introduction of these therapies alone and in combination, at different levels of coverage, in a variety of transmission settings and with varying efficiency of the referral system after administration of rectal artesunate. The model was used to predict the likely impact of a policy change to these therapies on the potential spread of artemisinin resistance and explore the effects of this resistance on malaria mortality and morbidity in Africa and Asia.

1268

SULPHADOXINE-PYRIMETHAMINE BASED COMBINATION THERAPY IN TREATMENT OF UNCOMPLICATED MALARIA IN MALI: A RANDOMIZED CLINICAL TRIAL IN TWO VILLAGES ON CHILDREN LESS THAN FIVE YEARS

Maiga Hamma, Beavogui Abdoul Habib, Toure Ousmane, Tekete Mamadou, Sangare Cheik Oumar Papa, Dara Antoine, Traore Zoumana Isaac, Traore Oumar Bila, N'Dong Christelle, Dama Souleymane, Niangaly Hamidou, Diallo Nouhoum, Dembele Demba, Doumbo Ogobara, Djimde Abdoulaye

MRTC, Bamako, Mali

Artemisinin-based combination therapies are now first line drugs in malaria treatment in Africa. Although they still remains effective in treatment of uncomplicated malaria in Africa, little cases of resistance rised in some areas in southern Asia. We initiated non ACT study to assess the effectiveness of SP+Amodiaquine (AQ) and SP+Artesunate (AS) vs. SP alone in two Malians villages. The purpose of this study was to assess the clinical and parasitological responses these SP combinations on uncomplicated *P. falciparum* malaria in Kolle and Bancoumana (both hyper-endemic areas). We conducted a prospective study from August to December 2004 and from July to December 2005 on children aged between 6 to 59 months. We used the *in vivo* standard follow up of 28 days of WHO for efficacy assessment. A total of 912 patients were included with 304, 306, and 302 in SP+AQ, SP+AS and SP alone respectively. We registered a total of 2% loss to follow-up. We observed only four (4) cases of early therapeutic failure (1.4%) all in the arm of SP alone. We found 1%, 1.7%, and 0% of late clinical failure (LCF) and 4.1%, 3.4% and 1.4% of late parasitological failures (LPF) in SP, SP+AS and SP+AQ respectively. We found that SP+AQ was less efficacy than SP+AS (p=0.024) and SP (p=0.043) regarding to LCF and LPF respectively. The rate of clinical and parasitological adequate response (RCPA) was 93.5%, 94.9% and 98.6% for SP, SP+AS and SP+AQ respectively. There was statistical significant differences between the three groups (p=0.007). After PCR correction using MSP2, the re-infection rates were 3.7%, 4.1%, and 1.4% in SP, SP+AS and SP+AQ group respectively. PCR-corrected cure rates at day 28 were 97.2%), 99%) 100%) in SP, SP+AS, and SP+AQ and the difference was statistically significant (0.01). In conclusion, sulphadoxine-pyrimethamine only and its combination with artesunate or amodiaquine are clinically and parasitologically effective in Mali.

1269

DIFFERENT MUTATION RATES IN THE DIHYDROFOLATE REDUCTASE AND DIHYDROPTEROATE SYNTHASE GENES IN *PLASMODIUM VIVAX* POPULATIONS FROM CHINA

Miao Miao[1], Zhaoqing Yang[2], Long Cui[1], Jessica Ahlum[1], Yaming Huang[3], Liwang Cui[1]

[1]Pennsylvania State University, State College, PA, United States, [2]Parasitology Department, Kunming Medical College, Kunming, Yunnan, China, [3]Malaria Department, Guangxi Centers for Disease Control and Prevention, Nanning, Guangxi, China

Plasmodium vivax genes encoding dihydrofolate reductase (*pvdhfr*) and dihydropteroate synthase (*pvdhps*) are considered to play a key role in folate biosynthesis. Point mutations in *pvdhfr/pvdhps* have been used to predict resistance to antifolates. In southern Asia and Africa, P. vivax

is considered highly resistant to sulfadoxine-pyrimethamine (SP) as an increasing use of SP in malaria treatment. In China, however, few pvdhfr/pvdhps mutants have been demonstrated until recently. We used direct sequencing to examine the prevalence of mutations in pvdhfr/pvdhps in 122 P. vivax clinical isolates collected from two areas in central (Anhui) and southern China (Guizhou). For pvdhfr, 36.9% were wild-type, whereas mutations were detected at four codons (57, 58, 61, and 117). S117N/T mutation was the most prevalent (48.4%), followed by the T61M mutation (18.9%). Six pvdhfr mutant alleles were found, ranging from 37.7 to 0.8%. The most prevalent mutant haplotype among all examined samples was F57S58T61N99S117 (36.9%). The dramatically different pvdhfr allele frequencies between the two P. vivax populations might be resulted from different drug histories or intrinsic difference between temperate and subtropical strains. In contrast, except polymorphisms within a repeat region, no resistance-conferring mutations were detected in pvdhps. Together with past clinical studies of pyrimethamine efficacy, our result suggests that P. vivax populations in China may be relatively susceptible to sulfadoxine-pyrimethamine.

1270

IN VITRO AMODIAQUINE RESISTANCE AND ITS ASSOCIATION WITH MUTATIONS IN PFCRT AND PFMDR1 GENES OF *PLASMODIUM FALCIPARUM* ISOLATES FROM NIGERIA

Onikepe A. Folarin[1], Carolina Bustamante[2], Olusola G. Gbotosho[1], Akin Sowunmi[1], Mariano G. Zalis[2], Ayoade M. Oduola[3], Christian T. Happi[1]

[1]Malaria Research Laboratories, University of Ibadan, Ibadan, Nigeria, [2]Laboratory of Molecular Infectiology and Parasitology, Clementino Fraga Filho University Hospital, Rio de Janeiro, Brazil, [3]Special Program for Research and Training in Tropical Diseases, World Health Organization/ TDR, Geneva, Switzerland

Amodiaquine (AQ) is currently being used as a partner drug in combination with artesunate for treatment of uncomplicated malaria in most endemic countries of Africa. In the absence of molecular markers of artemisinin resistance, molecular markers of resistance to AQ can be useful for monitoring the development and spread of parasites resistance to Artesunate-Amodiaquine combination. This study was designed to explore the potential role of polymorphisms on pfcrt and pfmdr1 genes and parasite *in vitro* susceptibility for epidemiological surveillance of amodiaquine resistance in *Plasmodium falciparum*. The modified schizont inhibition assay was used to determine *in vitro* susceptibility profiles of 98 patients' isolates of *P. falciparum* to amodiaquine. Polymorphisms on parasites pfcrt and pfmdr1 genes were determined with nested PCR followed by sequencing. The geometric mean of AQ 50% inhibitory concentration (IC50) in the 98 *P. falciparum* isolates was 21.287±3.61nM (range 1.25- 183.20nM). Molecular analysis showed presence of mutant pfcrtThr76, pfmdr1Tyr86 and double mutant pfcrtThr76+pfmdr1Tyr86 alleles in 76%, 48% and 35% of the isolates respectively. The geometric mean of IC50 of *P. falciparum* isolates harbouring both wild type pfcrtLys76+pfmdr1Asn86 were observed to be reduced (4.93nM) compared to isolates harbouring double mutant pfcrtThr76+pfmdr1Tyr86 (50.29nM). Reduced *in vitro* susceptibility of *P. falciparum* to amodiaquine was significantly associated with presence of mutant pfcrtThr76, pfmdr1Tyr86 or the double mutant pfcrtThr76+pfmdr1Tyr86 (p=0.0001). Results from this study suggest that polymorphisms in pfcrt and pfmdr1 genes are important for amodiaquine resistance and therefore may be useful for epidemiological surveillance of *P. falciparum* resistance to AQ.

1271

ANALYSES OF *PLASMODIUM FALCIPARUM* CHLOROQUINE RESISTANCE TRANSPORTER GENE (*PFCRT*) AND MICROSATELLITE DNA LOCI FLANKING THE GENE REVEALED GEOGRAPHICALLY DIFFERENT DISSEMINATION OF CHLOROQUINE RESISTANT MALARIA IN SOUTHEAST ASIA

Moritoshi Iwagami[1], Pannapa Susomboon[1], Noppadon Tangpukdee[2], Polrat Wilairatana[2], Sricha Krudsood[3], Le D. Dao[4], Shusuke Nakazawa[5], Muth Sinuon[6], Duong Socheat[6], Junko Yasuoka[7], Masamine Jimba[7], Shigeyuki Kano[1]

[1]Department of Tropical Medicine and Malaria, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan, [2]Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [3]Department of Tropical Hygiene, Mahidol University, Bangkok, Thailand, [4]Molecular Biology Department, National Institute of Malariology, Parasitology and Entomology, Hanoi, Vietnam, [5]Department of Protozoology, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan, [6]National Centre for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [7]Department of Community and Global Health, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

A mutation in *Plasmodium falciparum* chloroquine resistance transporter (*pfcrt*) gene in codon 76 (K76T) is associated with chloroquine (CQ) resistance and used to monitor the distribution and frequency of the CQ resistant malaria. Microsatellite (MS) DNA polymorphisms flanking the drug resistant genes can be used to study the evolution of the genes. In this study, we determined the frequency of the mutation in codon 72-76 in the *pfcrt* gene and the MS polymorphisms (2E10, 9B12, PE12A) flanking the gene, using *P. falciparum* isolates from Thailand (50 isolates), Vietnam (39 isolates) and Cambodia (26 isolates). All the isolates from Thailand were CQ resistant (CVIET). All the isolates from Cambodia were also CQ resistant (CVIET, CVIDT). In contrast, 27 of the 39 isolates (69%) from Vietnam were CQ susceptible (CVMNK), while the other 12 of them (31%) were CQ resistant (CVIET, CVIDT, CVMDT) or mixed. Expected heterozygosity (*H*: gene diversity) of the each MS locus showed that the Thai population (*H*: 0.08-0.61, average: 0.35) and the Cambodian population (*H*: 0.00-0.21, average: 0.11) were less divergent than both the Vietnamese CQ resistant population (*H*: 0.73-0.86, average: 0.79) and the CQ susceptible population (*H*: 0.37-0.97, average: 0.71). The sizes of each MS locus of the Cambodian population were identical or very close to those of the Thai population. However, the sizes of some of the MS loci of the Vietnamese population were different from those of the Thai and Cambodian populations. These results suggest that the Thai and Cambodian populations had been under strong CQ selective pressure but the Vietnamese population had not, and that the origin of CQ resistant mutation in Vietnam might be different from Thailand and Cambodia.

1272

MALARIA TRANSMISSION STUDIES: CAN THEY INDICATE NEW MARKERS OF DRUG RESISTANCE?

Rachel L. Hallett[1], Teun Bousema[1], Patrick Sawa[2], Seif Shekalaghe[2], Silas Otieno[2], Sabah Omar[2], Colin Sutherland[1]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]Kenya Medical Research Institute, Nairobi, Kenya

Transmission endpoints can be included in antimalarial clinical trials, where appropriate facilities for experimental mosquito feeding exist. We have demonstrated previously that, after drug treatment, *Plasmodium falciparum* parasites carrying molecular markers of drug resistance are associated with higher oocyst burdens in mosquitoes and therefore greater transmission success. We propose that candidate molecular markers of ACT resistance should also be validated in this way, which may be possible before resistance has progressed to the level where a serious reduction in clinical efficacy occurs. We will present data from a clinical trial of

artemether-lumefantrine vs dihydroartemisinin-piperaquine in Mbita, Kenya, where more than 50 successful membrane-feeds, 7 days after drug treatment, resulted in 320 infected mosquitoes. Candidate molecular markers of ACT resistance include well-studied mutations in genes pfcrt and pfmdr1, and less well described polymorphisms in genes pfmrp, pfnhe, pfubp-1 and pfatpase6. Associations between these markers and oocyst burden in the infected mosquitoes are currently under investigation and will be presented.

1273

A COMPARISON OF *IN VITRO* AND MOLECULAR MARKERS OF ANTIMALARIAL DRUG RESISTANCE IN NORTHERN AND WESTERN CAMBODIA

Stuart D. Tyner[1], David L. Saunders[1], Kritsanai Yingyuen[1], Panjaporn Chaichana[1], Paktiya Teja-isavadharm[1], Phisit Khemawoot[1], Sea Darapiseth[2], Panita Gosi[1], Delia B. Bethell[1], Youry Se[1], Nillawan Buathong[1], Worachet Kuntawunginn[1], Douglas S. Walsh[1], Duong Socheat[2], Chanthap Lon[1], Mark M. Fukuda[3]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [3]Armed Forces Health Surveillance Command, Washington, DC, United States

Decreasing artemisinin effectiveness in western Cambodian *Plasmodium falciparum* strains, characterized by elevated IC50s and increased parasite clearance times, is developing in Southeast Asia. While the focus of artemisinin resistance has been geographically limited to the Thai-Cambodian border in the Cambodian provinces of Battambang and Pailin, we are attempting to evaluate the evidence for decreasing effectiveness of artemisinins elsewhere in Cambodia. AFRIMS is currently conducting a multi-year *in vitro* parasite surveillance study in various regions of Cambodia to investigate *in vitro* and molecular markers of resistance in isolates from patients with uncomplicated *P. falciparum*. The results received during our surveillance study conducted in Anlong Veng, Oddar Meanchey province in 2009-10 (n=153) were compared with previous results obtained in our seven day artesunate mono-therapy trial in Samlot District, Battambang province in 2008-9 (n=141). Although patient enrollment data between the sites were similar, parasites from northern Cambodia appear to be more sensitive *in vitro* to artemisinins compared to those from Western Cambodia. Geomeans of *in vitro* drug sensitivity (IC50) results for DHA when compared between the two sites were significantly different by Mann-Whitney U test, with a geomean of 3.06ng/ml (Battambang) and 1.96ng/ml (Anlong Veng) respectively ($p<0.0002$). *In vitro* IC50 analysis for other antimalarial drugs; artesunate, mefloquine, quinine, chloroquine, and lumefantrine is ongoing. Comparative analysis of *in vitro* phenotype and molecular markers of parasite resistance will be presented and compared with clinical data. Despite the apparent parasite IC50 difference between provinces, the genetic basis for the apparent geographic differences in parasite susceptibility in Cambodia has yet to be determined.

1274

RELATIONSHIP BETWEEN CHLOROQUINE USE AND CHLOROQUINE RESISTANCE IN SUB-SAHARAN AFRICA

Meera Venkatesan, Anne E. Frosch, Miriam K. Laufer

University of Maryland School of Medicine, Baltimore, MD, United States

Despite policy changes from chloroquine (CQ) and sulfadoxine-pyrimethamine to artemisinin-combination therapy (ACT) as the primary treatment for uncomplicated malaria in most malaria-endemic countries, these compromised drugs are still widely used. We hypothesized that differences in CQ use would be reflected in the prevalence of the molecular marker for CQ resistance, the *Plasmodium falciparum* chloroquine resistance transporter (pfcrt) polymorphism at codon 76. To characterize the extent of recent CQ use, we compared data on reported drug treatment in children from national surveys conducted between 2006 and 2007 in 21 African countries. CQ use was particularly high in West Africa, where it was the most commonly reported antimalarial in 11 of 12 countries surveyed. Reported use of CQ varied in East Africa, ranging from < 1% to nearly 50%. To investigate the effect of continued CQ pressure on drug resistance, we compared drug use data from all available national surveys (between 2000 and 2007) with temporal trends in the prevalence of CQ resistance marker pfcrt 76T from published studies in 7 African countries. The proportion of resistant genotypes stayed stable over time in three countries with sustained high CQ use (Burkina Faso, Guinea Bissau and Uganda). The prevalence of pfcrt 76T increased in Niger, the only country reporting increased CQ use during the survey period, and declined in three countries exhibiting low or sharply decreasing CQ use (Malawi, Tanzania and Kenya). These findings suggest that with declining use of CQ, we may expect to find a decline in CQ-resistant malaria. As ACT availability continues to expand in the region, CQ-susceptible malaria may begin to predominate in sub-Saharan Africa in the near- to medium-term future.

1275

GENETIC DISSECTION OF THE ACCELERATED ACQUISITION OF DRUG RESISTANCE IN ARMD *PLASMODIUM FALCIPARUM*

Daniel L. Freeman, John White, Ramesh Gujjar, Karthikeyan Ganesan, Pradipsinh K. Rathod

University of Washington, Seattle, WA, United States

The in-field failure of most drugs against *Plasmodium falciparum* has contributed to the major global health burden of malaria. We have previously shown that parasites from Southeast Asia harbor the Accelerated Resistance to Multiple Drugs (ARMD) phenotype, as reported previously. Parasites from South East Asia consistently acquire resistance to new and unrelated antimalarials three to four orders of magnitude more frequently than parasites from other parts of the world. By assessing the ease with which progeny from an HB3xDd2 genetic cross (as reported previously) acquire resistance to the antifolate, 1843U89, we show that the ARMD phenotype is a complex, multigenic trait, with some progeny exhibiting intermediate phenotypes. Further, we show that this increased ability to acquire resistance to 1843U89 cannot be explained by external factors such as increased survivability in the presence of 1843U89 or our experimental setup. Using QTL analysis, we have identified at least four regions on chromosomes 4, 5, 7, and 13, acting in an additive or synergistic manner to confer the ARMD phenotype. Furthermore, to validate that our previously identified loci are involved in the accelerated acquisition of multiple drugs, we report on similar recent studies using an alternative antimalarial, BMS-388891, a protein farnesyl-transferase inhibitor.

1276

SELECTION FOR ARTEMISININ RESISTANCE IN *PLASMODIUM FALCIPARUM* UNDER LABORATORY CONDITIONS AND POTENTIAL MECHANISMS

Long Cui, Liwang Cui

The Pennsylvania State University, University Park, PA, United States

To select an artemisinin resistant line in the laboratory, we have subjected *P. falciparum* Dd2 strain to dihydroartemisinin (DHA) selection with step-wise increments of drug concentrations over 13 months. We have obtained three lines with more than 20-fold increase in IC50 DHA. However, the resistance phenotype was unstable and the parasites could regain susceptibility to DHA after three months of culture in the absence of the drug selection pressure. Phenotype analysis showed that the resistant parasite displayed cross-resistance to a number of the commonly used antimalarial drugs. Analysis of potential drug targets did not detect point mutations in *pfcrt, G7, G49, pfmrp* and *pfatp6* genes, but we identified increased copy number of *P. falciparum* multidrug resistance 1 (pfmdr1) gene associated with artemisinin resistance. In addition, the

DHA-resistant parasites also showed elevated activity of the antioxidant defense systems. Collectively, this study suggests that selection of artemisinin resistance under the laboratory conditions may be associated with multiple mechanisms

1277

SELECTION FOR RESISTANCE FOLLOWING A VARIETY OF ANTI-MALARIAL TREATMENT REGIMES

Silvie Huijben, Brian Chan, Derek Sim, Danielle Tomasello, Andrew Read

The Pennsylvania State University, University Park, PA, United States

Drug resistance is a serious problem in health care. In the case of malaria, resistance against most antimalarial drugs is widespread, except against the recently-deployed artemisinin derivatives. The effect of drug treatment regimens on the spread of resistance is largely unknown. Using the rodent model *Plasmodium chabaudi*, we compared the effects of a variety of 'patient' treatment regimes on infections consisting of resistant and sensitive parasites, testing the impact of each regime on host health, infectiousness and the transmission of resistant parasites. In untreated mixed infections, resistant parasites starting at low frequencies in the initial inoculation produced gametocytes at densities that were barely detectable by PCR. However, drug treatment resulted in a rapid increase of resistant parasites, causing recurrent parasitaemia, increased anaemia, and a much increased transmission potential of resistant parasites. Shorter drug courses or lower drug dosages significantly reduced the fitness of the resistant parasites without compromising host health. Conventional drug treatment aimed at radical cure resulted in the greatest fitness gain for drug resistant parasites. These results demonstrate the need for more research on the role of drug treatment regimens on the spread of drug resistance in malaria. Currently recommended regimes inadvertently impose maximal selection for resistance when resistant parasites are present in an infection. There is a need to empirically evaluate the public health consequences of treating in excess of clinical need.

1278

EFFECTS OF *PLASMODIUM FALCIPARUM* DIHYDROPTEROATE SYNTHASE MUTATIONS ON PARASITE FITNESS

Edwin O. Ochong, Philip J. Rosenthal

University of California at San Francisco, San Francisco, CA, United States

The antifolates sulfadoxine-pyrimethamine (SP) and trimethoprim-sulfamethoxazole (TMP/SMX) have potent activity against wild type *Plasmodium falciparum*, but activity is decreased due to resistance-mediating mutations in many areas. However, SP remains the drug of choice for intermittent preventive therapy in pregnant women and children, and TMP/SMX is widely used to prevent opportunistic infections in those with HIV infection. Resistance to antifolates is mediated by a series of mutations in the target enzymes dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS), key enzymes in the folic acid biosynthetic pathway. In East Africa, parasites commonly harbor 3 DHFR mutations (S108N, N51I and C59R) and 2 DHPS mutations (A437G and K540E) that mediate an intermediate level of resistance. Additional point mutations, seen more commonly outside Africa, mediate higher-level resistance. We are studying the impact of various resistance-mediating mutations in DHFR and DHPS on the relative fitness of *P. falciparum*. To this aim, we are exploring the growth of parasites under different conditions and in competition assays. We are studying D10-strain parasites engineered to express DHPS with 1-3 mutations. In initial competition experiments, D10 wild-type and single mutant (A437G) strains outcompeted parasites with 2 (A437G + A581G or S436A + A437G) or 3 (S436A + A437G + K540E) mutations over 2 months of culture, as assessed by strain-specific PCR. Experiments to more stringently characterize relative growth of these strains utilizing folate-limiting culture conditions and quantitative PCR and to evaluate parasites with mutations in DHFR are underway. Our preliminary results suggest that resistance-mediating mutations in DHPS engender a loss of fitness compared to that of wild type strains of *P. falciparum*.

1279

MOLECULAR ANALYSIS REVEALED ANTIMALARIAL DRUG RESISTANCE AT THE AMAZON BASIN OF ECUADOR

Francisco J. Morales[1], Mauricio Espinel[1], Mette Schousboe[2], Renato Leon[1], Ulla Abildtrup[2], Michael Alifrangis[2]

[1]Universidad San Francisco de Quito, Quito, Ecuador, [2]Center of Medical Parasitology, Copenhage, Denmark

Currently, the main strategy to control malaria in Ecuador relies on rapid diagnosis and treatment. Due to therapeutic failure, the scheme using Chloroquine drug has been replaced by Artesunate for *Plasmodium falciparum* non-complicated cases of malaria. No resistance to *P. vivax* has been reported to date, though further surveillance using molecular tools could contribute to a better use of antimalarial drugs. Through SSOP-ELISA, 9 *P. falciparum* and 17 *P. vivax* blood samples from 7 communities in Sucumbíos province were analyzed for Single Nucleotide Polymorphisms in the following genes: Chloroquine resistance transporter (crt); Dihidrofolate reductase (Pfdhfr) and Dihydropteroate Synthetase (Pfdhps) for *P. falciparum* and Dihidrofolate Reductase (Pvdhfr) for *P. vivax*. Further DNA sequence analysis was used to confirm the ELISA data. The following haplotypes were identified in all *P. falciparum* samples: CVMNT at the Pfcrt gene; CNCNI at the Pfdhfr gene and the wild-type SAK at the Pfdhps gene. For *P. vivax*, 11/17 samples showed the double mutated haplotype (L2R2TS) for the Pvdhfr gene. In addition, the haplotypes FR2TN, FR1TN and FR2TS were found in 4, 1 and 1 samples respectively. A low frequency of mutations related to antimalarial drug resistance was observed in the study. The K76T mutation at the Pfcrt gene, previously reported from Colombia and Peru is dominant in the Ecuadorian Amazon basin and explains the high rate of treatment failure using Chloroquine reported in the past. Despite the high pressure to Sulfadoxyne/Pyrimethamine (S/P), low resistant haplotypes were found in Pfdhfr and Pfdhps genes. A similar scenario was found in Pvdhfr gene, which carries only double and single mutations not related with resistance to S/P. Interestingly, 4 different genotypes in Pvdhfr gene were found, suggesting a high genetic diversity of *P. vivax*. Future studies increasing our sampling size will help to better understand the distribution and implications of these preliminary findings.

1280

REVISITING THE ROLE OF MICROSCOPY IN *PLASMODIUM FALCIPARUM* ANTIMALARIAL DRUG EFFICACY TRIALS

Maria E. Castellanos, Lucía Ortiz, Norma R. Padilla

Universidad del Valle de Guatemala, Guatemala, Guatemala

Although some *Plasmodium falciparum* antimalarial drug efficacy studies include molecular tools for the identification and discrimination of *Plasmodium* species, many of them just rely on microscopic diagnosis, as WHO recommends. Furthermore, treatment outcomes are partially assessed by the presence of post-treatment asexual parasites. The aim of this study was to determine if the sole use of microscopy in *in vivo* antimalarial drug efficacy trials might lead to the misclassification of both mono and mixed *P. falciparum* infections and an underestimation of the true number of treatment failures. A total of 250 paired blood samples (50 patients) from a 28-days *in vivo* study, conducted in Escuintla, Guatemala during 1998-1999 were analyzed. At each time-point (days 0, 3, 7, 14 and 28), blood was spotted on filter paper and a thick blood smear was prepared. Malaria diagnosis was performed through Giemsa slide examination and nested PCR of the *Plasmodium* small sub-until ribosomal gene (ssRNA). In addition to the standard methodology, quality of DNA was assessed incorporating an internal beta-globin PCR. Results show that in 87.5% of the cases, microscopy was able to correctly identify mono-infection with *P. falciparum*. However, PCR analyses show that 10.4% of the samples were mixed *P. falciparum-P. vivax* infections and 2.1% of

them were actually *P. vivax* mono-infection. Reclassification of *Plasmodium* species and treatment outcomes based on PCR results should be taken into consideration for the calculation of sample size and adjustment of end-points. Microscopy alone is not enough for the accurate identification and discrimination of *Plasmodium* species *in P. falciparum in vivo* antimalarial drug efficacy trials and the use of other techniques, such as molecular testing, are strongly recommended.

1281

ACTIVATION OF NADPH OXIDASE-REACTIVE OXYGEN SPECIES-INFLAMMATORY CYTOKINES PATHWAY CONTRIBUTE TO ACUTE MYOCARDIAL PATHOLOGY IN MICE INFECTED BY *TRYPANOSOMA CRUZI*

Monisha Dhiman, Nisha Jain Garg

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

Trypanosoma cruzi is the etiologic agent of Chagas disease. In this study, we investigated the crosstalk between *T. cruzi*-induced ROS and immune activation of pro-inflammatory response, and its role in myocardial pathology in Chagas disease. Splenocytes of infected mice, *in vitro* stimulated with *T. cruzi* antigenic lysate (TcL), exhibited a statistically significant increase in NADPH oxidase (NOX) activity, ROS production and expression level of proinflammatory and other cytokines, measured by catalytic staining, fluorometry using amplex red and H2DCFDA probes, and Bioplex-ELISA assay, respectively. Addition of apocynin (NADPH oxidase inhibitor), but not inhibitors of myeloperoxidase and xanthine oxidase, resulted in up to 98% inhibition of ROS production, and a significant decline in cytokine release (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and TNF-α) from TcL-stimulated splenocytes of infected mice. Likewise, RAW 264.7 macrophages incubated with live *T. cruzi* or TcL exhibited a substantial increase in NOX activity and cytokine (IL-1β, IL-4, IL-10, IFN-γ and TNF-α) release that was inhibited by apocynin and N-acetylcysteine (ROS scavenger) treatment. These data suggested that NOX-induced ROS signal cytokine production in macrophages and splenocytes of mice infected by *T. cruzi*. To determine the pathological significance of NOX/ROS, we treated infected mice with apocynin in drinking water. Apocynin-treated/infected mice exhibited a significant decline in endogenous and TcL-stimulated splenic cell proliferation, NOX/ROS production, and cytokines release as compared to that noted in infected/untreated mice. We observed no change in myocardial parasite burden, yet, symptoms of acute myocarditis, i.e., infiltration of inflammatory infiltrate (extensive presence of inflammatory foci or disseminated inflammation) and tissue oxidative damage (8-isoprostanes, protein carbonyls, 3-nitrotyrosine and 4-hydroxynonenal adducts) were significantly decreased in the myocardium of apocynin-treated/infected mice. We conclude that NOX-dependent ROS have an important role in regulation of splenic activation of inflammatory cells and cytokine production during acute infection, and contribute to infiltration of inflammatory infiltrate and oxidative injuries in Chagasic heart.

1282

DEFINING TARGET SPECIFICITY OF OXADIAZOLE COMPOUNDS ON REDOX PATHWAY MEMBERS OF THE HOOKWORM *ANCYLOSTOMA CEYLANICUM*

Jon J. Vermeire[1], Christopher D. Pool[1], Christina C. Wong[1], Lisa M. Harrison[1], Ganesha Rai[2], David J. Maloney[2], Anton Simeonov[2], Ajit Jadhav[2], Craig J. Thomas[2], David L. Williams[3], Michael Cappello[1]

[1]Yale University School of Medicine, New Haven, CT, United States, [2]National Institutes of Health Chemical Genomics Center, Rockville, MD, United States, [3]Rush University Medical Center, Chicago, IL, United States

Hookworms, parasitic nematodes that infect approximately 700 million people, are a major cause of anemia, malnutrition, and perinatal mortality in the tropic and subtropic regions of the developing world. Within an appropriate host, larval hookworms develop into blooding feeding adults, attaching to and lacerating the mucosa of the small intestine. Degradation of the ensuing bloodmeal leads to the production of reactive oxygen species (ROS), potentially hindering parasite development and survival. We hypothesize that adult hookworms possess fully functional thioredoxin (Trx) and glutathione (GSH) redox pathways that are essential for the breakdown of ROS produced during blood feeding and that neutralization of these redox systems will lead to reduction in hookworm induced pathogenesis. To characterize these pathways in the human hookworm *Ancylostoma ceylanicum,* we mined the NCBI *A. ceylanicum* expressed sequence tag (EST) database to identify sequences encoding representative members of the Trx and GSH pathways. Several full-length cDNAs were PCR amplified and expressed as recombinant proteins in *E. coli*. Utilizing a kinetic NADPH consumption assay, we determined the specific activity of a novel hookworm peroxiredoxin (AcePrx) and glutathione peroxidase (AceGPx). We also tested the effect of oxadiazoles, nitric oxide-donating compounds, on *A. ceylanicum* adult worms using an *ex vivo* survival assay. Exposure of adult *A. ceylanicum* to micromolar concentrations of select oxadiazole compounds resulted in the significant reduction in worm survival compared to controls treated with equivalent concentrations of albendazole or carrier alone. Furthermore, treatment of *A. ceylanicum*-infected hamsters with furoxan significantly diminished hookworm-induced anemia and worm burden in the host small intestine. This data suggests that oxadiazole compounds represent new lead drugs for the treatment of hookworm disease. Future experiments will investigate the impact of oxadiazoles on *A. ceylanicum* survival by determining the enzymatic activities targeted by these compounds in hookworms and their anthelminthic activity *in vivo*.

1283

METHIONYL-TRNA SYNTHETASE AS A DRUG TARGET FOR *TRYPANOSOMA BRUCEI*

Frederick S. Buckner, Sayaka Shibata, Zhongsheng Zhang, John R. Gillespie, Kuzma V. Kovzun, Ranae M. Pefley, Christophe L. Verlinde, Ethan A. Merritt, Wesley C. Van Voorhis, Wim G. Hol, Erkang Fan

University of Washington, Seattle, WA, United States

Aminoacyl-tRNA synthetases (aaRS) covalently attach amino acids to their corresponding tRNAs, and are required for protein translation. AaRS enzymes make excellent drug targets for antimicrobial drug development as evidenced by mupirocin (a topical antibiotic acting on the isoleucyl-tRNA synthetase) and REP8839 (a clinical Phase II antibiotic acting on the methionyl-tRNA synthetase [MetRS]). Sequence analysis of aaRS enzymes of trypanosomatid parasites indicates significant differences in active site residues between *Trypanosoma brucei* and human homologs of MetRS justifying additional effort to exploit this protein as a drug target. The MetRS has been subjected to RNAi experiments in bloodstream form *T. brucei* and confirm the essentiality of this protein. An aminoacylation assay has been developed to measure enzyme activity and inhibitory action of test compounds. A series of compounds related to REP8839 were synthesized and the most potent had an IC50 ~1 nM on recombinant *T. brucei* metRS. When tested on bloodstream form T. brucei, the EC50 of the most potent compound was 4 nM. Another series of compounds (pyrimidine derivatives) had EC50 values as low as 75 nM on *T. brucei* cultures. The compounds are non-toxic to mammalian cells at concentrations >20 µM. Our best compound (#1312) dramatically suppresses parasitemia in the murine model of acute *T. brucei* infection. The compounds appear to have poor penetration through the blood brain barrier as indicated by the MDR1-MDCK cell permeability assay. Thus, we are making analogs to try to improve this characteristic so as to make the compounds amenable for treating late stage *T. brucei* infection. Attempts to solve the crystal structure of the *T. brucei* met-RS bound to our inhibitors are underway so as to help guide rational drug development.

1284

A NOVEL FUNCTION OF THE *BRUGIA MALAYI* CATHEPSIN-LIKE CYSTEINE PROTEASES IN THE ENDOSYMBIOTIC INTERACTION WITH *WOLBACHIA*

Elena Melnikow[1], Tiruneh Hailemariam[1], Thomas R. Unnasch[2], Elodie Ghedin[3], Sara Lustigman[1]

[1]Lindsley F. Kimball Research Institute, New York, NY, United States, [2]Department of Global Health, University of South Florida, Tampa, FL, United States, [3]University of Pittsburgh School of Medicine, Pittsburgh, PA, United States

Wolbachia is essential for the development and reproduction of *B. malayi*, the causative agent of Lymphatic Filariasis, which opened up a search for novel anti-*Wolbachia* drug targets in addition to antibiotics. Our study was aimed at identifying proteins that potentially have an essential function in this endosymbiotic relationship. We first characterized the effects of tetracycline treatment on the regulation of *Brugia malayi* transcripts 7 and 14 days post treatment using a whole genome microarray studies. We observe primarily up-regulation of *B. malayi* transcripts encoding proteins and enzymes involved in amino acid synthesis and protein translation. Moreover, a bimodal regulation pattern of *B. malayi* transcripts encoding signaling genes and cysteine proteases was observed when tested further by *in vitro* treatment with tetracycline and qRT-PCR. This pattern may be representative of the worms' response to *Wolbachia* death in different tissues; earlier effect on embryogenesis and a later effect on the *Wolbachia* within the hypodermis of the adult worms. Filarial cysteine proteases are known to be involved in molting, embryogenesis and tissue remodeling, processes shown to be dependant on *Wolbachia*. To further elucidate the role cysteine proteases play during this symbiosis, we studied their time dependent expression pattern after anti-*Wolbachia* treatment. For example, the transcripts corresponding to *Bm-cpl-3* and *Bm-cpl-6* were found to be up-regulated 1 day post treatment, unchanged or down-regulated by day 3, but then up-regulated at day 6. Notably, RNAi knockdown of *Bm-cpl-5* that is detrimental for embryogenesis resulted in a specific reduction of *Wolbachia* in the hypodermis and microfilariae but not in the oocytes and embryos. This effect was further established by showing that in the *Bm-cpl-5* dsRNA RNAi treated worms, the transcript levels of a *Wolbachia*-specific ankyrin gene (*wBM0287*) were down-regulated by 10.2 fold. The possible role cysteine proteases play in the relationship between the endosymbiont and its *B. malayi* host will be further discussed.

1285

STAGE-SPECIFIC PATHWAYS OF *LEISHMANIA* ENTRY INTO MACROPHAGES

Nilda E. Rodriguez[1], Upasna Gaur[2], Lee-Ann H. Allen[1], Mary E. Wilson[1]

[1]Veterans Affairs Medical Center and The University of Iowa, Iowa City, IA, United States, [2]The University of Iowa, Iowa City, IA, United States

Leishmania spp. have a life cycle with two stages: the promastigote, found in the sand fly vector and the amastigote, found in macrophages. We showed that infection of susceptible BALB/c mice macrophages with *L. infantum chagasi* increased the expression of caveolae components. Caveolae, a subset of lipid rafts, are membrane microdomains enriched in ganglioside-M1 and cholesterol. Promastigotes co-localized with the caveolae markers caveolin-1 and GM1 during entry and up to 24h after phagocytosis. Transient depletion of macrophage membrane cholesterol by 1h exposure to methyl-β-cyclodextrin (MβCD), impaired the phagocytosis of virulent, but not attenuated promastigotes. In addition, virulent promastigotes experienced increased lysosome fusion ($P<0.001$), and impaired replication ($P<0.05$), even though macrophage cholesterol was replenished by 4h. Amastigotes reside in phagolysosomes, hence we hypothesized that the use of cholesterol-rich microdomains to delay lysosome fusion is promastigote-specific. Accordingly, the entry of promastigotes, but not of amastigotes, is decreased in MβCD-treated macrophages ($P<0.001$). Furthermore, for up to 48h, the intracellular survival of amastigotes was not affected by transient cholesterol depletion. Unexpectedly, by 72h, amastigotes in pre-treated macrophages were unable to replicate ($P<0.05$). By 1h of infection, the early lysosome marker LAMP-1 was recruited to 80% and 20% of amastigote and promastigote compartments, respectively. The promastigote-to-amastigote conversion takes 24 to 48h, consequently, by 24h, the recruitment of LAMP-1 in promastigote-infected macrophages increased to 46% ($P< 0.001$). Disruption of macrophage lipid rafts increases LAMP-1 recruitment to promastigote compartments ($P<0.001$). In contrast, amastigotes readily associated with LAMP-1, regardless of treatment. Our results support the hypothesis that: virulent promastigotes exploit a caveolae-mediated pathway to enter macrophages, and this cholesterol-rich route facilitates their survival by delaying lysosome fusion until their conversion into amastigotes.

1286

ANTENATAL INFECTION WITH LYMPHATIC FILARIASIS INCREASES SUSCEPTIBILITY TO MALARIA IN KENYAN CHILDREN

Indu Malhotra[1], Peter Mungai[1], Alex Wamachi[2], Eric Muchiri[3], Daniel Tisch[1], Christopher L. King[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Kenya Medical Research Institute, Nairobi, Kenya, [3]Division of Vector Borne Diseases, Nairobi, Kenya

We previously observed that antenatal exposure to schistosomiasis in women co-infected with malaria correlated with increased susceptibility to malaria during childhood. To examine the impact of lymphatic filariasis (LF) on malaria susceptibility we undertook a prospective cohort study of 700 newborns in a malaria endemic region of Kenya in which children were examined biannually from birth to age 3 for *Plasmodium falciparum* infection and the presence of malaria antigen-specific T cell responses. 13.6% of pregnant women were co-infected with LF and malaria and 32.6% were infected with LF alone. The risk of malaria increased by 58% (OR 1.58 [95% CI 1.05-2.38] P=0.027) in offspring of women infected with just malaria while the risk increased to almost 3-fold (OR 2.87 [95% CI 1.83-4.52] P=<0.0001) in offspring of women co-infected with malaria and LF as compared to offspring of women lacking any infection during pregnancy. We hypothesized that prenatal LF exposure impairs fetal immune responses to malaria antigens *in utero* thus acquiring a more tolerant phenotype. Cord blood mononuclear cells (CBMC) of newborns of women co-infected with LF and malaria had almost no malaria antigen-driven IFN-γ/IL-2 or IL-5/IL-13 (1 and 5% respectively) compared to malaria-specific responses in CBMC from women infected with malaria alone (24 and 25%, P=0.04). This impaired T cell response persisted into childhood. A greater proportion of CBMC had malaria-antigen-driven IL-10 from women with LF and malaria as compared to women with malaria alone (P=0.05). Co-infection with LF and malaria during pregnancy was associated with reduced ability to generate MSP1-specific IgG in newborns. Thus, filariasis and malaria co-infections during pregnancy enhance risk for malaria infection in their offspring, possibly through a mechanism of immune suppression to protective malaria blood stage antigens. Treatment of filariasis and other helminths in pregnant woman may reduce the risk of malaria for their children.

1287

THE IMPACT OF SCHISTOSOMIASIS AND MALARIA ON THE PATHOLOGY OF DISEASE AND THE IMMUNE RESPONSE IN NON-HUMAN PRIMATE MODELS

Amma A. Semenya, JoAnn S. Sullivan, John W. Barnwell, W. Evan Secor

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

Schistosomiasis and malaria are the two leading parasitic diseases worldwide. These diseases cause significant morbidity and mortality,

with malaria causing over 500 million clinical cases and up to a million deaths annually. Schistosomiasis affects an estimated 200 million people and causes approximately 280,000 deaths annually. Areas endemic for schistosomiasis and malaria overlap in sub-Saharan Africa as well as other parts of the world. Studies that have examined concurrent infections in mouse models have shown that the presence of helminth infections can adversely affect the protective immune response to malaria but similar studies in humans have been less clear. We hypothesized that in concurrently infected animals, a pre-existing schistosome infection would exacerbate a subsequent malaria infection. We percutaneously exposed four rhesus macaques to 500 cercariae of *Schistosomiasis mansoni*. At eight weeks of infection, these animals plus four additional macaques were exposed to the bites of *Anopheles dirus* mosquitoes infected with *Plasmodium coatneyi*. At day 12 and 13 following sporozoite challenge, parasitemia became patent and rapidly increased in both schistosomiasis positive and schistosomiasis negative animals. Both groups of animals were treated with anti-malarial drugs when parasitemia reached high levels around days 17, 18, or 19. In the presence of anti-malarial drug treatment, schistosomiasis positive animals obtained higher parasitemia levels for a longer time period than their malaria-only infected counterparts (p<0.0001). The mechanism underlying this difference in parasitemia is presumably due to a difference in the immune response. The cytokine and antibody responses are being studied to determine specific immune responses of the two groups of animals. These studies are critical for understanding the immunological and pathological consequences of concurrent infections and can aid in the design of vaccines, drug targets, and treatment regimens for individuals living in co-endemic areas.

1288

ANTIGEN-SPECIFIC B MEMORY CELL RESPONSES TO *PLASMODIUM FALCIPARUM* BLOOD STAGE MALARIA ANTIGENS AND SCHISTOSOMAL ANTIGENS IN MALIAN CHILDREN WITH AND WITHOUT *SCHISTOSOMA HAEMATOBIUM*

Kirsten E. Lyke[1], Amy Wang[1], Abdoulaye Dabo[2], Charles Arama[3], Modibo Daou[2], Issa Diarra[2], Sheetij Dutta[4], Evelina Angov[4], David E. Lanar[4], Christopher V. Plowe[5], Ogobara K. Doumbo[2], Marcelo B. Sztein[1]

[1]University of Maryland, Center for Vaccine Development, Baltimore, MD, United States, [2]University of Bamako, Bamako, Mali, [3]University of Stockholm, Stockholm, Sweden, [4]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [5]Howard Hughes Medical Institute, University of Maryland, Center for Vaccine Development, Baltimore, MD, United States

Polyparasitism is common in the developing world. We have previously demonstrated that schistosomiasis-positive (SP) Malian children, aged 4-8 years, have protection from malaria compared to matched schistosomiasis-negative (SN) children. Evidence of durable immunologic memory to malaria antigens is conflicting, particularly in young children, and the effect, if any, of concomitant schistosomiasis on acquisition of memory is unknown. We examined antigen-specific B memory (BM) cell frequencies (expressed as percentage of total numbers of IgG-secreting cells) in expanded peripheral blood mononuclear cells (PBMC) from Malian children aged 4-14 to malaria blood-stage antigens, apical membrane antigen 1 (AMA1) and merozoite surface protein 1 (MSP1) and to schistosomal antigens, soluble worm antigenic preparation (SWAP) and schistosoma egg antigen (SEA) during the malaria transmission season and again in the dry season. A ratio of greater than 0.01% was considered a positive response. Antigen-specific BM responses were detected in all age groups to all antigens. Memory B cell responses to SWAP, in SP children, were lower during the malaria transmission season compared to the subsequent dry season [4/16 (25%) vs. 10/16 (63%) responders, P= 0.04, X2 analysis]. Negligible SEA or SWAP-specific BM responses were detected in SN children. Enhanced MSP1-specific BM responses were noted across all age groups during the transmission season in SP versus SN children (10/16 [63%] vs. 4/16 [25%] responders, P= 0.04, X2 analysis; ratio 0.056 vs. 0.017, P=0.04) but equalized by the following dry season. AMA1-specific BM responses were greater in SP versus SN children, aged 9-14 years, during the malaria transmission season (6/8 [75%] vs. 2/8 [25%] responders, P=0.04, X2 analysis) and remained elevated at dry season follow-up (8/8 [100%] vs. 4/8 [50%], P=0.04). We conclude that detectable BM responses are present against both malaria and schistosomal antigens and that the presence of *S. haematobium* may be associated with enhanced BM responses to malaria antigens.

1289

MSP-1-SPECIFIC MEMORY B CELL RESPONSES ARE STABLE BUT B CELL PHENOTYPE FREQUENCIES CHANGES AFTER PROLONGED ABSENCE OF MALARIA TRANSMISSION IN HIGHLAND WESTERN KENYA

Cyrus Ayieko[1], Alexander Maue[2], Gideon N. Magak[3], Gregory Noland[4], Walter G. Jura[1], Ayub V. Ofulla[1], Rosemary Rochford[2], Chandy C. John[4]

[1]Maseno University, Kisumu, Kenya, [2]SUNY Upstate Medical University, Syracuse, NY, United States, [3]Moi University, Eldoret, Kenya, [4]University of Minnesota, Minneapolis, MN, United States

Reduction of malaria transmission could lead to decreased B cell stimulation and lack of induction of B cell memory to *Plasmodium falciparum* antigens. Conversely, a decrease in malaria transmission might reduce the frequency of "exhausted" memory B cells seen in populations with high levels of malaria transmission. To investigate changes in B cell immunity in the absence of malaria transmission, we assessed memory B cell responses in adults living in a highland area of Kenya that recently reported interruption in malaria transmission. Proportions and frequencies of MSP1-specific memory B cells and B cell phenotypes were measured in a cohort of 57 adults at a time after malaria interruption for a one year period (April 2008) and one year subsequently (April 2009). Antigen-specific memory B cells were assessed by B cell ELISPOT and B cell phenotyping by flow cytometry. During the one-year period, none of the individuals tested developed clinical malaria. MSP1-specific memory B cell responses were present in 55% and 60% of individuals respectively in 2008 and 2009. 75% of the participants who had MSP1-specific memory B cells in 2008 maintained the responses one year later. Proportions of MSP1-specific memory B cells increased over the one year period (p=0.02). B cell phenotyping demonstrated that at the second time point, the proportion of circulating CD19+ cells increased (p=0.0001) but the proportions of activated classical memory B cells (CD19+IgD-CD27+CD21-) (p=0.0001) and splenic marginal zone B cells (CD19+IgD+IgM+CD27+) decreased (p=0.0001). The proportion of atypical memory B cells (CD19+IgD-CD27-CD21-) and classical memory B cells remained the same across the two time points. The percentages of "exhausted" memory B cells (CD19+CD19+IgD-CD27-CD21-FcRL4+) were however very low at both time points (<5%). In conclusion, MSP-1 specific memory B cells persist in adults in areas of unstable transmission, even in the absence of transmission. However, the effects of a reduction in malaria transmission on B cell immunity and phenotype may not be restricted to malaria antigen-specific responses.

1290

ASSOCIATION BETWEEN *PLASMODIUM FALCIPARUM* ANTIBODY RESPONSES AND AMODIAQUINE-SULFADOXINE-PYRIMETHAMINE TREATMENT FAILURE IN KAMPALA, UGANDA

Chris Keh[1], Aashish Jha[1], Bridget Nzarubara[2], Philip J. Rosenthal[1], Grant Dorsey[1], Douglas F. Nixon[1], Bryan Greenhouse[1]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Makerere University, Kampala, Uganda

Elimination of *Plasmodium falciparum* after partially effective therapy is influenced by host immunity. We previously showed that responses

to treatment for uncomplicated malaria with amodiaquine-sulfadoxine-pyrimethamine (AQ+SP) were associated with surrogates of immunity, including age and proximity to a mosquito breeding site. To further assess associations between immunity and treatment response we studied humoral antimalarial responses in children in Kampala aged 1-10 years who received AQ+SP for treatment of uncomplicated malaria. We measured IgG responses to the following 8 *P. falciparum* antigens via ELISA in 207 pairs of serum samples collected on the day of therapy (Day 0) and 14 days after treatment (Day 14): circumsporozoite protein (CSP), liver stage antigen 1 (LSA1), apical membrane antigen 1 (AMA1), merozoite surface proteins 1, 2, and 3 (MSP 1, 2, 3), and the R0 and R2 domains of glutamine rich protein (GLURP). Results were standardized against pooled immune serum from adults living in Kampala. Our primary outcome was the genotype-adjusted risk of recrudescence within 63 days. Associations were estimated using generalized estimating equations. Age-adjusted IgG responses to AMA1 on Day 0 and Day 14 were significantly higher in those living closer to the breeding site ($p<0.02$). Overall risk of treatment failure was 12%. After adjusting for age and parasite polymorphisms associated with treatment failure, the risk of failing therapy was significantly lower in those with higher AMA1 responses on Day 0 (OR=0.79 / doubling of titer, $p=0.01$). IgG responses for the other antigens were not significantly associated with treatment response, however there was a trend for protection with higher Day 0 responses to MSP 2 (OR 0.79, $p=0.06$) and 3 (OR 0.81, $p=0.09$). Our findings demonstrate that antibody responses to AMA1 are associated with blood-stage immunity as measured by host clearance of parasites in the setting of partially effective therapy.

1291

EVALUATION OF *PLASMODIUM FALCIPARUM* MULTI-ANTIGEN ANTIBODY DYNAMICS IN INDIVIDUALS EXPERIENCING SUCCESSIVE ANNUAL INFECTIONS LIVING IN THE HYPOENDEMIC PERUVIAN AMAZON

Eva H. Clark[1], Onome Akpogheneta[2], Alexander Kayatani[3], Jean Hernandez[4], Diane W. Taylor[3], OraLee H. Branch[2]

[1]University of Alabama at Birmingham, Gurley, AL, United States, [2]New York University, New York, NY, United States, [3]University of Hawaii, Honolulu, HI, United States, [4]Universidad Nacional de la Amazonia Peruana, Iquitos, Peru

Years of exposure to *P. falciparum* are necessary for the development of immunity in high transmission areas, suggesting that protective humoral responses are disabled by parasite hyperexposure. However, in low transmission areas, such as the Peruvian Amazon, malaria-exposed individuals produce immune responses leading to clinical protection after only a few infections. To investigate the antigens responsible for these effective immune responses, this study used antigen-conjugated beads in a LUMINEX system to compare the pre-, during, and post-infection antibody responses to 8 antigens, including AMA1, CSP, EBA175, LSA1, MSP1, MSP2, MSP3, and MSP6. 34 adults and 21 children with samples from 2-3 successive infections spaced by ~1 year were evaluated. We found that adults maintained AMA1, EBA175, MSP1 and MSP3 responses for >300 days post-infection, while in children responses to only MSP1 and MSP3 lasted for >300 days. Although differences in the magnitude/longevity of responses among 1st, 2nd, and 3rd detected infections are recognizable, in adults there were no significant differences for any antigen. However, antibody levels to EBA175 and MSP-3 were significantly higher among children at the 3rd detected infection than at the 1st, suggesting that these responses are boosted earlier at each subsequent infection. After correlating each antigen response to the others, some antigen pairs were associated with more parasite exposure (AMA1&EBA175, AMA1&MSP3 and EBA175&MSP2), and some were associated with less exposure (EBA175&MSP1 and AMA1&MSP1). When comparing responses in asymptomatic versus symptomatic adults, responses to MSP1, MSP2, MSP3, and MSP6 were found to be significantly higher in asymptomatics than in symptomatics at the 2nd detected infection but not at the 1st. In summary, although MSP1 produced the largest post-infection responses even at the 1st detected infection, other blood-stage antigens, particularly MSP3 (to which even children elicited long-lived responses), were found to be important players in the anti-malarial immune response despite low transmission exposure.

1292

HAPLOTYPES OF FC GAMMA (FCΓ) RECEPTOR (FCΓRIIA AND FCΓRIIIB) PREDICT SUSCEPTIBILITY TO HIGH-DENSITY PARASITEMIA AND FUNCTIONAL CHANGES IN CIRCULATING IFN-Γ LEVELS IN CHILDREN WITH *PLASMODIUM FALCIPARUM* MALARIA IN WESTERN KENYA

Collins O. Ouma[1], Gregory C. Davenport[2], Samuel Kiplagat[1], Stephen Garcia[2], Prakasha Kempaiah[2], Ateefa Chaudhary[2], Tom Were[1], Samuel B. Anyona[1], Evans Raballah[1], James B. Hittner[3], John M. Vulule[4], John M. Ong'echa[1], Douglas Perkins[2]

[1]University of New Mexico/KEMRI, Kisumu, Kenya, [2]University of New Mexico, Albuquerque, NM, United States, [3]College of Charleston, Charleston, SC, United States, [4]KEMRI, Kisumu, Kenya

The development of protective immunity against *Plasmodium falciparum* is partially mediated through binding of malaria-specific IgG to Fc gamma (γ) receptors. Human FcγRIIA-H/R-131 and FcγRIIIB-NA1/NA2 exhibit polymorphic variability associated with differential binding to IgG subtypes and malaria disease outcomes. The role of FcγRIIA-H/R131 and FcγRIIIB-NA1/NA2 haplotypes in conditioning susceptibility to high-density parasitemia (HDP; ≥10,000 parasites/μL), a clinical manifestation of severe malaria in *P. falciparum* holoendemic areas, however, is largely undefined. As such, the role of FcγRIIA-H131R/FcγRIIIB-NA1/NA2 haplotypes was investigated in children (n=528) presenting with acute malaria at a rural hospital in western Kenya. Since variations in the FcγR may alter interferon gamma (IFN-γ) levels, a mediator of both innate and adaptive immune responses, additional functional analyses were carried out in the context of the FcγR haplotypes. Results reveal that circulating IFN-γ was negatively correlated with parasitemia levels (r=-1.740, P=0.005). Children with HDP also had lower circulating IFN-γ levels than the non-HDP group (P<0.001). Multivariate logistic regression analyses controlling for covariates revealed that carriage of the FcγRIIA-131R/FcγRIIIB-NA1 haplotype was associated with protection against HDP (OR; 0.48, 95%CI, 0.31-0.76; P=0.002), while carriage of FcγRIIA-131H/FcγRIIIB-NA1 haplotype increased susceptibility to HDP (OR; 1.49, 95%CI, 1.04-2.14; P=0.031) relative to individuals without these haplotypes. Carriers of the FcγRIIA-131H/FcγRIIIB-NA1 (131H/NA1) haplotype had significantly lower IFN-γ levels relative to non-carriers (P=0.046), while FcγRIIA-131R/FcγRIIIB-NA1 (131R/NA1) haplotype had elevated IFN-γ levels relative to non-carriers (P=0.067). These results demonstrate that variations at the FcγR gene are associated with functional changes in IFN-γ production, and susceptibility to HDP in children with *falciparum* malaria.

1293

SPATIAL DISTRIBUTION, HABITAT CHARACTERIZATION AND DYNAMICS OF *ANOPHELES GAMBIAE* MOLECULAR FORMS LARVAL BIOTOPES ALONG AN URBANIZATION GRADIENT IN THE CITY OF YAOUNDÉ, CAMEROON

Billy F. Tene[1], Christophe N. Antonio[2], Colince Kamdem[2], Philippe Bousses[3], Didier Fontenille[3], Nora J. Besansky[4], Carlo Costantini[1]

[1]IRD-UR016/OCEAC, Yaoundé, Cameroon, [2]LRP-OCEAC, Yaoundé, Cameroon, [3]IRD-UR016, Montpellier, France, [4]University of Notre Dame, Notre Dame, IN, United States

Increasing urbanization in Africa is drawing the attention of public health managers on urban malaria, and it raises the question of whether malaria vectors have the potential to adapt to the environmental stressors normally encountered in the most densely populated cities. In the forest domain of southern Cameroon, the molecular forms M and S of *Anopheles gambiae* segregate along urbanization gradients, suggesting that a

process of adaptation by the M form to the urban environment is under way (Kamdem et al., submitted). This process is presumably driven by the ability of M larvae to develop successfully in polluted urban habitats. To characterize the larval biotopes of M and S and their dynamics, we conducted a longitudinal survey of *An. gambiae* larval habitats to assess their distribution and relationship with human activities in the capital Yaoundé and peri-urban neighborhoods. A total of 2,449 potential mosquito breeding sites were examined, of which about 20% contained *An. gambiae* larvae. Anopheline larval habitats were more abundant in urban compared to rural or suburban areas. Seasonal fluctuations in breeding sites availability were more pronounced in the rural than urban habitat. Draining streams and swamps were associated with no or very low larval densities. Human activities such as vegetable market gardening, housing in swampy areas, and construction sites were associated with breeding sites of *An. gambiae*. Unexpectedly, *An. gambiae* larvae were collected from urban breeding sites highly polluted with organic matter. PCR identification revealed that only the M molecular form of *An. gambiae* was present in the most urbanized settings, whereas the S form was by far the most abundant in the rural sites, the suburban ones being transitional between these extremes. These findings provide evidence that the malaria vector *An. gambiae* s.s. is adapting to urban waste waters, and clearly partition the distribution of the molecular forms M and S between urban and rural areas.

1294

SPATIAL-TEMPORAL DISTRIBUTION OF IMMATURE AND ADULT MALARIA VECTORS IN FOUR ECOLOGICAL SETTINGS IN COASTAL KENYA

Francis M. Mutuku[1], Charles H. King[2], Peter L. Mungai[1], Eric M. Muchiri[1], Edward D. Walker[3], Uriel Kitron[4]

[1]Division of Vector-Borne and Tropical Neglected Diseases, Msambweni, Kenya, [2]Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH, United States, [3]Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, United States, [4]4Department of Environmental Studies, Emory University, Atlanta, GA, United States

The on-going malaria control activities in Kenya through use of Insecticide treated nets (ITN) and Indoor residual spraying (IRS) necessitate up-to-date information on malaria vectors. An ecological study of the spatial-temporal distribution of immature and adult malaria vectors was conducted in eight villages (two in each of four ecological settings) in the south coast of Kenya. Longitudinal larval surveys were conducted monthly in selected larval habitats from May 2009 to March 2010 using standard dipper. Additionally, adult malaria vectors were concurrently collected using pythremum spray collection (PSC) and clay pots in 10 houses from March 2009 to March 2010. A total of 285 Anopheline larvae were sampled during the 10 months of larval sampling. The number and quality of larval habitats sampled in each ecological setting fluctuated with rainfall. *Anopheles* larvae were found most frequently in larval habitats located in the estuarine habitats, accounting for 81% of the total larvae sampled. The majority of the larvae were *An. gambiae* s.l (88%), with *An. funestus* comprising the rest (12%). Mean density of *Anopheles* larvae was 3 times higher in the estuarine habitats compared to the other three ecological settings combined. Abundance and density of *Anopheles* larvae was highly associated with depth, pH and conductivity of aquatic habitats. Correspondingly, 69% (962/1386) of the adult mosquitoes were *An. gambiae* s.l with *An. funestus* comprising the remaining 31% (424/1386). An average of 7 *An. gambiae* s.l and 2 *An. fune*stus mosquitoes were collected each month in the estuarine environment compared to <1 mosquito of each species in the other ecological settings. Overall, densities of adult malaria vectors were low throughout the study period, and were highly dependent on rainfall throughout the 12 months. Both abundance and composition of malaria vectors was dependent on the ecological setting and modulated by rainfall. While these findings are not surprising, only limited data was available for the south coast of Kenya. The findings of study will be useful for the planning and implementation of control strategies for malaria vectors.

1295

ENVIRONMENTAL CHANGE AND THE MICROBIAL ECOLOGY OF *ANOPHELES GAMBIAE*

Thomas M. Gilbreath, III[1], Eliningaya Kweka[2], Guiyun Yan[1]

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

Recent studies suggest that land use changes, such as deforestation, strongly enhance the productivity of malaria vectors, and thus malaria transmission. This is because deforestation exposes aquatic habitats to sunlight, resulting in increased temperatures. Further, sunlight may induce changes in the microbial communities that mosquito larvae use for nutrition. This study utilized field-based microcosm approaches in combination with water chemistry analyses and pyrosequencing for microbial diversity in order to examine the impacts of environmental change in *An. gambiae* larval habitats and habitat vector productivity. Results of habitat productivity in different land use scenarios have demonstrated a significant effect of land use and canopy cover on larval malaria vector survivorship and habitat productivity. Survivorship in semi-forested and naturally forested areas was reduced 20% and 99%, respectively when compared to areas that had been deforested. Interestingly, when microcosm temperature in the field was controlled, temperature was shown to have the strongest effect on pupation rate while larval survivorship was more affected by algal biomass. Microbial diversity analyses show significant differences in bacterial communities from deforested, semi-forested, and forested habitats. Bacterial communities in the surface microlayers and larval guts from the same habitat also showed significant differences in composition and suggested a selective assimilation of photosynthetic microbes. These preliminary results suggest that *An. gambiae* ss preferentially feeds on photosynthetic microbes in the surface microlayer of their habitats and that the role of microbial community changes induced by light may play a more important role than temperature in some scenarios.

1296

PROFILING GUT MICROBIOTA IN MOSQUITO *ANOPHELES GAMBIAE* USING PYROSEQUENCING

Ying Wang[1], Thomas M. Gilbreath, III[2], Phanidhar Kukutla[1], Guiyun Yan[2], Jiannong Xu[1]

[1]New Mexico State University, Las Cruces, NM, United States, [2]University of California, Irvine, Irvine, CA, United States

Mosquito *Anopheles gambiae* is a major malaria vector. Vector competence is determined by the tripartite eco-interactions among microbes, malaria and mosquito immunity. Mosquito gut harbors diverse microbial communities. However, little is known about the dynamics of the gut microbiota from larva to adult and its impact on the gut eco-symbiotic interplays. Early studies of the gut bacteria relied on culture-and/or cloning-based low throughput techniques that characterize only a small fraction of the microbiota. Using pyrosequencing approach targeting the V1-3 hypervariable region of the 16S rRNA gene, we gained an unprecedented view of the diversity present in the gut microbiota and were able to detail the dynamics of the gut microbial community from larva to adult and assessed the effect of blood feeding on the gut community. Pyrosequencing yielded 79592 sequences from 14 samples of a lab reared *An. gambiae*. The sequences correspond to 260 genera belonging to 11 phyla with dominances of *Proteobacteria*, *Bacteroidetes, Actinobacteria* and *Firmicutes*. The structure of gut microbiota changes along the life stages. Among the tags obtained from larva, more than half (54.3%) belong to the Family *Enterobacteriaceae* (unable to classify to genus). *Microbacterium* (23.7%) is abundant only in larva. Pupa

harbors a complex community with the dominances of *Elizabethkingia* (26.1%), *Acidovorax* (10.5%) and *Enterobacteriaceae* (10.8%). In newly emerged adults, flora composition becomes simpler, dominated by *Elizabethkingia* (55.9%), *Staphylococcus* (8%), *Enterobacteriaceae* (7.5%) and *Leucobacter* (6.9%). At least 7 out 20 (35%) genera that were present in pupa did not rise in the adult, likely being cleaned up by the sterilization process during the metamorphosis. *Finegoldia, Serretia* and *Pantoea* seem to be newly established in the adult. Bloodmeal reduces the diversity of gut microbiota. After blood feeding, 6 out 17 (35.3%) genera disappeared. *Elizabethkingia* and *Enterobacteriaceae* dominate the community, accounting for up to 77% of the total tags. The diversity of gut communities reduced with operational taxonomic unit (OUT) dropping from 110 to 36. Richness of the communities was estimated by ACE and Chao implemented by the software Mothur. By picturing gut communities we have gone one step further towards a better understanding the mosquito gut ecosystem.

1297

SYMPATRIC *PLASMODIUM FALCIPARUM - ANOPHELES GAMBIAE* POPULATIONS PRODUCE LOWER INFECTION INTENSITIES IN AFRICA

Caroline Harris[1], Isabelle Morlais[2], Thomas S. Churcher[3], Louis Clement Gouagna[4], Roch D. Dabire[5], Didier Fontenille[1], Anna Cohuet[4]

[1]Institut de Recherche pour le Developpement, Montpellier, France, [2]Laboratoire de Recherche sur le Paludisme/Institut de Recherche pour le Developpement, Yaoundé, Cameroon, [3]Imperial College London, London, United Kingdom, [4]Institut de Recherche en Sciences de la Santé/Institut de Recherche pour le Developpement, Bobo Dioulasso, Burkina Faso, [5]Institut de Recherche en Sciences de la Santé, Bobo Dioulasso, Burkina Faso

Both *Plasmodium falciparum* and *Anopheles gambiae* show great diversity in Africa, in their own genetic makeup and malaria infection phenotypes. The genetics of the individual mosquito and parasite are known to play a role in determining the outcome of infection, but whether differences in infection phenotype vary between populations remains to be investigated. Here we conducted experimental infections using two recently established *An. gambiae* colonies from Cameroon and Burkina Faso and wild *P. falciparum* corresponding to their sympatric and allopatric populations. Infection phenotype was determined in terms of oocyst prevalence and intensity for at least nine infections for each vector-parasite combination and compared between infection types. We show that the mosquito colony used (sympatric or allopatric to the parasite) has no significant effect on infection prevalence, however has strong effects on infection intensity. Sympatric infections produced 25% fewer oocysts per midgut than allopatric infections, while prevalence was not affected by sympatric/allopatric interactions. The reduction in oocyst numbers in sympatric couples may benefit both the vector and parasite. It has been proposed that increasing the number of parasites ingested by a mosquito reduces its survival. If this is the case then a lower infection intensity, without affecting prevalence, would increase the fitness of infected mosquitoes while at the same time increasing the parasites chance of being transmitted. If the fitness costs are confirmed, this suggests local adaptation of the vector to the parasite and parasite to the vector, which has strong implications for malaria transmission dynamics.

1298

THE EVOLUTIONARY CONSEQUENCES OF HOST SPECIES CHOICE FOR AFRICAN MALARIA VECTORS: COULD UNTREATED BED NETS SELECT FOR A HOST SHIFT?

Issa Lyimo[1], Daniel Haydon[2], Tanya Russell[1], Kasian Mbina[1], Ally Daraja[1], Edgar Mbehela[1], Richard Reeve[2], Heather Ferguson[2]

[1]Ifakara Health Institute, Morogoro, United Republic of Tanzania, [2]University of Glasgow, Glasgow, United Kingdom

The host preference of malaria vectors is one of the key determinants of global transmission patterns. Here we conducted an experimental investigation of the major African malaria vectors Anopheles gambiae s.s and An. arabiensis to test whether their preference for humans over other commonly available animal hosts can be explained by the fitness benefits they derive from them, and whether the use of common interventions such as bednets can reduce the advantage of anthrophily to the point where selection for a host species shift could be generated. Experiments were conducted in which one host of either cow, human (exposed or protected by untreated net), dog, goat or chicken was placed inside an experimental hut set within a unique Semi-Field System (SFS) at Ifakara Health Institute in Tanzania. Groups of 200 insectary-reared An. gambiae s.s or An. arabiensis were released into the chamber at dusk and left overnight with the host. The next morning mosquitoes were recaptured and their blood feeding success and subsequent fecundity and survival measured (6 replicates of each host and vector species combination).

Whereas Anopheles arabiensis had a significantly greater feeding success on its naturally preferred bovid hosts, the more anthrophilic An. gambiae s.s. took considerably bigger blood meals and had greater survival after feeding on human than animal blood. The use of a bednet failed to completely prevent biting by either vector, but did reduce the expected fitness benefits from human hosts. By modeling the combined effect of all host species impacts on vector fitness, a mosquito life-history model predicted the lifetime egg production of An. arabiensis to be considerably higher on their naturally preferred bovids than all other host types. However, the lifetime reproductive success of the naturally anthrophilic An. gambiae s.s. was not predicted to be higher on humans than any other host species. Further study is required to identify the nature of selection favouring anthrophily in this vector, and possibilities for reducing it through vector control and/or environmental management.

1299

QUANTIFYING AND ANALYZING DANCE OF *ANOPHELES GAMBIAE* IN MATING SWARMS

Nicholas C. Manoukis[1], Sachit Butail[2], Derek Paley[2], Alpha S. Yaro[3], Moussa Diallo[3], Sekou F. Traoré[3], Adama Dao[3], José M. Ribeiro[1], Tovi Lehmann[1]

[1]Laboratory of Malaria and Vector Biology, National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States, [2]Department of Aerospace Engineering, University of Maryland, College Park, MD, United States, [3]Malaria Research and Training Center, Faculté de Médecine, de Pharmacie et d'Odontostomatologie, Université de Bamako, Bamako, Mali

We describe a technological breakthrough achieved through interdisciplinary collaboration that allows us to track individual swarming *Anopheles gambiae* males and females from stereoscopic video footage obtained from the field in Mali, Africa in August 2009. Mating behaviors of malaria vectors in nature are rarely described or studied, and even more rarely quantified. The actual movement of individual members of a mating swarm of mosquitoes over time has never before been measured. The principal reason this stage of the life history remains unexplored is that direct observation and quantification of mating in a swarm is very difficult due to the small size of mosquitoes, their relatively fast rate of movement and their habit of aggregating under conditions of low light. Despite our lack of knowledge in this area, determinants of male mating success in

medically important species are of major interest from a fundamental and applied perspective. We estimate three-dimensional position and velocity of individual mosquitoes through space and time by employing advanced image processing and probabilistic estimation techniques in a semi-automated computer tracking system. We verify the accuracy of our tracking system along several parameters by comparing it with manually created ground-truth data. We have used the system to quantify and analyze three instances of couple formation in *An. gambiae* swarms. These measurements reveal that female *An. gambiae* spend much more time in a swarm composed almost entirely of males than previously thought: more than six seconds in one instance. We will describe ongoing studies in which we are quantifying the differences in the flight patterns of males and females within the mating swarm and studying male flight patterns for determinants of male mating success. These data and the increasingly automated capacity in which we generate them promise to greatly extend our fundamental understanding of *An. gambiae* mating biology and have implications for any release-based approach to its control as a malaria vector.

1300

IMPROVING AVAILABILITY OF KEY ANTIMALARIAL COMMODITIES: PILOTING AN ESSENTIAL DRUG LOGISTICS SYSTEM IN ZAMBIA

Arturo Sanabria, Wendy Nicodemus, Gamariel Simpungwe, Rabson Zyambo, Walter Proper

United States Agency for International Development | DELIVER PROJECT, Lusaka, Zambia

The successful implementation of malaria programs depends on a continuous supply of malaria-related commodities: artemisinin-based combination therapy (ACTs), rapid diagnostic tests (RDTs), sulphadoxine-pyrimethamine (SP), and quinine. From January 2009 to January 2010, the United States Agency for International Development | DELIVER PROJECT supported the Ministry of Health of Zambia when they conducted an essential drugs/malaria logistics systems pilot to determine what key logistics factors impact commodity availability and to identify and implement the most effective elements to ultimately improve efficiency in the supply chain. To ensure statistical power, the districts were selected using a random stratified technique; eight districts per pilot system were selected. Twenty-four districts were involved, including a control group of eight districts. Some of the factors used in the sampling included prevalence of malaria, accessibility, urban/rural factor, and number of facilities. With support from the United States Agency for International Development | DELIVER PROJECT, a steering committee of key partners designed and piloted two logistics systems. In one system, the district serves as a pass-through to distribute commodities that are pre-packaged by the central medical stores. In the other, the district stores, packages, and supplies the facilities directly. The two pilot systems were rolled out in 16 districts and 347 service delivery sites. Specific variables tested were the availability of antimalarial drugs, transport from district to facility, storage capacity, and commodity management at the district- and health-facility level. To determine the impact on stock availability in each of the pilots, teams conducted a final evaluation visit to 259 sites, which were randomly chosen. The results of the assessment, completed in March 2010, showed that introducing a logistics system drastically reduced the stockout levels of antimalarial products, even when some products had stockouts at the central level. For example, pediatric ACTs were available in 88% of the pilot facilities, compared with 51% in the control; they were available 345 days a year, compared with 247 days in the control.

1301

FIVE-YEAR OVERVIEW OF STRENGTHENING REGIONAL CAPACITY IN MEDICINES QUALITY IN THE MEKONG SUBREGION: A FOCUS ON COUNTERFEIT AND SUBSTANDARD MEDICINES

Souly Phanouvong

United States Pharmacopeia, Rockville, MD, United States

In the Mekong subregion, an estimated 10-35% of medicines are improperly made or illegally imported and sold. To help country authorities in this region address medicine quality problems, in collaboration with its partners, the Promoting the Quality of Medicines (PQM) program - funded by the United States Agency for International Development (United States Agency for International Development) and implemented by the United States Pharmacopeia (USP) - has introduced a regional mechanism for Medicine Quality Monitoring (MQM). The MQM program focuses on early detection of poor-quality antimalarial, anti-TB, antibiotic, antiretroviral, and Avian Influenza medicines and strengthening the capacity of regulatory authorities for enforcement actions based on evidence obtained from the field. The ultimate goal is to reduce the prevalence of poor-quality medicines_counterfeit and substandard_available in the public, private, and informal sectors in Cambodia, Lao PDR, Thailand, and Vietnam. A well-designed framework and protocol for sampling, testing, data management and reporting were used. Data collected in 2004 revealed the wide availability of poor quality medicines: in some Mekong countries, up to 44% of artesunate (a commonly used, highly efficacious antimalarial) samples collected and tested contained no active ingredient. In 2008, this figure dropped to 11.2%. From 2005-2009 in the Mekong Subregion, the MQM program sampled 3,021 antibiotic, 6,176 antimalarial, 625 anti-tuberculosis, and 234 antiretroviral medicines. Antibiotics and antimalarials had the highest failure rate of 2.3% and 2% respectively. To present, the total number of samples collected and tested has reached almost 4,000. While initial failure rates in the region averaged around 6%, there was a steady trend toward a decreasing prevalence at the 42 monitoring sentinel sites in the region and in 2009, the overall failure was 1.3%. Medicines quality data were used to raise public awareness and shared with national law enforcement agencies for appropriate actions. They were also shared with international agencies like World Health Organization and INTERPOL, for collective investigations and actions at regional and international levels. Despite these progresses and achievements, many challenges remain to be addressed and the MQM needs to be scaled up to cover the whole region.

1302

CHANGING GUIDELINES FOR MALARIA CASE MANAGEMENT: CAN HEALTH FACILITIES KEEP UP?

Florence R. Nankya[1], Catherine Maiteki-Sebuguzi[1], Deborah DiLiberto[2], Miriam Kayendeke[1], Moses R. Kamya[3], Clare Chandler[2], Sarah G. Staedke[4]

[1]Infectious Diseases Collaboration Research, Kampala, Uganda, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom, [3]Infectious Diseases Collaboration Research, Department of Medicine, Makerere University, Kampala, Uganda, [4]Infectious Diseases Collaboration Research, London School of Hygiene and Tropical Medicine, Kampala, Uganda

The World Health Organization (WHO) has recently released new guidelines for treatment of malaria, recommending that suspected cases be confirmed by a parasitological test when possible. However, the current capacity for diagnosis and management of malaria and other febrile illnesses within the existing health system is unclear. To inform the design of future interventions, we conducted a situational analysis of government-run health centers in rural Eastern Uganda. Health workers stationed at centers in five sub-counties in Tororo district were approached for study participation. Structured questionnaires

addressing staffing, training and supervision, and knowledge of malaria case management were administered to consenting health workers. We interviewed 81 (88%) of 92 health workers stationed at the 17 local health centers, representing the spectrum of staff cadres. Staffing shortages were a problem at nearly all health centers. Unpaid volunteers with limited medical training constituted 26% of staff interviewed, and 48% at the lowest-level health centers. Most health workers (56 [69%]) were trained in management of malaria with artemether-lumefantrine, but only 29 (26%) had received training in rapid diagnostic tests (RDTs) for malaria. Overall, knowledge about malaria case management was poor; mean knowledge scores ranged from 18% (vaccinators) to 45% (nursing officers). The in-charges of health centers scored surprisingly low, as did volunteers (mean scores 34% and 20%, respectively). When asked how to confirm the diagnosis of malaria, only eight (10%) health workers mentioned microscopy and two (2%) RDTs. Additional gaps in knowledge included recognition of danger signs for severe malaria, differential diagnosis of non-malarial febrile illnesses, and key elements of managing and treating malaria. The shift towards universal diagnostic testing for malaria is a major step to ensuring appropriate management of malaria and other febrile illnesses. We found that health centers in Eastern Uganda currently have limited capacity to adhere to WHO's new guidelines. Poor staffing of health centres and gaps in knowledge urgently need to be addressed.

1303

VOLUNTEER COMMUNITY HEALTH WORKERS: TEMPORARY FIX OR LONG-TERM SOLUTION?

Kristin Banek[1], Joaniter Nankabirwa[2], Deborah DiLiberto[3], Lilian Taaka[4], Clare I. Chandler[5], Sarah G. Staedke[5]

[1]Uganda Malaria Surveillance Project, Kampala, Uganda, [2]Makerere University, Kampala, Uganda, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom, [4]Infectious Disease Research Collaboration, Kampala, Uganda, [5]London School of Hygiene and Tropical Medicine, London, United Kingdom

Community management of febrile children is evolving away from providing treatment of malaria only, toward a more integrated approach, focusing on malaria, pneumonia and diarrhea. However, more complex programmes place higher expectations on community health workers (CHWs). In Uganda, a policy of integrated community case management (iCCM) is being adopted. To investigate current challenges faced by CHWs, we conducted a situation analysis in rural Eastern Uganda. We interviewed 100 CHWs selected using convenience sampling from five sub-counties in Tororo district. A structured questionnaire addressing patient load was administered, and in-depth interviews were conducted. Focus group discussions were also conducted with health workers and community members. The CHWs reported receiving close to 20 patients per week (mean 19.5, SD 12.2, range 2-63), including 15 malaria patients (mean 15, SD 10.8). Only ten CHWs reported receiving specific incentives, such as payment, to motivate them to perform their duties. Interviews revealed that over 80% of CHWs were motivated by non-monetary incentives including altruistic reasons, good relationships with health workers, and self benefit, with the strongest theme focusing on respect or 'becoming someone important' through their work. However, over half of CHWs felt demotivated due to limited support from communities and the health system, unrealistic expectations of caregivers, lack of drugs and supplies, and lack of compensation. Although communities appeared to understand the role of CHWs, they often inappropriately attributed the logistical constraints and lack of CHW motivation to personal rather than system shortfalls. Our results suggest that many volunteer CHWs in Uganda are motivated by altruistic and/or self-serving motives. However, the non-monetary benefits of becoming a CHW, including social status, appear to wane after one year. Relying on minimally trained volunteers to deliver community programmes may temporarily address gaps in the health system but is unlikely to be a sustainable solution. Focus on community programmes should not divert attention away from longer-term interventions to improve health care infrastructure and delivery of good quality health care by trained practitioners.

1304

DEMYSTIFYING DRUG DELIVERY: A ONE-PAGE SOLUTION

Deborah DiLiberto[1], Florence Nankya[2], Miriam Kayendeke[2], Christine Nabirye[2], Clare I. Chandler[1], Sarah G. Staedke[1]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]Infectious Disease Research Collaboration, Uganda Malaria Surveillance Project, Kampala, Uganda

Drug stock-outs due to poorly functioning delivery systems are a major barrier to providing good quality health care in many resource-poor settings. In Uganda, stock-outs of artemether-lumefantrine (AL) threaten strategies to reduce malaria-related morbidity and mortality. Complex, large-scale initiatives have been suggested to improve health systems; however, few inexpensive, simple strategies exist to improve drug delivery at the periphery. To investigate challenges and possible solutions for drug delivery in Uganda, structured questionnaires were administered to representatives of 17 government-run health centers in Tororo district. We also conducted a literature review, in-depth interviews, and focus group discussions (FGDs) with health workers and key informants. Drug stock-outs are a major problem at most health centers, particularly at the lowest level. Two (12%) centers never stock AL, and only 13 (76%) had AL in stock on the day of the interview. Stock-outs of other antimalarial drugs, such as oral and injectable quinine and artesunate + amodiaquine, and other medications including panadol, amoxicillin, mebendazole, oral rehydration solution, and iron were also reported in most health centers. The literature review revealed several challenges to drug delivery including managerial inefficiencies, limitations in human resources, and logistical constraints. The interviews and FGDs revealed that health workers lack knowledge about the drug delivery system and are confused about the roles and responsibilities of staff involved in drug procurement. Challenges including inadequate transport, lack of funding, and delays in processing orders were cited as reasons for stock-outs. Most health workers agree that failure to provide drugs results in unmet expectations and poor quality health care. The challenges identified represent logistical nodes which should be operational to ensure effective and efficient drug delivery. We have developed a simple one-page tool based on formative evaluation and logistics measurement methodologies to help identify, communicate and resolve drug distribution issues. We plan to implement and evaluate the tool in a cluster-randomized control trial in Uganda.

1305

ANTIBIOTIC USE IN INFANTS IN A SETTING WHERE ANTIBIOTICS ARE AVAILABLE WITHOUT PRESCRIPTION: WHOSE RESPONSIBILITY?

Lucie Ecker[1], Liset Olarte[2], Gustavo Vilchez[1], Theresa J. Ochoa[2], Isabel Amemiya[1], Ana I. Gil[1], Claudio F. Lanata[1], Thomas G. Cleary[3]

[1]Instituto de Investigación Nutricional, Lima, Peru, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]University of Texas Health Science Center at Houston, Houston, TX, United States

Antibiotics are the most commonly prescribed drugs in children. However their misuse has been associated with the development of resistant pathogens. Generally, it has been assumed that the availability of antibiotics without medical advice has been one of the most important causes of misuse. The aim of this study was to describe the use of antibiotics in Peruvian infants in a setting where antibiotics are available without prescription. Within a cohort study of 1023 children of peri-urban Lima, antibiotic use data were recorded in the clinical records at a study clinic. Children less than 2 months of age were enrolled and followed until 12 months of age. History of previous illnesses and drug use prior to enrollment and between the scheduled visits was recorded in the medical

record as well as the treatment offered by the study physician. Clinical records were reviewed and descriptive analysis was performed. During a one year period, 770 of 1023 (75.3%) children took 2085 courses of antibiotics. There were two courses/child/year, range (0-12). The mean age of usage was 6.5 months (range 9 days to 12.9 months). Higher rates of antibiotic use were found in children from 3 to 6 months of age (37.2%). Antibiotics were given to children in 8.5% of common colds, 59.1% of all pharyngitis, 68.9% of bronchitis, 64.4% of diarrheas, 23% of dermatitis, and 12% of bronchial obstruction. Physician prescription was the most common reason for antibiotic use (90.8%), self-medication was found in 6.9% and was preceded by a physician antibiotic prescription in 63.9%. The most frequently used antibiotics were penicillins (32.9%) and macrolides (23.4%). Upper respiratory tract infections were treated mainly with penicillins (56.4%), diarrhea with macrolides (49.6%) and bronchial obstruction with penicillins (44.6%). Based on the diagnoses 83.1% of the antibiotic prescribed drugs were inappropriate. In conclusion, infants are often exposed to antibiotics early in life in this peri-urban area. Antibiotic use is typically inappropriate (83.1% of courses) based on the most common etiologies for this age-group. Since physicians prescription was the most common reason for antibiotic use, interventions to improve use of antibiotics should focus on them.

1306

FEASIBILITY OF WEB-BASED TELECONFERENCING FOR REMOTE ULTRASOUND TRAINING AND QUALITY ASSURANCE IN REMOTE SETTINGS

Walter (Ted) Kuhn, Matthew Lyon

Medical College of Georgia, Augusta, GA, United States

Ultrasound (US) is an important adjunct to diagnosis and management in resource-limited settings. However, US is highly operator dependent and in remote locations, ongoing training is difficult. The objective of this study was to determine the feasibility of remote US training and quality assurance (QA) in a remote, resource-limited setting. Ultrasound training was provided to physicians working in the developing world using a SonoSite 180 Plus US machine with a C60 broadband curved array transducer. The investigators reviewed selected exams obtained on patients remotely as well as conducting on-line real time QA and educational sessions. Cases were reviewed from remote locations in South America and South-East Asia using two methods of web-based video conferencing: Skype and ooVoo. Skype worked well for one-on-one training using ultrasound images only, but did not allow for ultrasound video conferencing. However, the ultrasound equipment only allowed for still image archive. Skype also did not allow for PowerPoint based presentations. OoVoo allowed for video conferencing at more than one site. In this case, 4 sites were imaged simultaneously in 2 countries with streaming-video. OoVoo allowed for PowerPoint based presentations to be viewed by all participants. OoVoo required a faster Internet connection and one site had a long delay in the video images (Cambodia). Video conferencing allowed for training in patient positioning, sonographic windows, and also suggestions in improvement in scanning techniques. Review of one scan resulted in a change in diagnosis, which was significant, diagnosis of gallbladder cancer. The video conferencing using Skype from South America went well without loss of signal. With multiple video conferencing using ooVoo, there was a transmission delay, which was most pronounced in the South-East Asia site and resulted in loss of connection 3 times over the 90-minute session. In conclusion, web-based QA and ongoing training in US with remote, resource-limited sites is possible using commercially available programs.

1307

MOSQUITO CELLS SURVIVE FROM DENGUE 2 VIRUS INFECTION THROUGH AN ANTI-OXIDANT DEFENSE

Tien-Huang Chen, Petrus Tang, Ching-Kai Chuang, Yu-Tzu Shih, Chao-Fu Yang, **Wei-June Chen**

Chang Gung University, Tao-Yuan, Taiwan

Both mosquito and mammalian cells are essential for the propagation of dengue (Den) viruses during alternate transmission of the viruses. The viruses generally induce apoptosis in mammalian cells but cause only minor damages in mosquito cells, leading to persistent infection in most cases. In order to find genes involved in determining the cell fate, datasets derived from expressed sequence tags (ESTs) of C6/36 cells with and without infection were established. A total of 3876 unigenes which contained 875 contigs and 3001 singletons were obtained. Of which, 2267 and 2189 unigenes were respectively expressed in mock- and infected complementary DNA (cDNA) libraries. Among the generated unigenes, 600 (in addition to 7 viral proteins) were only found in infected cells, while 642 were only found in mock-infected cells. Chaperone protein including GRP78/BiP and endoplasmin were found to be significantly upregulated in C6/36 cells infected by Den-2 virus for 24 h. This suggests that Den-2 virus infection in mosquito cells, as in mammalian cells, activates the unfolded protein response (UPR) to cope with the endoplasmic reticulum (ER) stress at the early stage of infection. Changes of mitochondria membrane potential (MMP) and generation of superoxide provided further evidence that Den-2 virus induce the oxidative stress in spite most infected cells remain intact. Due to significant elevation of the superoxide dismutase (SOD) activity, mosquito cells are able to rescue themselves from viral infection through antioxidant defenses. The findings of this study have shed lights on interactions between the virus and host cells, particularly mosquito cells.

1308

DENGUE-2 ALTERS SALIVARY GLAND PROTEIN EXPRESSION IN INFECTED *AEDES AEGYPTI* MOSQUITOES

Daniel M. Chisenhall, Berlin L. Londono, Rebecca C. Christofferson, Christopher N. Mores

Louisiana State University, Baton Rouge, LA, United States

The principle arthropod vector of dengue virus is the *Aedes aegypti* mosquito, and in that vector the virus must disseminate to the salivary glands prior to being transmitted to the vertebrate host via salivation during probing and/or feeding. As has been shown previously, the saliva of mosquitoes contains a diverse cocktail of pharmacologically active compounds that are deposited simultaneously with the virus to the bite site of the vertebrate host. It is here that the mosquito's saliva modifies the local environment, perhaps in a way that facilitates the establishment of an infection. In order to determine whether dengue virus infection alters the protein composition of the saliva in the mosquito, we have analyzed the proteins expressed in *Aedes aegypti* salivary glands in infected mosquitoes and uninfected control mosquitoes via 2-D gel electrophoresis. Briefly, *Aedes aegypti* (Rockefeller) were orally exposed to bovine blood in Alsever's with and without dengue-2 (strain 1232). Mosquitoes were held for 9 days at 28° C before salivary glands were dissected; total proteins were extracted, and separated by 2-D electrophoresis. Mass spectroscopy analysis (LC-MS/MS) revealed that several proteins were differentially expressed between the two cohorts. In particular are several down-regulated proteins involved in ATP synthesis. We also have observed a decrease in a 30kD allergen protein and an increase in D7 in infected mosquito salivary glands, leading to an altered salivary composition that may create a more receptive environment for a successful viral infection This research indicates the need to not only review the components of mosquito saliva that are being inoculated with the virus, but also highlights the potential direct effects the virus may have on the composition of the saliva due to salivary gland structural and functional changes in the infected invertebrate.

1309

VIRAL AND IMMUNOLOGICAL DETERMINANTS OF DENGUE VIRUS FITNESS AND VIRULENCE

Molly OhAinle[1], Matthew Henn[2], Yolanda Tellez[3], Alexander Macalalad[2], Aubree Gordon[1], Andrea Nuñez[3], Saira Saborío[3], Niall Lennon[2], Bruce Birren[2], Angel Balmaseda[3], Eva Harris[1]

[1]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States, [2]Broad Institute, Cambridge, MA, United States, [3]Departamento de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua

Four serotypes of dengue virus (DENV1-4) circulate in humans, causing more illness than any other arthropod-borne virus. Despite decades of epidemiological research, we lack a sufficient understanding of the relative importance of host genetics, pre-existing immunity and viral evolution in dengue virus pathogenesis. We report here a significant increase in the incidence of severe dengue disease caused by DENV-2 in two independent studies of pediatric dengue in Managua, Nicaragua. Through full-length genome sequencing of viruses isolated from patients across several years (2005-2008), the increase in severity was found to be correlated with a clade replacement event occurring in DENV-2 circulating during the same time frame. Clade assignment by genotyping methods of additional viruses whose full-length sequence was not available increased the initial sample size substantially. Association analyses including clade and year suggests that a shift in viral genetics does not explain the increased severity observed in the later years of the studies. However, viral isolates derived from the replacing clade ("Clade 2") replicate more productively *in vitro* in human and mosquito cells, indicating that clade replacement involved the evolution of more fit DENV-2 viruses. Thus, our findings support a model in which increased viral fitness is not necessarily linked to increased pathogenesis. Consistent with this model, more in-depth analyses of clinical indicators of severity, such as low platelet count and hemoconcentration, suggest that less fit viruses ("Clade 1") are associated with more severe disease outcomes when stratified by year. Finally, we are exploring the alternative hypothesis that waning cross-reactive immunity in the population resulting from infection with a heterologous serotype (DENV-1, which circulated in Managua in 2003-5) is sufficient to explain the increased severity associated with DENV-2 infections in later years (2006-8) and are testing this by analyzing neutralization profiles of serum samples collected from our pediatric cohort study in 2004-2008. Our findings provide the first in-depth analysis of the contribution of relatively small genetic changes in viral sequence to viral fitness and pathogenicity and suggest that pre-existing immunity is the major determinant of dengue virus pathogenesis.

1310

GENETIC AND PHENOTYPIC CHARACTERIZATION OF SYLVATIC DENGUE VIRUS TYPE 4 STRAINS

Nikos Vasilakis, Farooq Nasar, Shannan L. Rossi, Sandra V. Mayer, Robert B. Tesh, Scott C. Weaver, Mark D. Estes

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

The four serotypes of endemic dengue viruses (DENV) circulate between humans and peridomestic *Aedes* mosquitoes. At present endemic DENV infect 100 million people per year, and a third of the global population is at risk. In contrast, sylvatic DENV strains are maintained in a transmission cycle between nonhuman primates and sylvatic *Aedes* species, and are evolutionarily and ecologically distinct from endemic DENV strains. Phylogenetic analyses place sylvatic strains basal to each of the endemic serotypes, supporting the hypothesis that each of the endemic DENV serotypes emerged independently from sylvatic ancestors. We utilized complete genome analyses of both sylvatic and endemic DENV serotype 4 (DENV-4) to expand our understanding of their genetic relationships. A high degree of conservation was observed in both the 5'- and 3'-untranslated genome regions, whereas considerable differences at the nucleotide and amino acid levels were observed within the open reading frame. Additionally, replication of the two genotypes was compared in mammalian (Vero and Huh-7) and mosquito (C6/36) cultured cells. Understanding the genetic relationships and phenotypic differences between endemic and sylvatic DENV genotypes may provide valuable insight into DENV emergence and guide monitoring of future outbreaks.

1311

FAST GROWTH DENGUE-LIKE CHIMERIC VIRUSES

Karen Boroughs, Betty Luy, Janae Stovall, **Claire Y. Huang**

Centers for Disease Control and Prevention, Fort Collins, CO, United States

Four chimeric West Nile/dengue viruses were engineered by expressing the premembrane-envelope (prM-E) gene region of dengue serotypes 1-4 (DEN 1-4) in the genetic backbone of West Nile virus. These viruses were stabilized by incorporating mutations which enhanced the fitness of the chimeras in Vero cells. They exhibited DEN serotype-specific antigenic properties and produced clear immune foci and plaques in Vero cell monolayers within 1 and 3 days, respectively. Compared to wild-type DEN viruses, these DEN-like chimeras replicated rapidly and reached peak titers at 3-5 days earlier in Vero cells and 2 days earlier in C6/36 cells. They achieved similar or higher titers than wild-type DEN viruses in the cell cultures. Despite their higher replication efficiency, these DEN-like chimeric viruses were attenuated in mice and had poor dissemination rates in mosquitoes. Due to their fast growth and attenuated properties, these viruses may be useful in various aspects of vaccine development, in diagnostic assays, and in DEN virus research.

1312

ACCELERATED PLATELET APOPTOSIS IS ASSOCIATED WITH PLATELET PHAGOCYTOSIS AND THROMBOCYTOPENIA IN SECONDARY DENGUE VIRUS INFECTION

Maria Terrese Alonzo[1], Talitha Lea Lacuesta[2], Lady-anne Suarez[3], Dan Takeuchi[1], Cynthia Mapua[3], Takeshi Kurosu[4], Yukihiro Akeda[1], Efren Dimaano[2], Filipinas Natividad[3], Kazunori Oishi[1]

[1]Laboratory for Clinical Research on Infectious Diseases, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan, [2]Department of Blood Borne Diseases, San Lazaro Hospital, Manila, Philippines, [3]Research and Biotechnology Division, St. Luke's Medical Center, Quezon City, Philippines, [4]Department of Virology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

An increased platelet phagocytosis was demonstrated during the acute phase of patients with secondary dengue virus (DV) infection, as reported previously. To determine the role of apoptosis in the phagocytosis of platelets, the relationship between platelet phagocytosis by differentiated THP-1 macrophages and platelet apoptosis was examined by flowcytometry using freshly isolated platelets from 32 patients clinically diagnosed with DV infection at San Lazaro Hospital, Manila, Philippines in year 2009.

The levels of platelet apoptosis from patients were significantly increased during the acute and early convalescent phase of infection compared with those of the convalescent phase and healthy controls. In addition, a significant inverse correlation was found between the peripheral platelet counts, the levels of platelet apoptosis (by Annexin V binding: $r = -0.491$, $p = 0.001$; by caspase-3 activation: $r = -0.507$, $p = 0.001$) and and the levels of platelet phagocytosis ($r = -0.455$, $p = 0.002$) among these patients. Furthermore, a significant direct correlation between the levels of platelet phagocytosis and platelet apoptosis (against Annexin V binding: $r = 0.395$, $p = 0.007$; against caspase-3 activation: $r = 0.453$, $p = 0.002$) was also found in these patients. Meanwhile, no effects were observed upon utilizing anti-Fc receptor IgG or anti-CR3 IgG as inhibitors in the phagocytosis of platelets by macrophages.

Collectively, our data suggest that accelerated phagocytosis of apoptotic platelets is involved in the mechanisms of thrombocytopenia in secondary DV infection. Further studies on the mechanisms of platelet apoptosis and platelet phagocytosis during the acute phase of secondary DV infection are warranted.

1313

PERMISSIVENESS OF BONE MARROW CELLS FOR DENGUE VIRUS INFECTION IS AGE-DEPENDENT

Guey Chuen Perng, Sansanee Noisakran, Hui-Mien Hsiao, Francois Villinger, Aftab Ansari

Emory University School of Medicine, Atlanta, GA, United States

Dengue is one of the most important mosquito-borne viral diseases affecting humans, with over half of the world's population living in areas at risk. Bone marrow suppression has been observed in dengue patients during the acute stage of infection associated with reduction of megakaryocytes. Studies of bone marrow biopsies from patients during acute infection indicate dengue virus infection induces bone marrow progenitor cells hypocellularity. Results from early attempts to investigate the possible underlying mechanisms leading to bone marrow suppression *in vitro* have been inconclusive. A systematic investigation on this subject was performed with bone marrow from 10 rhesus monkeys of various ages. Freshly collected bone marrow aspirates were infected with low dose of dengue virus, strain 16881 grown in Vero cells, at MOI=0.1. Cell smears were performed and supernatant fluids were collected daily for 10 consecutive days. Quantitative real-time RT-PCR was used to measure the viral titers in the supernatant fluids and immunohistochemistry staining with antibodies for cellular surface markers and dengue viral antigen was performed on smears. Results revealed that bone marrow from i) young monkeys (under 5 years old) were highly permissive to infection and able to support dengue virus replication with viral titers peaking at 2-3 days after infection; ii) older monkeys (over 5 years old) generated two patterns; viral titers declined from day 1 to day 10 and occasionally, viral titer peaked at 1 day after infection. Surface markers staining of sequential daily samples indicated that progenitor cells expressing CD235a and CD41 markers were positive for dengue viral antigen early (1-3 days) post infection, while cells with markers typical for dendritic cells or macrophages were positive for dengue viral antigen at later days (5-8) of infection. The significance of the findings will be discussed.

1314

COMMUNITY-BASED DELIVERY OF HEALTH CARE: WHAT IS THE CAPACITY FOR EXPANDING INTERVENTIONS?

Joaniter I. Nankabirwa[1], Kristin Banek[2], Deborah DiLiberto[3], Lilian Taaka[2], Clare Chandler[3], Sarah Staedke[3]

[1]Makarere University Kampala, Kampala, Uganda, [2]Uganda Malaria Surveillance Project, Infectious Disease Research Collaboration, Kampala, Uganda, [3]London School of Hygiene and Tropical Medicine, London, United Kingdom

Prompt treatment with effective antimalarial drugs is one of the key strategies for reducing the burden of malaria, and community-based programs are advocated to improve access to treatment. In 2002, Uganda adopted a policy of home management of malaria (HMM), which is now scaling-up to integrated community case management (iCCM). Community health workers (CHWs) will provide presumptive treatment for malaria, pneumonia, and diarrhea to febrile children. To investigate challenges faced by CHWs in the existing HMM programme, we conducted a situation analysis in rural Eastern Uganda. We interviewed 100 CHWs selected using convenience sampling from five sub-counties in Tororo district. A structured questionnaire addressing training and supervision and a knowledge questionnaire were administered, and in-depth interviews were conducted. We identified major gaps in CHW training, knowledge, and supervision. Overall, CHWs scored poorly on the knowledge questionnaire (mean score 22%). Only 74% CHWs correctly identified fever as the most common symptom of malaria in children, and recognition of danger signs of severe malaria was poor. Although 61% of CHWs had received training on management of malaria with artemether-lumefantrine (AL), few CHWs correctly described how AL should be administered. Only 23% said that they would refer a child who was not improving after two days. Recognition of non-malarial causes of fever in children was also poor. Only four CHWs reported receiving support supervision in the last six months. Interviews revealed that CMDs are involved in implementing multiple programmes led by different stakeholders, which are not integrated. Community-based programs provide opportunities to improve access to treatment, but their success depends on the capacity of CHWs to implement strategies. In Uganda, CHWs knowledge of appropriate management of malaria is limited, despite training, and they may be overstretched by stakeholders attempting to deliver community-based interventions. CHWs may lack capacity to deliver complex interventions successfully and sustainably. To ensure interventions are implemented appropriately, attention should be paid to training and support supervision of CHWs, and evaluation of the health impact of programmes.

1315

SUBOPTIMAL MANAGEMENT OF SEVERE MALARIA CASES IN UGANDAN HEALTH FACILITIES: A CROSS SECTIONAL SURVEY

Jane Achan[1], James Tibenderana[2], Daniel Kyabayinze[2], Henry Mawejje[2], Rukaaka Mujizi[2], Betty Mpeka[2], Ambrose Talisuna[3], Umberto D'Alessandro[4]

[1]Makerere University, School of Medicine, Kampala, Uganda, [2]Malaria Consortium, Kampala, Uganda, [3]Medicines for Malaria Venture, Makerere University, School of Public Health, Kampala, Uganda, [4]Institute of Tropical Medicine, Antwerp, Belgium

Malaria morbidity and mortality in Africa remains unacceptably high, partly due to sub-optimal case management. We evaluated the management practices of severe malaria in Ugandan health facilities by conducting a cross sectional survey using multi-stage sampling methods. Health facilities were selected in 11 districts in the eastern and mid-western parts of the country. The study instruments were adapted from the WHO hospital care assessment tools. Between June and August 2009, 105 health facilities were surveyed and 181 health workers and 868 patient/caregivers were interviewed. None of the inpatient facilities had all seven components of the basic care package for the management of severe malaria consistently available during the 3 months prior to the survey. Referral practices were appropriate in less than 10% (18/196) of the patients, while prompt care was reported by 29% (247/868) of the patients. Severe malaria was correctly diagnosed in 27 % (233/868) of the patients. Though the quinine dose and regimen was correct in the majority of patients (611/868, 70%), it was administered in the correct volumes of 5 % dextrose in only 18% (147/815) of the cases. Most patients (80%) had several doses of quinine administered in one single 500ml bottle of 5% dextrose. Medications were purchased by 385 (44%) patients and medical supplies by 478 patients (71%). These results highlight the challenges of correctly managing severe malaria in Uganda and other resource limited settings. Several priority areas need improvement, including triage and emergency care, referral practices, quality of diagnosis and treatment, health worker training, zero tolerance for stock outs for recommended medicines and ancillary treatments, functional referral systems, adequate infrastructure, better organization of hospital services, and regular supervision and clinical audits.

1316

AN ASSESSMENT OF ADHERENCE TO ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN PHALOMBE, MALAWI, 2009

Kimberly E. Mace[1], Dyson Mwandama[2], James Jafali[2], Madalitso Luka[2], S. Patrick Kachur[1], Scott J. Filler[1], Don Mathanga[2], Jacek Skarbinski[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Malaria Alert Center, Malawi College of Medicine, Blantyre, Malawi

Malaria causes substantial morbidity and mortality in Malawi. Prompt and effective treatment is a cornerstone of malaria control. In 2007, due to increasing parasite resistance to the first-line treatment for uncomplicated malaria, Malawi replaced single-dose sulphadoxine-pyrimethamine treatment with a six-dose, three-day treatment regimen of artemether-lumefantrine (AL). Given concerns about the complex AL regimen, we assessed patient adherence to AL for the treatment of uncomplicated malaria in Phalombe District, Malawi. Adults and children with uncomplicated malaria were recruited at three health centers. To assess adherence, we conducted pill counts and in-home interviews on medication consumption 72-hours after patients received AL. Complete adherence was defined as correctly taking all six doses of AL as assessed by pill count and patient recall of number of doses, number of pills per dose, and timing for each dose. We recruited 427 patients, completed in-home interviews on 414 (97%), and analyzed 368 (86%) patients with complete data. Among 368 patients, 238 (65%) were completely adherent. Classifications of non-adherence included skipping doses, taking incorrect number of pills per dose, or taking doses at incorrect times. Factors significantly associated with adherence were a preference for AL over other medications (odds ratio (OR) 2.7, p<0.001), use of AL package for instructions, (OR 2.5, p=0.02), and direct observation of the first dose of AL (OR 2.4, p<0.01). In contrast, being <5 years old was associated with non-adherence (OR 0.5, p=0.05). In conclusion, two-thirds of patients assessed were completely adherent to a six-dose AL regimen for the treatment of uncomplicated malaria. Efforts to improve adherence should focus on children <5 years old, the age-group most vulnerable to malaria. Interventions including direct observation of the first dose, utilization of the AL package for instructions, and enhancing patient preference for AL have the potential to increase adherence and therefore improve cure rates, and possibly mitigate antimalarial drug resistance.

1317

MAPPING THE PRIVATE SECTOR SUPPLY CHAIN TO UNDERSTAND THE RETAIL PRICES AND AVAILABILITY OF ANTIMALARIALS IN SIX LOW-INCOME COUNTRIES IN AFRICA AND SOUTH EAST ASIA

Catherine Goodman[1], Edith Patouillard[2], Sarah Tougher[2], Benjamin Palafox[2], Immo Kleinschmidt[2], Martine-Esther Tassiba[3], Louis-Daniel A. Tshisungu[4], Sochea Phok[5], Arogundade D. Ekundayo[6], Peter Buyungo[7], Felton Mpasela[8], Desmond Chavasse[9], Kate O'Connell[9], Kara Hanson[2]

[1]London School of Hygiene & Tropical Medicine/KEMRI-Wellcome Trust, Nairobi, Kenya, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom, [3]PSI, Cotonou, Benin, [4]Association de Santé Familiale, Kinshasa, The Democratic Republic of the Congo, [5]PSI, Phnom Penh, Cambodia, [6]Society for Family Health, Abuja, Nigeria, [7]PACE, Kampala, Uganda, [8]Society for Family Health, Lusaka, Zambia, [9]PSI, Nairobi, Kenya

In many low-income settings the private sector plays a crucial role in the delivery of antimalarials, often complementing the formal public health system. In the context of the introduction of a global subsidy to improve access to artemisinin-based combination therapies (ACTs) it is important to understand private sector supply chains and how they vary across countries, as these have an important impact on price and availability, and therefore equitable access. As part of the ACTwatch study, we undertook surveys of nationally representative samples of private sector antimalarial wholesalers and retailers, collecting volume, mark-up and provider characteristic data in 6 countries (Benin, Cambodia, DR Congo, Nigeria, Uganda, and Zambia). In total, structured interviews were completed with 688 wholesalers and 7048 retailers across the 6 countries between February 2009 and April 2010. We will present maps of the private sector antimalarial supply chains in each country, descriptions of their composition and characteristics, and estimates of availability and price mark-ups for antimalarials. For example, 81.8% of Zambian wholesalers had antimalarials available, while only 64.7% had an ACT in stock, 40.0% had an artemisinin monotherapy (AMT) and 60.0% had a non-artemisinin therapy (e.g. chloroquine) in stock at the time of interview. In terms of percentage price mark-up on ACTs, median wholesaler mark-ups (26.7%) were observed to be generally lower than median retail level mark-ups, which ranged from 42.9% in private pharmacies to 150% in grocery stores. Median percentage price mark-ups in Zambia for AMTs were similar to those for ACTs, 26.1% among wholesalers, and a range of 42.9% to 168% among private sector retailers. Results across countries will be contrasted and implications for interventions to improve ACT access through the private sector, such as the Affordable Medicines Facility for Malaria, will be explored.

1318

CHANGES IN MALARIA IN SOUTHERN SENEGAL WITH THE INTRODUCTION OF ARTESUNATE PLUS AMODIAQUINE AND PARASITOLOGICAL DIAGNOSIS

Philippe Brasseur[1], Oumar Gaye[2], Malick Badiane[3], Moustafa Cisse[4], Patrice Agnamey[5], Michel Vaillant[6], **Piero L. Olliaro**[7]

[1]IRD, Dakar, Senegal, [2]Faculté de Médecine, Université Cheikh Anta Diop, Dakar, Senegal, [3]District Medical D'Oussouye, Oussouye, Senegal, [4]PNLT Ministère de la Santé et de la Prévention, Dakar, Senegal, [5]Laboratoire de parasitologie - mycologie CHU, Amiens, France, [6]Unité d'Epidémiologie Clinique et de Santé Publique, CRP-Santé, Luxembourg, Luxembourg, [7]World Health Organization, Geneva, Switzerland

In Senegal, antimalarial treatment policy changed from chloroquine or quinine on clinical grounds to artesunate/amodiaquine (ASAQ) on parasitological confirmation in 2006. RDTs are provided from 2007. In the District of Oussouye, the new policy was staggered in since 2000 in Mlomp and later elsewhere. Malaria is meso-endemic with transmission peaking July-December (EIR 25 in 2000). Data for 1996-2008 were extracted from the clinic registries of the referral health centre and the four peripheral dispensaries of the District of Oussouye. Data over time were analysed by logistic regression. Pluviometry and bednet distribution were also accounted. Over the entire 13-year period a total of 363,966 consultations occurred (mean 1.2 person/year) with 139,144 antimalarial treatments (on either clinical or parasitological grounds; 38% of all attendances) and a projected 32,384 true cases of malaria (~77% of antimalarial treatments were redundant.) In Mlomp (early implementation) compared to 1996, the number of consultations, antimalarial treatments and estimated malaria incidence increased initially until 1998/2000 and then decreased steadily to reach in 2008 OR (95%CI) of 0.39 (0.38-0.41), 0.18 (0.17-0.20) and 0.09 (0.08-0.11) respectively vs. 1996. The age of malaria patients changed over time; while the proportion of patients 0-5 and 6-10 years old decreased steadily and 11-15 remained overall constant, all categories >15 increased. In 2008, all categories >10 were all at higher risk than the 0-5 years (in particular, the RR(95%CI) for >30 years was 14 (1.5-131.7) Details on the comparative data from the other sites will be presented. In conclusion, there is a temporal correlation between the implementation of ASAQ and parasitological confirmation and decreasing consultations to the health facilities, fevers diagnosed and treated as malaria and estimated malaria incidence in this meso-endemic area. The age profile changes are compatible with decreased transmission intensity.

1319

AN EVALUATION OF THE EFFECT OF TRIMETHOPRIM-SULFAMETHOXAZOLE PROPHYLAXIS ON GAMETOCYTEMIA AND ASYMPTOMATIC PARASITEMIA IN HIV-EXPOSED CHILDREN IN RURAL UGANDA

Abel Kakuru[1], Humphrey Wanzira[1], Emmanuel Arinaitwe[1], Victor Bigira[1], Jaco Homsy[2], Moses Kamya[3], Jordan W. Tappero[4], Grant Dorsey[5], Taylor Sandison[6]

[1]Infectious Diseases Research Collaboration, Kampala, Uganda, [2]Institute for Global Health, University of California, San Francisco, San Francisco, CA, United States, [3]Department of Medicine, Makerere University, Kampala, Uganda, [4]GAP, Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]Department of Medicine, University of California, San Francisco, San Francisco, CA, United States, [6]Department of Medicine, University of Washington, Seattle, WA, United States

Treatment with sulfadoxine-pyrimethamine (SP), an antifolate antimalarial, has been associated with increased gametocytemia, especially in the presence of antifolate resistance. However, data are limited regarding the effect of trimethoprim-sulfamethoxazole (TS) prophylaxis on gametocytemia. We previously reported that TS prophylaxis has a 40% protective efficacy against malaria among HIV-exposed (HIV-uninfected infants born to HIV-infected mothers) children, but data are lacking concerning the effect of TS on asymptomatic parasitemia (AP). Here, we examine the effects of TS prophylaxis on gametocytemia and AP. In an area of high malaria endemicity and antifolate resistance in rural Uganda, we randomized 185 HIV-exposed infants (median age= 9.6 months), following breastfeeding and a negative HIV PCR test, to discontinue or continue TS prophylaxis through age 2 years. Routine smears were obtained every 30 days, and time-at-risk was divided into calendar months. Gametocytemia and AP were diagnosed by microscopy (and absence of fever for AP). All smears performed within 7 days of a malaria episode and during malaria follow-up were censored in assessing for prevalence of AP. Among 98 infants randomized to continue TS, there were 28 episodes of gametocytemia over 1,068 months (2.6%), and among 87 infants randomized to stop TS, there were 8 episodes of gametocytemia over 845 months (1.0%) (RR=2.83, p=0.07). Among children taking TS, there were 77 AP episodes over 812 months (9.5%), and among children who discontinued TS, there were 78 AP episodes over 606 months (12.9%) (RR=0.74, p=0.18). Compared to AP in participants not taking TS, AP in children taking TS prophylaxis was less likely to progress to malaria within 7 days (RR=0.41, p=0.001). Though the overall prevalence of gametocytes was low, TS prophylaxis was associated with a trend toward increasing gametocytemia. Compared to children not taking TS, AP episodes among children taking TS prophylaxis were less likely to progress to clinical malaria, indicating that TS may prevent malaria at the erythrocytic stage.

1320

INTERMITTENT PREVENTIVE THERAPY IN PREGNANCY WITH SULPHADOXINE-PYRIMETHAMINE (SP); 42 DAY *IN-VIVO* FOLLOW-UP STUDY AMONG ASYMPTOMATIC PARASITEMIC PREGNANT WOMEN IN AN AREA WITH HIGH SP RESISTANCE IN SOUTHERN MALAWI

Linda Kalilani[1], Ebbie Chaluluka[1], Gertrude Kalanda[1], Kassoum Kayentao[2], Steve Taylor[3], Steve Meshnick[3], Feiko ter Kuile[4]

[1]College of Medicine, University of Malawi, Blantyre, Malawi, [2]Malaria Research and Training Centre, Department of Epidemiology of Parasitological Diseases, Faculty of Medicine, Pharmacy and Dentistry, University of Bamako, Bamako, Mali, [3]University of North Carolina, Gillings School of Global Public Health, Chapel Hill, NC, United States, [4]Department of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands; Liverpool School of Tropical Medicine, Liverpool, Liverpool, United Kingdom

Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) is recommended by the World Health Organization for the control of malaria during pregnancy in sub-Saharan Africa. Malawi was the first country to introduce IPTp with SP in 1993. Parasite resistance has compromised the efficacy of SP in the case-management of symptomatic children, but SP has remained effective for IPTp in many areas of Africa. We conducted a 42-day in-vivo assessment of the parasitological response to IPTp-SP in women scheduled to receive their first dose of IPTp, to study the effect of SP resistance on the efficacy of IPTp-SP in clearing parasites and preventing new infections. HIV-negative asymptomatic parasitemic women of all gravidity were eligible if they provided written informed consent. Recruitment is ongoing in two antenatal clinics within one hour drive south-west of Blantyre. Between December 2009 and April 2010, 79 women were successfully followed weekly until day 42, or until the day of re-occurrence of parasitemia: 46 primi- and secundi-gravidae (G1+2) and 33 multigravidae (G3+). By day 42, 34 (43.0%) experienced a re-appearance of parasitemia; This was 3, 9, and 29 by day 7 (3.3%), day 14 (11.4%), and day 28 (36.7%), respectively. Primi-, and secundi-gravidae (29/46: 63.0%) were more likely to be parasitaemic by day 42 than multigravidae (5/33: (15.2%); RR 4.16, 95% CI 1.80-9.61). Molecular analyses for SP resistance-associated mutations in dhps 436, 437, 540 and 581, dhfr 51, 59 and 164 and pfmrp1 1466, and genotyping to differentiate between recrudescent and new infections is ongoing. A study of the impact of IPTp-SP on placental malaria and birth outcome is also ongoing.

These preliminary results of the in-vivo follow-up suggest high rates of recrudescence and reinfection in primi-, and secundi-gravidae receiving IPTp with SP. This raises concern about the longevity of IPTp-SP in southern Malawi and stresses the need to explore alternative drugs to replace SP or alternative strategies to replace IPTp.

1321

ALANYL-GLUTAMINE PREVENTS SMALL INTESTINAL EPITHELIAL APOPTOSIS *IN VITRO* AND IN A MURINE MODEL OF WEANLING MALNUTRITION

Priscilla M. Ueno[1], Tara Willson[1], David Wu[1], Simon Hogan[1], Orleancio G. de Azevedo[2], Marjorie M. Guedes[2], Aldo A. Lima[2], Reinaldo B. Oriá[2], Richard L. Guerrant[3], D. Brent Polk[4], Lee A. Denson[1], **Sean R. Moore**[1]

[1]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, [2]Federal University of Ceará, Fortaleza, Brazil, [3]University of Virginia, Charlottesville, VA, United States, [4]University of Southern California and Childrens Hospital Los Angeles, Los Angeles, CA, United States

Malnutrition contributes to over half of all child deaths in developing countries and is associated with an enteropathy characterized by villous atrophy and increased gut permeability. Ala-Gln, a stable glutamine

dipeptide, has recently been shown to enhance catch-up growth and gut integrity in underweight children from Northeast Brazil. We sought to test the hypothesis that Ala-Gln mediates these effects via anti-apoptotic mechanisms *in vitro* and *in vivo.* Colorimetric viability assays were performed in mouse small intestine epithelial (MSIE) cells in the presence of varying concentrations of Ala-Gln. Apoptosis was assessed by annexin and 7-AAD staining and flow cytometry. To determine Ala-Gln's *in vivo* effects, we randomized dams of 10-day old C57/B6 mice to standard chow or an isocaloric, Northeast Brazil "regional" diet (low protein, low fat). On day of life 21, pups were weaned to their dam's diet and randomized to Ala-Gln solution or plain drinking water. At 6 weeks of age, mice were sacrificed to obtain jejunal specimens for morphological, immunohistochemical, and Ussing chamber analyses. Ala-Gln promoted MSIE viability in a dose-response manner and reduced early apoptosis. Pups of dams that received the regional diet exhibited failure to thrive and villous blunting, as well as decreased epithelial proliferation and increased epithelial apoptosis (as measured by BrdU and caspase-3 staining, respectively). Despite no differences in catch-up growth, undernourished pups randomized to Ala-Gln showed significant improvements in villous height and epithelial proliferation/apoptosis. In conclusion, the regional diet induces failure to thrive and environmental enteropathy-like changes in weanling mice. Ala-Gln promotes enterocyte survival and normal villous architecture in the setting of malnutrition, independent of changes in weight. Further studies are needed to define the signaling pathways by which Ala-Gln mediates these cellular responses, which are critical to recovery from the reciprocal cycle of diarrhea and malnutrition.

1322

POLYMORPHISMS IN INFLAMMATORY MEDIATOR GENES CONFER PROTECTION FROM SYSTEMIC BACTERIAL INFECTIONS IN *PLASMODIUM FALCIPARUM*-INFECTED CHILDREN

Gregory C. Davenport[1], Tom Were[2], Collins O. Ouma[2], Prakasha Kempaiah[1], Samuel B. Anyona[2], Evans Raballah[2], John M. Ong'echa[2], Jeremy Martinson[3], Douglas Perkins[1]

[1]University of New Mexico, Albuquerque, NM, United States, [2]University of New Mexico/KEMRI, Kisumu, Kenya, [3]University of Pittsburgh, Pittsburgh, PA, United States

Malaria and bacteremia both cause significant morbidity and mortality in Kenyan children living in holoendemic *P. falciparum* regions. We have recently reported decreased parasitemia levels in these co-infected children without exacerbation of anemia, despite elevated levels in a number of pro- and anti-inflammatory mediators in co-infected children. To further explore these findings from a genetic perspective, we examined the following single nucleotide polymorphisms (SNPs) in IL-12 (T-1188C), TNF-alpha (G-238A, C-308T, A-376T, T-1031C), IFN-gamma (receptor G-56A, G+2200A), IL-4 (C-589T, C-1335T), and IL-10 (C-592A, C-819T, T-1082C) in relation to infection status (malaria mono-infected, *Pf*[+], n=294; Gram[-] bacteremia and malaria co-infected, G[-]/*Pf*[+], n=17; and Gram[+] bacteremia and malaria co-infected, G[+]/*Pf*[+], n=9). Pearson's chi-square analysis identified associations between infection status and IFN-gammaR G-56A (*P*=0.071), IFN-gamma G+2200A (*P*=0.033), TNF-alpha C-308T (*P*=0.009), and IL-4 C-589T (*P*=0.024). Multinomial logistic regression analyses of these four SNPs, with infection status as the dependent variable (0, *Pf*[+]; 1, Bacteremia/*Pf*[+] co-infected), and sickle cell status, HIV status, G6PD status, gender, and age as covariates, revealed significant protection from bacteremia in malaria parasitemic children for IFN-gammaR G-56A [AA, odds ratio (OR)=0.394, *P*=0.029], TNF-alpha C-308T [TT, OR=0.422, *P*=0.037], and IL-4 C-589T [CT, OR=0.455, *P*=0.034]. Another set of regression analyses using high-density parasitemia (HDP, >10,000 parasites/μL) as the dependent outcome variable, demonstrated that heterozygosity at the IFN-gammaR G-56A locus was associated with increased susceptibility to HDP (GA, OR=1.607, *P*=0.050). Thus, homozygous A alleles at IFN-gammaR -56 are associated with protection from bacteremia in children with malaria, while heterozygosity at the same locus predisposes children to HDP. Taken together, these findings suggest that many of the SNPs important in malaria disease severity also play a significant role in acquisition of systemic bacterial infections in Kenyan children.

1323

RISK FACTORS OF *STREPTOCOCCUS SUIS* INFECTION IN VIETNAM: A CASE-CONTROL STUDY

Nghia Ho Dang Trung[1], Tu Le Thi Phuong[2], Thai Cao Quang[2], Hoang Nguyen Van Minh[2], James Campbell[2], Hien Tran Tinh[3], Jeremy Farrar[2], Constance Schultsz[2]

[1]Hospital for Tropical Diseases, Ho Chi Minh, Vietnam, [2]Oxford University Clinical Research Unit, Ho Chi Minh, Vietnam, [3]Hospital for Tropical Diseases, Ho Chi Minh, Vietnam

Streptococcus suis, an emerging zoonotic infection, is the most common bacterial cause of adult bacterial meningitis in Vietnam. The explosive outbreak of *S.suis* infection in China in 2005 with hundreds of human cases and 39 deaths and emergence across South East Asia makes this an increasingly public health problem. We conducted a case-control study to identify the risk factors of *S. suis* infection in Vietnam. A standard case-control study with appropriate hospital and matched community controls for each patient. The study was conducted between May 2006 and June 2009 at Hospital for Tropical diseases in Ho Chi Minh City, Vietnam. Patients were confirmed by blood culture or cerebrospinal fluid (CSF) culture or real-time PCR. Risk factors were assessed by a standard questionnaire, including socio-demographic and cultural characteristics, medical history, and assessment of potential risk factors. We investigated whether the bacterial is carried by patients and healthy individuals using real-time PCR and culture of throat and rectal swab samples. We recruited 101 cases *S. suis* meningitis, 303 hospital controls and 300 community controls. By multivariate analysis, we found that the risk factors of *S. suis* infection included occupations related to pigs (OR1=3.83; 95%CI=[1.33-11.04] and OR2=5.56; 95%CI=[1.51-20.57]), exposures to pigs or pork in the previous 2 weeks with skin injuries (OR1=7.29; 95%CI=[1.92-27.64] and OR2=15.76; 95%CI=[2.94-84.45]) and eating "high risk" dishes in the last 2 weeks (OR1=2.32; 95%CI=[1.21-4.46] and OR2=4.67; 95%CI=[2.26-9.62]). *S. suis* DNA was detected in rectal and throat swabs of 7 patients and was cultured from 2 of these samples, but was not detected in such samples of 1522 healthy individuals or patients without *S. suis* infection. *S. suis* is an important and emerging public health issue in Asia and one with the potential for both endemic transmission and for explosive epidemics. This case control study, the largest prospective epidemiological assessment of this disease, has identified the most important risk factors associated with *S. suis* bacterial meningitis to be occupational exposure to pigs and pig products, preparation of pork and eating 'high risk' dishes popular in parts of Asia.

1324

HANSEN'S DISEASE (LEPROSY) AMONG UNITED STATES-RESIDING MICRONESIANS AND MARSHALLESE

Patricia A. Woodall

Tulane University, New Orleans, LA, United States

From 2004-2008, 13% of Hansen's Disease cases reported in the United States occurred among migrants from the Federated States of Micronesia and the Republic of the Marshall Islands, countries with high HD prevalence of 10-20/10,000. Citizens of these countries (former U.S. Trust Territory of the Pacific Islands) may freely enter and work in the US, not subject to immigration restrictions. Due to economic and climatologic factors, migration is increasing; ~2400 Marshallese/Micronesians move to the U.S. annually. This study included consolidation of data from various published reports of HD indicators in the countries of origin, analysis of 1990-2009 National Hansen's Disease Program surveillance and clinical data, and collection/analysis of qualitative data relevant to disease control issues among US-resident clusters. HD prevalence and case detection rates in the two source countries remain the highest in the world, with

fluctuations due to program activity, but with little progress toward the WHO leprosy elimination target of <1/10,000. Local community rates as high as 5% have been reported. 55% of US-resident Micronesian/ Marshallese cases occurred in Hawaii. Among 74 US mainland cases in 26 states, 50% were diagnosed within 3 years of US entry, 63% did not complete treatment, and 80% had at least 1 complication, including advanced neuropathic disease. Since 1996, 17 cases have been reported in a single community in Arkansas, primarily young adult males with lepromatous disease. Comparison with rates in the Marshall Islands and in Hawaii suggests that fewer than half of cases in this community have been identified, with women, children, and tuberculoid disease under-represented. Cultural, socio-economic, and health-system barriers to HD care in this community are identified. With the goal of decreasing health disparity and preventing disability, case-finding and case-management interventions are needed in US-resident Marshallese/Micronesian communities as well as in the countries of origin.

1325

POLYMORPHISMS ILE655VAL IN ERBB2/HER-2/NEU RECEPTOR IS ASSOCIATED WITH HANSEN'S DISEASE (LEPROSY) IN A BRAZILIAN POPULATION

Sérgio R. Araujo[1], Kate Dupnik[2], Maurício L. Nobre[3], Adriana F. Barbosa[1], Glória M. Monteiro[1], Márcia S. Dias[1], Pedro B. Trindade[1], Carlos M. Gomes[1], Sarra Jamieson[4], Jenefer Blackwell[4], Selma M. Jerônimo[5]

[1]Universidade Federal do Rio Grande do Norte, Natal, Brazil, [2]Weill Cornell Medical College, New York, NY, United States, [3]Secretaria de Saude, Governo do Rio Grande do Norte, Natal, Brazil, [4]University of Western Australia, Perth, Australia, [5]Universidade Federal do Rio Grande do Norte and INCT Doenças Tropicais, Natal, Brazil

Leprosy is an infectious disease caused by *Mycobacterium leprae* which can lead to severe permanent disability. *M. leprae* induces nervous degeneration by linkage to Schwann cell in periphery nervous system in part resulting from the interaction of the ErbB2 receptor and *M. leprae*. The objective of this study was to evaluate whether the polymorphisms in *ERBB2* gene is associated with leprosy. A total of 216 leprosy patients and 226 controls were genotyped for six markers located in *ERBB2* gene. The markers were rs2517955 and rs2517956 in the promoter region; rs1810132 and rs2952156 in intron regions, and rs1801200 and rs1058808 in exons of the *ERBB2* gene. Statistical analysis was performed by using Web-based tool Snpstats (http://bioinfo.iconcologia.net/index.php?module=Snpstats). Two SNPs were associated with Hansen's disease, respectively, SNP rs1801200 (p = 0.036, OR = 1.61, CI = 0.90 - 2.88) and the SNP rs2517956 (p = 0.047, OR = 1.53, CI = 0.84 - 2.76). The base change from A to G in the allele marker rs1801200 results in amino acid change in position 655 from isoleucine to valine. This amino acid is located in the transmembrane domain of the ErbB2 receptor, which is involved in the dimerization of ErbB2 monomers and its activation. The presence of valine induces a tighter linkage of ErbB2 monomers than isoleucine does, and results in greater stability and increased catalytic activity of ErbB2. In conclusion, presence of the polymorphic alleles in the markers rs1801200 and rs2517956 in *ERBB2* gene was found to be associated with Hansen's disease. Presence of Val in position 655 (rs1801200) results in greater stability of the two ErbB2 monomers and might increases its catalytic activity.

1326

A QUICK AND COST-EFFECTIVE METHOD FOR THE DIAGNOSIS OF *MYCOBACTERIUM ULCERANS* DISEASE

Dziedzom K. de Souza, Charles Quaye, Lydia Mosi, Daniel A. Boakye

Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana

Mycobacterium ulcerans causes the painless necrotizing skin disease Buruli ulcer, highly endemic in rural West and Central Africa. The mode of transmission remains unknown largely due to the lack of early case diagnosis and late treatment seeking behavior of infected individuals. Diagnosis is based on clinical presentation and microscopy followed by PCR confirmation in reference laboratories. Complete diagnosis is therefore usually late, inefficient, time consuming and very expensive. Early diagnosis is important for effective treatment to prevent the morbid effects of the disease on affected individuals. We report the development of a simple and inexpensive test that could be used in poorly to medium resourced settings at point of care facilities based on the loop mediated isothermal amplification (LAMP) method. Four sets of primers, targeting various sections of the *M. ulcerans* genome, were designed for the reaction and the assay was developed and tested on five *M. ulcerans* strains from patients in Ghana and two American Type Culture Control (ATCC) reference isolates; Ghana #970321 (D19F9) and Benin #990826 (D27D14). To determine specificity, the assay was tested on the closely related *M. marinum* 1218 and other mycolactone producing mycobacteria; *M. marinum* DL240490*, M. liflandii* and *M. pseudoshotsii*. The assay was finally tested on DNA obtained from biopsy samples from infected laboratory animals, prepared using either boil preparation or Qiagen kit extraction methods. The test was successful for DNA obtained by both methods although the latter provided the best results. Our results revealed a high specificity of the LAMP assay for selectively detecting *M. ulcerans.* Compared to the conventional PCR, the new assay is cheaper and simpler and does not require the use of a thermal cycler or electrophoresis. Results are obtained within one and a half hours and visually observed under UV light. These observations indicate that the BU-LAMP assay is suitable for early disease diagnosis and application in low resource health facilities.

1327

LANDSCAPE AND ENVIRONMENTAL INFLUENCES ON PRESENCE/ABSENCE OF *MYCOBACTERIUM ULCERANS* IN GHANA

Shannon McClintock[1], Julie Clennon[1], Ellen Whitney[1], Edwin Ampadu[2], William Opare[2], Eric Benbow[3], Richard Merritt[4], Pamela Small[5], Heather Williamson[5], Daniel Boakye[6], Jiaguo Qi[4], Lindsay Campbell[4], Jenni van Ravensway[4], Lance Waller[1]

[1]Emory University, Atlanta, GA, United States, [2]National Buruli Ulcer Control Program, Accra, Ghana, [3]University of Dayton, Dayton, OH, United States, [4]Michigan State University, East Lansing, MI, United States, [5]University of Tennessee, Knoxville, TN, United States, [6]Noguchi Memorial Institute for Medical Research, Accra, Ghana

Buruli ulcer is a neglected tropical skin disease caused by *Mycobacterium ulcerans* (MU) that is highly endemic in West Africa. While the mode of transmission is unknown, many epidemiological studies have found Buruli ulcer to be associated with different types of exposure to water sources. Sixty-eight aquatic sites used for daily domestic purposes by communities in the greater Accra and Ashanti regions of Ghana were sampled in 2005-2007 to test for the presence of *M. ulcerans*. We explored the spatial distribution of the MU positive and negative water bodies as well as identified site, water, land cover, and landscape characteristics associated with MU presence using logistic regression with model selection by AIC. Kulldorff's Bernoulli spatial scan statistic using circular windows found no significant local clusters of MU positive sites, and Ripley's K function showed no significant global clustering of MU positive sites compared to negative sites. The best fitting logistic regression model identified region, elevation, region by elevation interaction, presence of urban land cover within 100m, presence of forest land cover within 1km, water hardness, and region by water hardness interaction to be associated with the presence of MU. An empirical semivariogram of the residuals from the final model revealed no significant residual spatial autocorrelation. These results support the notion that MU is an environmental organism that exists in specific niches, but whose distribution in nature may not necessarily reflect the distribution of the disease. Understanding the spatial distribution of MU as well as factors associated with its presence can further research on modes of transmission as well as identify areas in need of surveillance for Buruli ulcer.

1328

THE SENSITIVITY OF STANDARD CIRCULATING FILARIAL ANTIGEN TESTS AND ULTRASONOGRAPHY FOR INDIVIDUAL DIAGNOSTICS AND EPIDEMIOLOGICAL SURVEILLANCE OF BANCROFTIAN FILARIASIS

Achim Hoerauf[1], **Ute Klarmann**[1], Alexander Y. Debrah[2], Linda Batsa[3], Ohene Adjei[4], Sabine Mand[1]

[1]Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany, [2]Faculty of Allied Health Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, [3]Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kumasi, Ghana, [4]Komfo Anokye Teaching Hospital (KATH), Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

Significant advances were made in the diagnosis of lymphatic filariasis (LF) in the past years using two tests for the detection of circulating filarial antigen (CFA) in individuals with LF: the Og4C3-ELISA (TropBio®) and the immunochromatographic test (ICT; NOW® Filariasis). These tests have been mainly used in microfilariae (Mf) carriers and both resulted in high sensitivity. To verify parasitic infection also in amicrofilaremic individuals, ultrasonography (USG) of the scrotal area is frequently used. In this study Mf-load and CFA-status (Og4C3) were assessed in healthy adult volunteers (n=1976), 535 samples were additionally analysed with ICT. All men (n=1132) underwent ultrasound examination of the scrotum. Altogether 324 were Mf+ and 1652 were Mf-. Both tests, Og4C3 and ICT, showed a high sensitivity for detection of CFA in the Mf+ samples (99% and 100% respectively) but there was a significant difference between both tests regarding the Mf- samples (consistency only in 410/483 (85%) cases). USG revealed that 201 men were FDS+/Mf+, 151 FDS+/Mf-, 74 FDS-/Mf+ and 706 FDS-/Mf-. The sensitivity of Og4C3 and ICT was high in microfilaremic patients (99% or 100%). The sensitivity of the Og4C3 for FDS+/Mf- men was 91%, that of the ICT 82%. There was a significant difference between both tests in the assessment of the FDS-/Mf- patients (consistency in 113/140 (81%)). In 74/275 (27%) Mf+ men, life adult worms could not be detected by USG. In conclusion, confirmative to a former trial, in 73% of the Mf+ individuals life adult worms were detected by USG. The lower detection of the USG is presumably caused by adult worms located in sites of the body other than the scrotum. Og4C3 and ICT both show a high and comparable sensitivity in the detection of Mf+ individuals while in FDS+/Mf- cases the sensitivity of antigen detection is lower. Particularly in absence of Mf and FDS, Og4C3 and ICT show a lack of consistency. Therefore antigen results from Mf- individuals should be interpreted taking this caveat into account.

1329

IDENTIFICATION OF A *WUCHERERIA BANCROFTI* LARVAL STAGE SPECIFIC STAGE PROTEIN THAT IS BOTH SENSITIVE AND SPECIFIC IN DETECTING ANTIBODIES IN *W. BANCROFTI* INFECTED PATIENTS

Joseph Kubofcik, Doran L. Fink, Thomas B. Nutman

National Institutes of Health, Bethesda, MD, United States

The current antibody tests used for mapping the distribution of lymphatic filariasis (LF) and for monitoring progress in elimination programs suffer from poor specificity because of considerable cross-reactivity with antibodies induced by other filarial infections such as *Loa loa*, *Onchocerca volvulus*, and *Mansonella* spp. Using the dCAS bioinformatics package, we assembled 2048 expressed sequence tags (EST) from the L3 infective larvae of *W. bancrofti* into non-redundant contigs which were then assessed for homology to protein and nucleotide databases as well as head-to-head against contig sets assembled from L3 larval ESTs of *B. malayi* (Bm - 5068 ESTs), *O. volvulus* (Ov - 4166 ESTs), and *Loa loa (*Ll- 3315 ESTs). Nineteen potential L3- and Wb-specific antigens were identified and expressed as fusion proteins with Renilla luciferase in mammalian cells. Screening of cell lysates by a Luciferase Immunopreciptation System (LIPS) assay revealed that only 1 of the 19 antigens (Wb-123) was both highly immunogenic and Wb-specific. Using a broad panel of well-defined sera from normal North Americans (n=53) and patients infected exclusively with Wb (n=43), Ll (n= 70), Ov (n=43), or intestinal helminths (n= 21), the Wb-123 based LIPS assay could identify sera from all of the Wb-infected individuals (MF+ or CAg+ from diverse geographic regions) with 100% sensitivity and 100% specificity compared to sera from uninfected controls and those with intestinal helminths. When specificities and sensitivities were assessed using sera from Ll-infected or Ov-infected individuals as the comparator, the sensitivities ranged between 98-100% and the specificities between 97-98%. Thus, we have identified an L3- and Wb-specific antigen that can be used not only as a rapid and specific tool to diagnose individual *Wb* infections but also as a sensitive, high-throughput, and potentially point-of care method for early detection of recrudescent infections in areas of control and for mapping new areas of Wb transmission.

1330

CARDIAC LESIONS IN AN AREA HYPERENDEMIC FOR LOIASIS IN CAMEROON

Jean Tenaguem[1], Edwine Wawo-Yonta[2], Raceline Ngounou Fotsing[3], Joseph Kamtchum Tatuene[2], Hugues Nana Djeunga[4], Jean Bopda[5], Adrian Hopkins[6], Thomas B. Nutman[7], Christopher Kuaban[2], Michel Boussinesq[8], Amy Klion[9], **Joseph Kamgno**[1]

[1]Filariasis Research Center and Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon, [2]Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon, [3]Faculty of Sciences University of Yaounde I, Yaounde, Cameroon, [4]Filariasis Research Center and Faculty of Sciences, University of Yaounde I, Yaounde, Cameroon, [5]Filariasis Research Center, Yaounde, Cameroon, [6]Mectizan Donation Program, Decatur, GA, United States, [7]Helminth Immunology Section, National Institutes of Health, Bethesda, MD, United States, [8]Unité Mixte de Recherche 145, Institut de Recherche pour le Développement and Université Montpellier 1, Montpellier, France, [9]Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Although the majority of patients with loiasis are asymptomatic despite high levels of blood microfilariae, characteristic symptoms include migratory angioedema and subconjunctival migration of the adult worm. Serious complications, including endomyocardial fibrosis (EMF), have been described; however, the prevalence of such complications in endemic areas is unknown and likely underestimated. To assess the cardiac complications related to loiasis, we performed a cross-sectional, study of 297 adult (>15 years of age) residents of a hyperendemic focus of loiasis in Cameroon. Subjects with evidence of onchocerciasis or lymphatic filariasis, a history of cardiovascular disease prior to their settlement in the study area or any antifilarial treatment taken during the last two years were excluded from the study. All subjects underwent a detailed clinical examination, assessment of microfilaremia by calibrated thick smear of daytime blood, *Loa loa* serology (SXP LIPS), and echocardiography performed by an experienced cardiologist. Of the 297 subjects, 180 had detectable *Loa* microfilaremia, 39 had both *Loa* and *Mansonella perstans* microfilaremia and 63 had no serologic or parasitologic evidence of *Loa* infection. Echocardiography was abnormal in a high percentage (84.5%) of patients and included valvular or endocardial calcifications (70%), diastolic dysfunction (35.7%), cavity dilatation (34.3%), valvular insufficiency (18.5%), left ventricular hypertrophy (9.8%), pericardial lesions (2.4%) and EMF (1.01%). Although the frequency and distribution of these abnormalities was not statistically different between subjects with and without loiasis, the number of uninfected subjects was small. Of note, all 3 subjects with EMF had detectable *Loa* infection, negative stool examination for intestinal helminths and marked eosinophilia. Although these data are consistent with an increased prevalence of cardiac

abnormalities, including EMF, in an area hyperendemic for loiasis, the role of loiasis in the pathogenesis of these abnormalities remains to be elucidated.

1331

TARGETING *WOLBACHIA* ENDOSYMBIONTS IN *ONCHOCERCA VOLVULUS* EFFECTIVELY CLEARS PERSISTENT MICROFILARIAE IN THE SKIN OF ONCHOCERCIASIS PATIENTS IN WHOM REPEATED IVERMECTIN TREATMENT HAD FAILED TO CLEAR

Alexander Y. Debrah[1], Sabine Specht[2], Sabine Mand[2], Linda Batsa[3], Ute Klarmann[2], Yeboah Marfo-Debrekyei[3], Alexander Kwarteng[3], Mike Osei-Atweneboana[4], Daniel Boakye[4], Ohene Adjei[5], Achim Hoerauf[2]

[1]Faculty of Allied Health Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, [2]Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany, [3]Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kumasi, Ghana, [4]Noguchi Memorial Institute, Accra, Ghana, [5]School of Medical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

Ivermectin (IVM) has been the drug of choice for the treatment of onchocerciasis since 1987. However, there have been reports of persistent microfilariae (Mf) in the skin of some people after many rounds of IVM treatment in some districts in Ghana. These indications are consistent with the emergence of drug resistance or sub-optimal response to IVM. To assess the effect of targeting Wolbachia endosymbionts in O. volvulus on onchocerciasis patients in whom repeated IVM treatment had failed to mediate Mf clearance, 149 patients were recruited in 2 districts in Ghana where IVM resistance has been reported. They were treated with either 100mg/d doxycycline (Doxy) or matching placebo for 6 weeks. Three and 12 months after Doxy treatment, all patients took part in ongoing IVM mass treatment. Patients were snipped before, 12 and 20 months after treatment to assess the levels of Mf that IVM could not clear. Entomological work was also carried out in all the studied villages before and after Doxy treatment.

Before treatment, of the 73 patients allocated for doxycycline, 66% had persistent Mf in the skin and 34% had only nodules but no skin Mf, and of the 76 patients allocated for placebo, 63% had persistent Mf in the skin and 37% had only nodules (P=0.74). However, at 12 months after Doxy treatment, of the 72 Doxy-treated patients snipped, 10% still had low numbers of Mf in the skin and 90% had no Mf at all. Of the 71 placebo patients snipped, 58% still had Mf in the skin while 42% had no Mf (P<0.001). At 20 months post therapy, only 3% of the 69 Doxy patients had low Mf and 97% were Mf negative. In contrast, of the 71 placebo patients, 69% still had Mf while only 31% had no Mf. This difference between the Doxy and placebo groups was significant (P<0.001). Doxy cleared Wolbachia significantly compared to placebo group and shows embryostatic effect in the adult worms compared to placebo patients. A comparison between pre-treatment and post treatment transmission parameters indicated a significant reduction after intervention in most areas. Doxycycline clears Wolbachia from O. volvulus worms, and resulted in embryogenesis blockade. Therefore, 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-Wolbachia l treatment to control the re-emergence of onchocerciasis in areas where infections persist despite the use of IVM.

1332

HIGH DOSE BIANNUAL ALBENDAZOLE AND IVERMECTIN SUPPRESS *WUCHERERIA BANCROFTI* MICROFILARIAL LEVELS MORE EFFECTIVELY THAN STANDARD DOSE ANNUAL TREATMENT

Benoit Dembele[1], Yaya I. Coulibaly[1], Siaka Konate[1], Housseini Dolo[1], Siaka Y. Coulibaly[1], Dramane Sanogo[1], Lamine Soumaoro[1], Michel E. Coulibaly[1], Salif S. Doumbia[1], Abdallah A. Diallo[1], Sekou F. Traore[1], Adama D. Keita[2], Thomas B. Nutman[3], Amy D. Klion[3]

[1]University of Bamako, Bamako, Mali, [2]Hospital of Point G, Bamako, Mali, [3]Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD, United States

Annual mass treatment with albendazole and ivermectin is the mainstay of current strategies to interrupt transmission of *Wuchereria bancrofti* (Wb) in Africa. More effective microfilarial suppression could reduce the time necessary to interrupt transmission and ease the economic burden of such programs in countries with limited resources. To determine the effect of increased dose and frequency of albendazole/ivermectin (A/I) treatment on microfilarial (mf) clearance, 40 Wb microfilaremic residents of an endemic area in Mali were randomized to receive three doses of standard annual A/I therapy (400 mg/150 mcg/kg; n=21) or six doses of twice-yearly increased dose A/I therapy (800 mg/400 mcg/kg; n=19). Mf levels were assessed by Nuclepore filtration of 1 ml of blood and circulating antigen (CAg) levels by TropBio™ ELISA. We have previously reported increased efficacy of twice-yearly high dose treatment in reducing mf counts at 12, 18 and 24 months as compared to standard dose annual therapy with no mf detected in subjects in the twice-yearly group at any time point after 6 months. At 30 months, only 1/17 subjects in the annual group and 0/17 subjects in the twice-yearly group had detectable mf (p=NS). As at prior time points, a significant and comparable decrease in CAg levels was seen in the annual and twice-yearly treatment groups at 30 months with geometric mean (GM) % pre-treatment levels of 74% and 54%, respectively. Thirty-six month followup is planned for July 2010. These findings suggest that increasing the dose and frequency of A/I treatment leads to more rapid suppression of microfilaremia than standard annual therapy and that this effect is not due to an enhanced adulticidal effect. Consequently, twice-yearly high dose treatment is likely to have the greatest benefit in accelerating transmission interruption in regions where mass treatment has been non-existent or suboptimal. Additional studies examining the independent effects of dose and frequency are clearly needed.

1333

A HYDROCELECTOMY PROGRAM FOR LYMPHATIC FILARIASIS IN LÉOGANE, HAITI: CLINICAL INFORMATION AND SURGICAL OUTCOMES

Nicole Kosacz[1], Melanie Strahm[1], Yves Laurissaint[2], Luccene Desir[2], Jacquelin Roberts[3], David Addiss[4], Thomas Streit[5], LeAnne Fox[3]

[1]Emory Rollins School of Public Health, Atlanta, GA, United States, [2]Lymphatic Filariasis Program, Hospital Sainte Croix, Leogane, Haiti, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]The Fetzer Institute, Kalamazoo, MI, United States, [5]The University of Notre Dame, Notre Dame, IN, United States

Lymphatic filariasis (LF) has been endemic in Haiti for over 250 years with a current estimate of 8 million people at risk of infection. In Léogane Commune, up to 30% of adult males suffer from hydrocele, the most common manifestation of chronic LF. Since 2001, a surgical program providing hydrocelectomy has been in operation at Hôpital Sainte Croix and Hôpital Cardinal Légère in Léogane. We assessed clinical data and surgical outcomes for 491 men who underwent hydrocelectomy between 2001 and 2008. Patients ranged in age from 14-85 years (mean, 42 years) and reported an average of 5.6 years with the hydrocele (range, 3 days

- 26 years). Over this eight year period, a total of 792 hydrocelectomies were performed (bilateral hydrocelectomies were counted as two procedures) with the majority (98%) of these procedures utilizing the 'excision technique' where complete excision of the tunica vaginalis is performed. The average hydrocele volume was 510 cc (range 3-2,100 cc) and 116/390 (30%) men were positive for filarial antigen by ICT card test prior to surgery. Variability of hydrocele fluid types were noted intraoperatively including pure hydrocele (n=478) (60.4%), lymphocele (n=269) (34.0%), chylocele (n=23) (2.9%), hematochylocele (n=17) (2.1%), and hematocele (n=5) (0.6%). Only 32/491 (6.5%) men had a negative outcome following surgery, defined as hydrocele recurrence, development of a new hydrocele, post-operative infection, or hematoma formation. Potential predictors of negative clinical outcome will be highlighted and discussed. These results illustrate the clinical variability of filarial hydrocele and the success of the Léogane hydrocelectomy program.

1334

ECONOMIC AND PSYCHOSOCIAL IMPACT OF HYDROCELE AND THE BENEFITS OF HYDROCELECTOMY

Nathaniel Yellu[1], John O. Gyapong[1], Dominique Kyelem[2], Molly Brady[2], Mark Bradley[3], Eric Ottesen[2]

[1]Research and Development Division, Ghana Health Service, Accra, Ghana, [2]Lymphatic Filariasis Support Centre, Task Force for Global Health, Atlanta, GA, United States, [3]GlaxoSmithKline, Brentford, United Kingdom

Hydrocele is a major public health problem in Lymphatic Filariasis (LF) endemic countries. It causes disability and negatively impacts on productivity, quality of life and sexuality. Global estimates suggest 80 countries are endemic for LF, affecting over 120 million people. In highly endemic countries, hydrocele can affect up to 20% of adult males. Globally, little quantitative data exist on the economic and psychosocial effects of hydrocele and the benefits surgery can provide. This study was undertaken in Ghana to provide greater understanding of the psychosocial and economic burden of hydrocele and to facilitate policy formulation. This was a longitudinal study, comprising of pre-surgical, surgical and a series of post surgical evaluation over a two year period. The pre-surgical phase involved identification and recruitment of respondents and confirmation of the presence of hydrocele and the surgical phase involved the provision of surgery. The post surgical phase involved evaluating the clinical outcome of surgery and assessing the economic and psychosocial effects of surgery at predetermined timelines. A modified time series evaluation scheme, where patients are assessed at six points: before surgery, and 3, 6, 12, 18 and 24 months after surgery was adopted. Data collection was done with semi-structured questionnaires and Focus Group Discussions. Of 1,201 men reporting scrotal swellings, 392 were confirmed to have hydrocele. Of these, 323 gave informed consent and were recruited into the study. Post-surgical evaluation identified significant improvement in economic situation (66.8%), performance of daily activities (95.0%), ability to work/engage in income generating activities (88.2%), family life (67.9%), sexual performance (35.2%), quality of life and self reliance among the respondents. The findings also showed significant reduction in personal health related costs as very few respondents sought medical treatment after surgery.

Filariasis predominantly affects rural communities that depend almost entirely on subsistence agriculture. Household economies are extremely fragile and unable to extend to surgery for hydrocele repair. Results of our study clearly show the economic and social benefits of hydrocelectomy and argue strongly for providing access to surgical repair in all programs targeting LF and the Neglected Tropical Diseases.

1336

PLASMODIUM RHOMBOID PROTEASE, ROM3, IS NECESSARY FOR DEVELOPMENT WITHIN THE MOSQUITO VECTOR

Iset Medina Vera[1], Wandy Beatty[2], Photini Sinnis[3], Kami Kim[1]

[1]Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY, United States, [2]Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, United States, [3]Department of Medical Parasitology, New York University School of Medicine, New York, NY, USA

Transmission of the malaria parasite into the mosquito vector occurs when the mosquito ingests gametocytes during a blood meal from an infected host. Within the midgut, ingested gametocytes transform into motile ookinetes that traverse the midgut epithelia and settle within the basal lamina. Nestled in this safe and nutrient-rich environment, the ookinete becomes sessile, rounds up and transforms into the oocyst. The oocyst becomes a spherical syncitium and enlarges to accommodate the thousands of nuclei which will be repartitioned to daughter sporozoites. The process of sporozoite budding within the oocyst is called sporogony. We characterized a rhomboid protease, ROM3 that is specifically expressed in the sexual stages of the malaria parasite. Using the rodent malaria model, *Plasmodium yoelii*, deletion mutants of pyROM3 were generated. We find that *pyrom3-/-* parasites progress normally through gametocytogenesis and gametogenesis leading to oocyst development, but are unable to undergo sporulation. ROM3 is necessary for the production of infectious sporozoites since *pyrom3-/-* parasites are unable to transmit disease to mice. Ultrastructural analysis of *pyrom3-/-* mutant oocysts reveals a defect in the early stages of sporulation prior to sporoblast formation and membrane retraction. The defect is characterized by accumulation of membranous whorls, unusually enlarged nuclei, and an inability to initiate membrane retraction for the onset of sporulation. This study provides novel insights into the function of a rhomboid protease in *Plasmodium* parasites during intracellular development. Further studies into how ROM3 exerts its function for the proper maturation and onset of sporulation should be helpful in the identification of potential substrates whose processing is required for transmission of malaria parasites.

1337

3D ANALYSIS REVEALS COUPLED DYNAMICS OF CHROMATIN AND NUCLEAR PORES IN THE HUMAN MALARIA PARASITE *PLASMODIUM FALCIPARUM*

Noa Dahan-Pasternak[1], Allon Weiner[2], Eyal Shimoni[3], Vera Shinder[3], Michael Elbaum[2], Ron Dzikowski[1]

[1]Department of Microbiology and Molecular Genetics, Hebrew University, Jerusalem, Israel, [2]Department of Materials and Interfaces,Weizmann Institute of Science, Rehovot, Israel, [3]Electron Microscopy Unit, Weizmann Institute of Science, Rehovot, Israel

The deadliest form of human malaria is caused by the protozoan parasite *Plasmodium falciparum*, which is believed to be responsible for millions of death cases each year. The parasite virulence is attributed to its ability to modify the infected erythrocyte by means of antigenic variation, in order to evade immune attack and maintain long-term chronic infections. The complex life cycle of malaria parasites is associated with tight transcriptional regulation of gene expression. Nuclear positioning may play an important role in the regulation of *P. falciparum* virulence genes. In order to investigate the nuclear dynamics in *P. falciparum* we have applied an emerging technique of electron microscopy that provides automated acquisition of serial section images as thin as 10 nm. Thus a single nucleus is spanned typically by 150 sections, allowing direct generation of a 3D model without requiring tomographic reconstruction. We generated 3D models of the parasite nucleus at distinct stages of development within the infected red blood cell. We found dramatic changes in chromatin organization coinciding to a previously-described pattern of gene expression during the mid- to late schizont phase. We also found a clear correlation between euchromatin positioning at the nuclear

envelope and the local distribution of nuclear pores, as well as a dynamic nuclear polarity during schizogony. Our results suggest that dynamics in nuclear architecture during parasite development are correlated with gene expression.

1338

4D-LIFE CELL MICROSCOPY OF ASEXUAL *PLASMODIUM FALCIPARUM* DEVELOPMENT AND HOST CELL MODIFICATIONS

Christof Gruring, Arlett Heiber, Tim-Wolf Gilberger, Tobias Spielmann

Molecular Parasitology, Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany

The visualization of growing *Plasmodium falciparum* blood stage parasites is crucial for the understanding of dynamic cellular processes such as protein transport. However, this has not been achieved so far. We established 4D-imaging (time lapse of 3D reconstructions) of individual *P. falciparum* parasites across the entire asexual blood cycle. Our time lapse movies show an unexpectedly dynamic parasite. It provides a reference for the asexual development cycle that includes indicators for the build up and completion of host cell modifications, onset of feeding and active preparation for egress. Using 4D-imaging we analysed parasite-induced host cell modifications termed Maurer's clefts, structures important for the export of parasite proteins. Our data show that the Maurer's clefts pass through three distinct phases during parasite maturation. Further we show that Maurer's clefts are present much earlier in the cycle than generally thought and that no clefts are generated thereafter. This contradicts the widespread view that there is continued formation of clefts from the parasitophorous vacuole membrane surrounding the parasite. Consequently we find no indication for the proposed protein export via nascent clefts but show that different membrane associated proteins reach already formed clefts located in the host cell cytoplasm. Thus, in addition to providing a new view of the asexual blood development, our work challenges generally held views regarding the formation of Maurer's clefts and protein export in malaria parasites.

1339

SYSTEMIC AND LOCAL CONTROL OF DENDRITIC CELL (DC) POPULATIONS DURING GUT-DWELLING HELMINTH INFECTION: INSIGHTS INTO LOCAL VERSUS BYSTANDER EFFECTS OF CHRONIC INFECTIONS

John Grainger, Jason Hall, Elizabeth Wohlfert, Shruti Naik, Yamine Belkaid

National Institutes of Health, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States

Parasitic worms (helminths) have co-evolved alongside their host's immune system to establish long-term infections. Understanding the immunological basis of these interactions is important given the strong correlations between presence of infection and diminished local (e.g. Crohn's disease), and systemic, allergic or autoimmune conditions; known as the "hygiene hypothesis". Regulatory T-cells. (Treg) play a key role in mediating protection, however, the contribution of other immune cell types, particularly antigen presenting cells (APC), remains unresolved. Using a murine model of chronic gut helminth infection, *Heligmosomoides polygyrus*, we characterize changes to local and systemic APC. We found that the most dramatically increased population in the gut are serosal macrophages. Despite this change, CD103+ lamina propria DC are maintained and the CD103+-DC to CD103--macrophage ratio is unaltered. This correlates with maintenance of Foxp3+ Treg conversion to food antigens even in the presence of Th2-cells, which are considered to be counter-regulatory to this process. Moreover, the CD103+ DC retain their ability to metabolize vitamin A to retinoic acid in the altered environment providing one explanation for the maintenance of Treg conversion. Systemic changes to CD103 expression are also evident; in the spleen CD103 is upregulated on the CD8α+ DC. These data add to our understanding of how APC are altered during establishment of a chronic infection, and suggest that changes in DC populations and function can extend beyond the primary site of parasitism. This provides a previously unrecognized mechanism by which APC-mediated bystander suppression may contribute to inhibition of allergic and autoimmune reactivity.

1340

LARVAL EXCRETORY / SECRETORY PRODUCTS OF THE HELMINTH *TRICHURIS SUIS* MODULATE THE ONSET OF INFLAMMATORY DISEASES

Matthew Hepworth[1], Ping Shen[2], Eckard Hamelmann[3], Simon Fillatreau[2], Richard Lucius[1], Susanne Hartmann[1]

[1]Humboldt University, Berlin, Germany, [2]German Arthritis Center (DRFZ), Berlin, Germany, [3]Charite Hospital, Berlin, Germany

Infections with parasitic helminths are highly prevalent in the developing world and are of particular importance as they have the ability to complicate vaccination and drug efficacy in endemic areas due to their potent immunomodulatory capacity. In recent years epidemiological studies have identified a strong correlation between loss of helminth infections in the western world and the development of autoimmune and inflammatory diseases - leading to a revision of the hygiene hypothesis to include helminths as a critical regulator of immune homeostasis. The ability of helminth-derived molecules to manipulate the immune system and interfere with the development of unrelated diseases thus provides the potential for development of new treatment strategies.

Utilising first stage larvae of the porcine helminth Trichuris suis we isolated potent parasite derived products, which demonstrated an immunodulatory capacity in vitro. Furthermore, treatment with the T. suis products in a mouse model of airway hyperreactivity led to a significant reduction of disease parameters including reduced airway eosinophilia and lymphocyte infiltration, suppressed antigen specific cytokine responses and reduced antigen specific IgE. Similarly T. suis products were effective in the suppression of the development of Experimental Autoimmune Encephalomyelitis (EAE). Thus, products of Trichuris suis represent promising candidates for the development of drugs based on helminthic therapy.

1341

GUT MICROFLORA REGULATES GUT MACROFAUNA: EXPLOITATION OF THE INTESTINAL ECOSYSTEM BY A PARASITIC NEMATODE

K.S. Hayes, **Allison Bancroft**, M. Goldrick, C. Portsmouth, I.S. Roberts, R.k. Grencis

University of Manchester, Manchestr, United Kingdom

Trichuris muris lives in close association with the host, embedding into the caecal epithelium, burrowing through cells and forming syncitial tunnels. We demonstrate for the first time that the *T. muris* life cycle is also intricately linked to the host gut microflora. *T. muris* eggs can be induced to hatch naturally *in vitro* by culturing with bacteria. We have identified the surface protein on gram negative bacteria that can facilitate this hatching. Using genetically modified *E. coli,* Fim H, an adhesin present on type I fimbrae was shown be of major importance. In order to ascertain whether these types of interactions are required *in vivo*, broad spectrum antibiotic treated mice were infected with *T. muris*. We found that antibiotic treatment substantially reduced the number of bacteria in the gut and significantly reduced the establishment of these worms, indicating an effect on hatching. Furthermore, treatment also influenced the normal infection induced immune response mounted by the host, skewing towards a Th2 phenotype. Increased levels of IL-4 and IL-13 and reduced levels of pro inflammatory cytokines and IL-17 were observed. Critical interactions between bacteria (microflora), parasite (macrofauna) and

the host introduce a new and important dynamic to the intestinal niche which has fundamental implications for our current concepts of intestinal homeostasis and regulation of immunity.

1342

HOST IMMUNE RESPONSES TO INFECTION WITH LEISHMANIA GUYANENSIS

Annette Ives, G. Ruzzante, F. Prével, P. Launois, M. Breton, N. Fasel, S. Masina

University of Lausanne, Epalinges, Switzerland

Infections with *L.guyanensis* are distinguished by their ability to disseminate from the initial site of infection to the nasopharyngeal tissues forming destructive secondary lesions. Mucocutaneous Leishmaniasis (MCL) patients have hyper-inflammatory responses with a high degree macrophage and T cell infiltration into the lesion. We show that hamster derived metastatic (M+) or patient derived MCL *L.guyanensis* parasites induce elevated levels of chemokines and cytokines in infected BMMf, namely CXCL10, CCL5, TNF-α, IL-6, and IFN-β as compared to BMMf infected with M-, or (Cutaneous Leishmaniasis) CL or *L. major* LV39 parasites. The induction of these cytokines and chemokines by M+ parasites was completely abrogated in infected TLR3-/- BMMf. *L. guyanensis* parasites can be infected with *Leishmaniavirus* (LRV1) and we detected the 5.3Kb dsRNA genome of LRV1 by gel electrophoresis in M+ or MCL promastigotes and showed by PCR with that M+ and MCL promastigotes have a higher viral load (LRVhigh) than M- or CL (LRVlow). Purified LRV1 dsRNA stimulated increased cytokine and chemokine transcripts in C57BL/6 BMMf, which was significantly diminished in TLR3-/- BMMf. Finally, we show by *in vivo* infection experiments that TLR3-/- mice were more resistant to infection with LRV1high M+ parasites than C57BL/6 mice as shown by a decreased footpad swelling peak associated with a lower parasitemia, whilst there were no observable difference in disease evolution in LRV1low M- parasites. This study shows that there is a correlation between the disseminating capability of parasites, presence of high LRV1 burden, and the induction of inflammation in the mammalian host. Could the presence of high LRV1 viral burden be involved in MCL development in humans?

1343

MULTIPLE ARGININE METHYLTRANFERASES AND METHYLPROTEINS IN THE PARASITIC PROTOZOAN *TRYPANOSOMA BRUCEI*

John C. Fisk[1], Jun Li[2], Danielle L. Tomasello[1], Jun Qu2, Laurie K. Read[1]

[1]Microbiology and Immunology, University at Buffalo, Buffalo, NY, United States, [2]Department of Pharmaceutical Sciences, University at Buffalo, Buffalo, NY, United States

Arginine methylation is a common posttranslational modification catalyzed by a family of enzymes termed protein arginine methyltransferases (PRMTs). In kinetoplastid parasites, gene expression is modulated post-transcriptionally by RNA stabilization, translation, and mitochondrial RNA editing. These processes rely on a large number of RNA binding proteins (RBPs), a class of proteins that are commonly methylated on arginine (arg) residues in yeast and mammals, suggesting a prominent role for PRMTs in kinetoplastids. Kinetoplastids stand out among single celled eukaryotes in that their genomes encode five putative PRMTs, a relatively large number. *In vitro* studies reveal both homologues of yeast and mammalian PRMTs, as well a novel, extraordinarily active Type III PRMT. *In vivo* RNAi studies in *T. brucei* highlight the Type I TbPRMT6 as an essential PRMT with a role in cytokinesis. Other PRMT knockdowns fail to exhibit a growth phenotype in PF, and studies are ongoing in BF. Lack of a growth phenotype may reflect redundancy between PRMTs, as observed in other organisms. We are currently investigating redundancy and cooperation between PRMTs by simultaneous RNAi studies. Regarding PRMT substrates, immunoblotting with anti-methylarg antibodies reveals arg methylproteins enriched in the cytosol, nucleus, and mitochondrion of *T. brucei*. Currently, we are employing combined ETD/CID mass spectrometry to define the *T. brucei* arg methylproteome, and we have already identified methylproteins from many functional classes of proteins. *In vivo* studies are also underway involving the essential RNA editing factor TbRGG2, which is extensively methylated *in vitro* and *in vivo* to determine the role of methylation in its function. Together, these studies will provide insight into the role of arg methylation in kinetoplastid gene regulation.

1344

CELLULAR LOCALIZATION AND FUNCTIONAL CHARACTERIZATION OF A VOLTAGE-DEPENDENT POTASSIUM CHANNEL IN *TRYPANOSOMA CRUZI*

Veronica Jimenez[1], John Wagner[2], Roberto Docampo[1]

[1]Cellular Biology, University of Georgia, Athens, GA, United States, [2]Physiology and Pharmacology, University of Georgia, Athens, GA, United States

Ion homeostasis is a dynamic mechanism that mediates adaptation to environmental and intracellular variations. In *Trypanosoma cruzi*, changes in potassium equilibrium seem to be involved in plasma membrane potential regulation, pH homeostasis, and osmotic balance. We identified, cloned and expressed a *T. cruzi* gene (Tc00.1047053511301.140) encoding a voltage-dependent potassium channel (TcKv). The predicted structure possesses a tetramerization domain and two transmembrane domains, characteristic of inwardrectifier potassium channels. TcKv is expressed in the three life cycle stages of the parasite, with a slightly different subcellular localization, being flagellum-related in trypomastigotes. When expressed in mutant yeast, TcKv restored the normal phenotype, suggesting its function as a K+ permeability pathway. To further characterize this channel the His-tagged protein was expressed in *E. coli*, purified and fused with liposomes in a reconstituted system. Using patchclamp technique we established that TcKv behaves as an inwardrectifier channel, with conductances of 54pS and 112pS at +80 and -80 mV, respectively. The selectivity sequence for monovalent cations is K>Cs>NH4>Rb>Na>Li. The relative permeability ratio K+/Na+ is about 3 and K+/Cl–is close to five, indicating a weak selectivity filter. Permeability for divalent cations was low, showing strong blockage by Ba2+ and Ca2+. Interestingly, when parasites are exposed to hyperosmotic conditions, TcKv localization changes. In epimastigotes it translocates to the plasma membrane, whereas in trypomastigotes is released to the medium, suggesting a differential role of TcKv in *T.cruzi* osmotic response of different stages. In conclusion, we have developed a reliable strategy to characterize ion channels in trypanosomatids, which could contribute to elucidate their physiological roles. [This work was funded in part by the American Heart Association and the NIH]

1345

THE SECRETED PSEUDOKINASE, ROP5, IS CRITICAL TO *TOXOPLASMA* PATHOGENESIS

Michael L. Reese[1], Jon P. Boyle[2], John C. Boothroyd[1]

[1]Microbiology and Immunology, Stanford University, Stanford, CA, United States, [2]Biological Science, University of Pittsburgh, Pittsburgh, PA, United States

Toxoplasma gondii secretes a variety of effector proteins into its host cell cytoplasm during invasion. We have identified one of these factors, the pseudokinase ROP5, as absolutely critical to pathogenesis in mice; while ablation of the ROP5 locus (ΔROP5) causes no phenotype to *Toxoplasma* growth *in vitro*, ΔROP5 parasites are completely unable to cause pathology in a mouse. Furthermore, allelic variation of ROP5 is responsible for a >104 difference in virulence (LD50) in a mouse model of disease. ROP5 appears to exert its profound effect on disease outcome through subversion of the innate immune system; ΔROP5 parasites elicit a significantly stronger pro-inflammatory response during early infection than do wild-type parasites, and appear to be cleared within 10 days post-infection. We

have solved the crystal structure of the pseudokinase domain of ROP5 and have found that polymorphisms in ROP5 between the avirulent and virulent alleles cluster almost exclusively in the substrate binding regions and former active site of the kinase domain, which we have verified as catalytically inactive, though it still maintains its ability to bind ATP. This strongly suggests that differences in virulence are mediated by differences in interaction of binding partners with ROP5's pseudokinase domain. We hypothesize that ROP5 is acting to dysregulate one or more host signaling networks, thereby influencing the outcome of disease. To address this question, we are using phospho-flow cytometry to compare the cellular activation states of the immune systems of mice infected with either wild-type or ΔROP5 parasites. In addition, we are interrogating ROP5's binding partners by co-immunoprecipitation and mass spectrometry.

The number(s) following author name refers to the abstract number.

Author Index

The number(s) following author name refers to the abstract number.

B

Author Index

The number(s) following author name refers to the abstract number.

Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

D

Author Index

The number(s) following author name refers to the abstract number.

E

The number(s) following author name refers to the abstract number.

F

Author Index

The number(s) following author name refers to the abstract number.

G

The number(s) following author name refers to the abstract number.

H

Author Index

The number(s) following author name refers to the abstract number.

I

J

K

Author Index

The number(s) following author name refers to the abstract number.

Author Index

The number(s) following author name refers to the abstract number.

L

The number(s) following author name refers to the abstract number.

M

Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

N

Author Index

The number(s) following author name refers to the abstract number.

O

The number(s) following author name refers to the abstract number.

P

Author Index

The number(s) following author name refers to the abstract number.

Q

R

The number(s) following author name refers to the abstract number.

S

Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

T

Author Index

The number(s) following author name refers to the abstract number.

U

V

The number(s) following author name refers to the abstract number.

W

X

Y

Author Index

The number(s) following author name refers to the abstract number.

Z